ACTA PHARMACEUTICA SCIENCIA

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Formerly: Eczacılık Bülteni / Acta Pharmaceutica Turcica
Founded in 1953 by Kasım Cemal GÜVEN

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Contents

Aims and Scope of Acta Pharmaceutica Sciencia
Gülden Zehra OMURTAG ..... **V**

Instructions for Authors ..... **VI**

EDITORIAL ..... **XXIV**

Exosomes as drug delivery system
F. Julide AKBUGA 1

REVIEW ARTICLE **5**

Historical eponyms of vitamin A (1863–1954)
Halil TEKİNER, Steven H. YALE, Eileen S. YALE 6

ORIGINAL ARTICLES **29**

The effect of decolonization-decontamination prophylaxis versus traditional prophylaxis in orthopedic surgery in Kosovo
Donjetë AHMETAJ, Nilay AKSOY, Barkın BERK 30

Levels of adiponectin, malondialdehyde and lipid profile in women with polycystic ovary syndrome
Zainab FATHI, Zaid YOUNUS, Sameer MAHMOOD, Jehan MOHAMMAD 42

Cladode and fruit anatomy of Opuntia ficus-indica (L.) Miller in Türkiye
Gülsen KENDİR, Ayşegül KÖROĞLU 54

Metabolic profiling of Brachychiton rupestris (T.Mitch. ex Lindl.) K. Schum. leaves using UPLC-ESI-MS and their antimicrobial potential
Heba Raafat MOHAMED, Eman Ahmed EL-WAKIL, Maher Mahmoud EL-HASHASH, Mohamed SHEMIS, El-Sayed Saleh ABDEL-HAMEED 69

Banana peels a contemptible source of dietary fiber and natural antioxidants
Muhammad Khalid SAEED, Naseem ZAHRA 89

Assessment of patients' adherence to antihypertensive therapy in a teaching hospital in Ogun State, Nigeria
Olutayo ADELEYE, Tolulope AREMU, Henry OKERI 104

Physical/chemical modifications of Oryza glaberrima and Digitaria exilis starches: Effect on packing and compression properties of Ibuprofen tablet formulations
Omobolanle OMOTESO, Michael ODENIYI 122

The impact of an education program on the appropriate prescription of proton pump inhibitors in hospitalized internal medicine services patients
Yunus AYHAN, Cüneyd ENVER, Betül OKUYAN, Çağlayan KEKLİKKIRAN, Abdulmünir AZIZY, Tuba Kıratlı YOLCU, Osman Cavit ÖZDOĞAN, Mesut SANCAR 145

Examination of the relationship between skin autofluorance and lifestyle habits in young adults
Burak ERİM, Gülgün ERSOY.....159

Phytochemical profile and antioxidant activity potential of Lotus sanguineus (Vural) D.D.Sokoloff
Fatma TOSUN, Sümeyye ALBAYRAK, Esra ACAR ŞAH, Ömer ÇEÇEN.....172

In vitro anti-inflammatory activities of Tanacetum parthenium L. extract and its major metabolite parthenolide
Rengin BAYDAR, Ayşe Esra KARADAĞ, Sevde Nur BİLTEKİN, Etil GÜZELMERİÇ, Fatih DEMİRCİ.....183

Dietary habits, physical activity, sleep duration, and their association with overweight and obesity among children aged 6-10
Halime Pulat DEMİR, Hatice Merve BAYRAM.....193

The relationship between healthy living-style behaviors and type-2 diabetes risk of students of health sciences
Ayşe Hümeyra İSLAMOĞLU, Gamze SELÇUK, Meliha AKPINAR, Berfin ARAS, Zehra Margot ÇELİK, Fatma Esra GÜNEŞ.....210

Monitoring the charge variant profile of antibody-tomaymycin conjugates by icIEF method
Ayat ABBOOD.....226

Aims and Scope of Acta Pharmaceutica Sciencia

Aims and Scope of Acta Pharmaceutica Sciencia

Acta Pharmaceutica Sciencia is a continuation of the former "Eczacılık Bülteni" which was first published in 1953 by Prof. Dr. Kasım Cemal GÜVEN's editorship. At that time, "Eczacılık Bülteni" hosted scientific papers from the School of Medicine-Pharmacy at İstanbul University, Türkiye.

In 1984, the name of the journal was changed to "Acta Pharmaceutica Turcica" and it became a journal for national and international manuscripts, in all fields of pharmaceutical sciences in both English and Turkish. (1984-1995, edited by Prof. Dr. Kasım Cemal GÜVEN, 1995-2001, edited by Prof. Dr. Erden GÜLER, 2002-2011, edited by Prof. Dr. Kasım Cemal GÜVEN)

Since 2006, the journal has been published only in English with the name, "Acta Pharmaceutica Sciencia" which represents internationally accepted high-level scientific standards. The journal has been published quarterly except for an interval from 2002 to 2009 in which its issues were released at intervals of four months. The publication was also temporarily discontinued at the end of 2011 but since 2016, Acta Pharmaceutica Sciencia has continued publication with the reestablished Editorial Board and also with the support of you as precious scientists.

Yours Faithfully
Prof. Dr. Gülden Zehra OMURTAG
Editor

INSTRUCTIONS FOR AUTHORS

Manuscripts must be prepared using the manuscript template.

Manuscripts should contain the following elements in the following order:

Title Page

Abstract

Keywords

Introduction (without author names and affiliations)

Methodology

Results and Discussion

Statement of Ethics

Conflict of Interest Statement

Author Contributions

Funding Sources (optional)

Acknowledgments (optional)

References

It is best to use the Times New Roman font, 11 font size, and all kinds of articles must be 1.5 spaced including text, references, tables, and legends.

The title should be concise and informative. Avoid abbreviations and formulae, where possible. The title page should include full title, author names and affiliations, present addresses, corresponding author, and ORCID numbers for every author. Also, the full manuscript should include a full title page.

Abstracts should not be separated into categories; it should be written in a paragraph format.

Keywords: Max. 5

Graphics may be included with both in the text and uploaded as separate files.

Sections: (Capital letters should be used in) Introduction, Methodology, Results and Discussion, Statement of Ethics, Conflict of Interest Statement, Author Contributions, Funding Sources (optional), Acknowledgments (optional).

Table and figure titles should not be abbreviated exp. fig. is not acceptable. It should be written as; Table 1. .... Figure 1. .....

Figure captions: A caption should comprise a brief title (not on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used. Figure captions should be written on the bottom.

Titles: Number tables consecutively by their appearance in the text and place any table notes below the table body. Table captions should be written on the top.

References in the text should be identified using Arabic numerals. Years of the references should not be written boldly. More than one reference from the same author(s) in the same year must be identified by the letters “a”, “b”, “c”, etc., placed after the year of publication. References should conform to Vancouver style and be numbered consecutively in the order in which they are cited in the text.

*Obligatory files are manuscript main document, title page and copyright form for submission. If exist, supplementary files should also be added.

1. Scope and Editorial Policy

1.1 Scope of the Journal

Acta Pharmaceutica Sciencia (Acta Pharm. Sci.), formerly known as Bulletin of Pharmacy and Acta Pharmaceutica Turcica is a peer-reviewed scientific journal publishing current research and reviews covering all fields of pharmaceutical sciences since 1953.

The original studies accepted for publication must be unpublished work and should contain data that have not been published elsewhere as a whole or a part. The reviews must provide critical evaluation of the state of knowledge related with the subject.

All manuscripts have to be written in clear and concise English.

Including the October 2023 issue, the journal has started to be published online only. It will also publish special issues for national or international scientific meetings and activities in the interested field.

1.2 Manuscript Categories

Manuscripts can be submitted as Research Articles.

Research Articles are definitive accounts of significant, original studies. They are expected to present important new data or provide a fresh approach to an established subject.

1.3 Prior Publication

Authors should submit only original work that has not been previously published and is not under consideration for publication elsewhere. Academic theses, including those on the Web or at a college Web site, are not considered to be prior publication.

1.4 Patents and Intellectual Property

Authors need to resolve all patent and intellectual property issues. Acceptance and publication will not be delayed for pending or unresolved issues of this type. Note that Accepted manuscripts and online manuscripts are considered published documents.

1.5 Professional Ethics

Editors, reviewers, and authors are expected to adhere to internationally accepted criteria for scientific publishing. Helsinki declaration is applied and accepted for the ethical standards of the journal.

World Medical Association. (2001). World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. Bulletin of the World Health Organization, 79(4),373-374.

1.5.1 Author Consent

Submitting authors are reminded that consent of all coauthors must be obtained prior to submission of manuscripts. If an author is removed after submission, the submitting author must have the removed author consent to the change by e-mail or faxed letter to the assigned editor.

1.5.2 Plagiarism

Manuscripts must be original with respect to concept, content, and writing. It is not appropriate for an author to reuse wording from other publications, including one's own previous publications, whether or not that publication is cited. Suspected plagiarism should be reported immediately to the editorial office. Report should specifically indicate the plagiarized material within the manuscripts. Acta Pharmaceutica Sciencia uses iThenticate or Turnitin software to screen submitted manuscripts for similarity to published material. Note that your manuscript may be screened during the submission process.

1.5.3 Use of Human or Animal Subjects

For research involving biological samples obtained from animals or human subjects, editors reserve the right to request additional information from au-

thors. Studies submitted for publication approval must present evidence that the described experimental activities have undergone local institutional review assessing safety and humane usage of study subject animals. In the case of human subjects, authors must also provide a statement that study samples were obtained through the informed consent of the donors, or in lieu of that evidence, by the authority of the institutional board that licensed the use of such material. Authors are requested to declare the identification or case number of institution approval as well as the name of the licensing committee in a statement placed in the section describing the Material and Methods utilized in the studies.

World Medical Association. (2001). World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. Bulletin of the World Health Organization, 79(4),373-374.

1.6 Issue Frequency

The Journal publishes 4 issues per year.

2. Preparing the Manuscript

2.1 General Considerations

Manuscripts should be kept to a minimum length. Authors should write in clear, concise English, employing an editing service if necessary. For professional assistance with improving English and/or the figures, or formatting in the manuscript before submission please contact to editorial office by e-mail for suggestions.

The responsibility for all aspects of manuscript preparation rests with the authors. Applying extensive changes or rewriting of the manuscript will not be undertaken by the editors. A standard list of Abbreviations, Acronyms, and Symbols is in section 5.

It is best to use the font “Times New Roman”. Other fonts, particularly those that do not come bundled with the system software, may not translate properly. Ensure that all special characters (e.g., Greek characters, math symbols) are present in the body of the text as characters and not as graphic representations. Be sure that all characters are correctly represented throughout the manuscript—e.g., 1 (one) and l (letter l), 0 (zero) and O (letter o).

All text (including the title page, abstract, all sections of the body of the paper, figure captions, scheme or chart titles, and footnotes and references) and tables should be in one file. Graphics may be included with the text or uploaded as separate files. Manuscripts that do not adhere to the guidelines may be returned to authors for correction.

2.1.1 Articles of All Kind

Use page size A4. Vertically orient all pages. Articles of all kind must be double-spaced including text, references, tables, and legends. This applies to figures, schemes, and tables as well as text. They do not have page limitations but should be kept to a minimum length. The experimental procedures for all experimental steps must be clearly and fully included in the experimental section of the manuscripts.

2.1.2 Nomenclature

It is the responsibility of the authors to provide correct nomenclature. It is acceptable to use semisynthetic or generic names for certain specialized classes of compounds, such as steroids, peptides, carbohydrates, etc. In such a case, the name should conform to the generally accepted nomenclature conventions for the compound class. Chemical names for drugs are preferred. If these are not practical, generic names, or names approved by the World Health Organization, may be used.

Authors may find the following sources useful for recommended nomenclature:

- The ACS Style Guide; Coghill, A. M., Garson, L. R., Eds.; American Chemical Society: Washington DC, 2006.
- Enzyme Nomenclature; Webb, E. C., Ed.; Academic Press: Orlando, 1992.
- IUPHAR database of receptors and ion channels (http://www.guidetopharmacology.org/).

2.1.3 Compound Code Numbers

Code numbers (including peptides) assigned to a compound may be used as follows:

- Once in the manuscript title, when placed in parentheses AFTER the chemical or descriptive name.
- Once in the abstract.
- Once in the text (includes legends) and once to label a structure. Code numbers in the text must correspond to structures or, if used only once, the chemical name must be provided before the parenthesized code number, e.g., “chemical name (JEM-398).” If appearing a second time in the text, a bold Arabic number must be assigned on first usage, followed by the parenthesized code number, e.g., “1 (JEM-398).” Subsequently, only the bold Ara-

bic number may be used. All code numbers in the text must have a citation to a publication or a patent on first appearance.

Compounds widely employed as research tools and recognized primarily by code numbers may be designated in the manuscript by code numbers without the above restrictions. Their chemical name or structure should be provided as above. Editors have the discretion of determining which code numbers are considered widely employed.

2.1.4 Trademark Names

Trademark names for reagents or drugs must be used only in the experimental section. Do not use trademark or service mark symbols.

2.1.5 Interference Compounds

Active compounds from any source must be examined for known classes of assay interference compounds and this analysis must be provided in the General Experimental section. Many of these compounds have been classified as Pan Assay Interference Compounds (PAINS; see Baell & Holloway, J. Med. Chem. 2010, 53, 2719-2740). These compounds shown to display misleading assay readouts by a variety of mechanisms by forming reactive compounds. Provide firm experimental evidence in at least two different assays that reported compounds with potential PAINS liability are specifically active and their apparent activity is not an artifact.

2.2 Manuscript Organization

2.2.1 Title Page

The title of the manuscript should reflect the purposes and findings of the work in order to provide maximum information in a computerized title search. Minimal use of nonfunctional words is encouraged. Only commonly employed abbreviations (e.g., DNA, RNA, ATP) are acceptable. Code numbers for compounds may be used in a manuscript title when placed in parentheses AFTER the chemical or descriptive name.

Authors' Names and Affiliations: The authors' full first names, middle initials, last names (with capital letters for only last names), and affiliations with addresses at time of work completion should be listed below the title. The name of the corresponding author should be marked with an asterisk (*).

2.2.2 Abstract and Keywords

Articles of all types must have an abstract following the title page. The maximum length of the Abstract should be 200 words, organized in a findings-oriented format in which the most important results and conclusions are sum-

marized. Code numbers may be used once in the abstract. After the abstract, a section of Keywords not more than five has to be given. Be aware that the keywords, chosen according to the general concept, are very significant during searching and indexing of the manuscripts.

Keywords: instructions for authors, template, journal

2.2.3 Introduction

The Introduction should argue the case for the study, outlining only essential background, and should not include the findings or the conclusions. It should not be a review of the subject area but should finish with a clear statement of the question being addressed. Authors should use this template when preparing a manuscript for submission to the ACTA Pharmaceutica Sciencia.

2.2.4 Methodology

Materials, synthetic, biological, demographic, statistical or experimental methods of the research should be given detailed in this section. The authors are free to subdivide this section in the logical flow of the study. For the experimental sections, authors should be as concise as possible in experimental descriptions. General reaction, isolation, preparation conditions should be given only once. The title of an experiment should include the chemical name and a bold Arabic identifier number; subsequently, only the bold Arabic number should be used. Experiments should be listed in numerical order. Molar equivalents of all reactants and percentage yields of products should be included. A general introductory section should include general procedures, standard techniques, and instruments employed (e.g., determination of purity, chromatography, NMR spectra, mass spectra, names of equipment) in the synthesis and characterization of compounds, isolates and preparations described subsequently in this section. Special attention should be called to hazardous reactions or toxic compounds. Provide analysis for known classes of assay interference compounds.

The preferred forms for some of the more commonly used abbrevations are mp, bp, ^{o}C, K, min, h, mL, μL, g, mg, μg, cm, mm, nm, mol, mmol, μmol, ppm, TLC, GC, NMR, UV, and IR. Units are abbreviated in table column heads and when used with numbers, not otherwise. (See section 4 for more abbreviations)

2.2.5 Results and Discussion

This section could include preparation, isolation, synthetic schemes and tables of biological and statistical data. The discussions should be descriptive. Authors should discuss the analysis of the data together with the significance of results and conclusions. An optional conclusions section is not required.

2.2.6 Ancillary Information

Include pertinent information in the order listed immediately before the references.

PDB ID Codes: Include the PDB ID codes with assigned compound Arabic number. Include the statement "Authors will release the atomic coordinates and experimental data upon article publication."

Homology Models: Include the PDB ID codes with assigned compound Arabic number. Include the statement "Authors will release the atomic coordinates upon article publication."

Corresponding Author Information: Provide telephone numbers and email addresses for each of the designated corresponding authors.

Present/Current Author Addresses: Provide information for authors whose affiliations or addresses have changed.

Author Contributions: Include statement such as "These authors contributed equally."

Acknowledgments: Authors may acknowledge people, organizations, and financial supporters in this section.

Abbreviations Used: Provide a list of nonstandard abbreviations and acronyms used in the paper, e.g., YFP, yellow fluorescent protein. Do not include compound code numbers in this list. It is not necessary to include abbreviations and acronyms from the Standard Abbreviations and Acronyms listed in section 4.

2.2.7 References and Notes

Vancouver style is used in the reference list and citations. List manuscripts as "in press" only accepted for publication. Manuscripts available on Web with a DOI number are considered published. For manuscripts not accepted, use "unpublished work" after the names of authors. Incorporate notes in the correct numerical sequence with the references. Footnotes are not used. List submitted manuscripts as "in press" only if formally accepted for publication. Manuscripts available on the Web with a DOI number are considered published. For manuscripts not accepted, use "unpublished results" after the names of authors. Incorporate notes in the correct numerical sequence with the references. Footnotes are not used. In-text citations should be given superscript numbers (see examples) according to order in the manuscript.

References

Please check with your faculty for any specific referencing or formatting requirements.

- References are listed in numerical order, and in the same order in which they are cited in text. The reference list appears at the end of the paper.
- Begin your reference list on a new page and title it 'References'.
- The reference list should include all and only those references you have cited in the text. (However, do not include unpublished items such as correspondence.)
- Use Arabic numerals (1, 2, 3, 4, 5, 6, 7, 8, 9) as a superscripts.
- Abbreviate journal titles in the style used in the NLM Catalog.
- Check the reference details against the actual source – you are indicating that you have read a source when you cite it.
- Use of DOI URL at the end of reference is strongly advised.

Examples

For printed articles

• Article with 1-6 authors:

Author AA, Author BB, Author CC, Author DD. Title of article. Abbreviated title of journal, Date of publication YYYY;volume number(issue number):page numbers.

Sahin Z, Ertas M, Berk B, Biltekin SN, Yurttas L, Demirayak S. Studies on non-steroidal inhibitors of aromatase enzyme; 4-(aryl/heteroaryl)-2-(pyrimidin-2-yl)thiazole derivatives. Bioorg Med Chem, 2018; 26(8): 1986–1995. https://doi.org/10.1016/j.bmc.2018.02.048.

• Article with more than 6 authors:

Author AA, Author BB, Author CC, Author DD, Author EE, Author FF, et al. Title of article. Abbreviated title of journal, Date of publication YYYY Mon DD;volume number(issue number):page numbers.

For electronic journal articles

Author AA, Author BB, Author CC, Author DD, Author EE, Author FF. Title of article. Abbreviated title of Journal [Internet], Year of publication [cited YYYY Mon DD];volume number(issue number):page numbers. Available from: URL DOI

For books and book chapters

Book: a.) Print book OR b.) Electronic book

a.) Author AA. Title of book. # edition [if not first]. Place of Publication: Publisher; Year of publication. Pagination.

b.) Author AA. Title of web page [Internet]. Place of Publication: Sponsor of Website/Publisher; Year published [cited YYYY Mon DD]. Number of pages. Available from: URL DOI: (if available)

2.2.8 Tables

Tabulation of experimental results is encouraged when this leads to more effective presentation or to more economical use of space. Tables should be numbered consecutively in order of citation in the text with Arabic numerals. Footnotes in tables should be given italic lowercase letter designations and cited in the tables as superscripts. The sequence of letters should proceed by row rather than by column. If a reference is cited in both table and text, insert a lettered footnote in the table to refer to the numbered reference in the text. Each table must be provided with a descriptive title that, together with column headings, should make the table self-explanatory. Titles and footnotes should be on the same page as the table. Tables may be created using a word processor's text mode or table format feature. The table format feature is preferred. Ensure each data entry is in its own table cell. If the text mode is used, separate columns with a single tab and use a return at the end of each row. Tables may be inserted in the text where first mentioned or may be grouped after the references.

2.2.9 Figures, Schemes/Structures, and Charts

The use of illustrations to convey or clarify information is encouraged. Structures should be produced with the use of a drawing program such as ChemDraw. Authors using other drawing packages should, in as far as possible, modify their program's parameters so that they conform to ChemDraw preferences. Remove all color from illustrations, except for those you would like published in color. Illustrations may be inserted into the text where mentioned or may be consolidated at the end of the manuscript. If consolidated, legends should be grouped on a separate page(s). Include as part of the manuscript file.

To facilitate the publication process, please submit manuscript graphics using the following guidelines:

1. The preferred submission procedure is to embed graphic files in a Word document. It may help to print the manuscript on a laser printer to ensure all artwork is clear and legible.

2. Additional acceptable file formats are: TIFF, PDF, EPS (vector artwork) or CDX (ChemDraw file). If submitting individual graphic files in addition to them being embedded in a Word document, ensure the files are named based on graphic function (i.e., Scheme 1, Figure 2, Chart 3), not the scientific name. Labeling of all figure parts should be present and the parts should be assembled into a single graphic.

EPS files: Ensure that all fonts are converted to outlines or embedded in the graphic file. The document settings should be in RGB mode. NOTE: While EPS files are accepted, the vector-based graphics will be rasterized for production. Please see below for TIFF file production resolutions.

3. TIFF files (either embedded in a Word doc or submitted as individual files) should have the following resolution requirements:

- Black & White line art: 1200 dpi
- Grayscale art (a monochromatic image containing shades of gray): 600 dpi
- Color art (RGB color mode): 300 dpi
- The RGB and resolution requirements are essential for producing high-quality graphics within the published manuscript. Graphics submitted in CMYK or at lower resolutions may be used; however, the colors may not be consistent and graphics of poor quality may not be able to be improved.
- Most graphic programs provide an option for changing the resolution when you are saving the image. Best practice is to save the graphic file at the final resolution and size using the program used to create the graphic.

4. Graphics should be sized at the final production size when possible. Single column graphics are preferred and can be sized up to 240 points wide (8.38 cm.). Double column graphics must be sized between 300 and 504 points (10.584 and 17.78 cm's). All graphics have a maximum depth of 660 points (23.28 cm.) including the caption (please allow 12 points for each line of caption text).

Consistently sizing letters and labels in graphics throughout your manuscript will help ensure consistent graphic presentation for publication.

2.2.10 Image Manipulation

Images should be free from misleading manipulation. Images included in an account of research performed or in the data collection as part of the research require an accurate description of how the images were generated and produced. Apply digital processing uniformly to images, with both samples and

controls. Cropping must be reported in the figure legend. For gels and blots, use of positive and negative controls is highly recommended. Avoid high contrast settings to avoid overexposure of gels and blots. For microscopy, apply color adjustment to entire image and note in the legend. When necessary, authors should include a section on equipment and settings to describe all image acquisition tools, techniques and settings, and software used. All final images must have resolutions of 300 dpi or higher. Authors should retain unprocessed data in the event that the editors request them.

2.3 Specialized Data

2.3.1 Biological Data

Quantitative biological data are required for all tested compounds. Biological test methods must be referenced or described in sufficient detail to permit the experiments to be repeated by others. Detailed descriptions of biological methods should be placed in the experimental section. Standard compounds or established drugs should be tested in the same system for comparison. Data may be presented as numerical expressions or in graphical form; biological data for extensive series of compounds should be presented in tabular form.

Active compounds obtained from combinatorial syntheses should be resynthesized and retested to verify that the biology conforms to the initial observation. Statistical limits (statistical significance) for the biological data are usually required. If statistical limits cannot be provided, the number of determinations and some indication of the variability and reliability of the results should be given. References to statistical methods of calculation should be included.

Doses and concentrations should be expressed as molar quantities (e.g., mol/kg, μmol/kg, M, mM). The routes of administration of test compounds and vehicles used should be indicated, and any salt forms used (hydrochlorides, sulfates, etc.) should be noted. The physical state of the compound dosed (crystalline, amorphous; solution, suspension) and the formulation for dosing (micronized, jet-milled, nanoparticles) should be indicated. For those compounds found to be inactive, the highest concentration (*in vitro*) or dose level (in vivo) tested should be indicated.

If human cell lines are used, authors are strongly encouraged to include the following information in their manuscript:

- the cell line source, including when and from where it was obtained;
- whether the cell line has recently been authenticated and by what method;

- whether the cell line has recently been tested for mycoplasma contamination.

2.3.2 Purity of Tested Compounds

Methods: All scientifically established methods of establishing purity are acceptable. If the target compounds are solvated, the quantity of solvent should be included in the compound formulas. No documentation is required unless asked by the editors.

Purity Percentage: All tested compounds, whether synthesized or purchased, should possess a purity of at least 95%. Target compounds must have a purity of at least 95%. In exceptional cases, authors can request a waiver when compounds are less than 95% pure. For solids, the melting point or melting point range should be reported as an indicator of purity.

Elemental Analysis: Found values for carbon, hydrogen, and nitrogen (if present) should be within 0.4% of the calculated values for the proposed formula.

2.3.3 Confirmation of Structure

Adequate evidence to establish structural identity must accompany all new compounds that appear in the experimental section. Sufficient spectral data should be presented in the experimental section to allow for the identification of the same compound by comparison. Generally, a listing of 1H or 13C NMR peaks is sufficient. However, when the NMR data are used as a basis of structural identification, the peaks must be assigned.

List only infrared absorptions that are diagnostic for key functional groups. If a series contains very closely related compounds, it may be appropriate merely to list the spectral data for a single representative member when they share a common major structural component that has identical or very similar spectral features.

3. Submitting the Manuscript

3.1 Communication and Log in to Author's Module

All submissions to Acta Pharmaceutica Sciencia should be made by using e-Collittera (Online Article Acceptance and Evaluation) system on the journal main page (www. actapharmsci.com).

3.2 Registration to System

It is required to register into the e-Collittera system for the first time while entering by clicking "Create Account" button on the registration screen and the

fill the opening form with real information. Some of the information required in form is absolutely necessary and the registration will not work if these fields are not completely filled.

After the registration, a "Welcome" mail is sent to the user by the system automatically reminding user name and password. Authors are expected to return to the entry screen and log on with their user name and password for the submission. Please use only English characters while determining your username and password.

If you already registered into the e-Collittera system and forget your password, you should click on "Forgot My Password" button and your user name and password will be mailed to your e-mail in a short while.

3.3 Submitting a New Article

The main page of author module consists of various parts showing the situation of manuscripts in process. By clicking the New Manuscript button, authors create the beginning of new submission, a process with a total of 9 consecutive levels. In first 7 levels, information such as the article's kind, institutions, authors, title, summary, keywords etc. are asked respectively as entered. Authors can move back and forth while the information is saved automatically. If the is transaction is discontinued, the system move the new submission to "Partially Submitted Manuscripts" part and the transaction can be continued from here.

3.1.1 Sort of Article Authors should first select the type of article from the drop-down menu.

Warning. If "Return to Main Page" button is clicked after this level, the article automatically assigned as "Partially Submitted Manuscripts".

3.2.2 Institutions Authors should give their institutional information during submission.

3.2.3 Authors The authors' surnames, names, institutional information appear as entered order in the previous page. Filling all e-mail addresses are required. Institutional information is available in Manuscript Details table at the top of the screen. After filling all required fields, you may click the Continue button.

3.2.4 Title should be English, explaining the significance of the study. If the title includes some special characters such as alpha, beta, pi or gamma, they can easily be added by using the Title window. You may add the character by clicking the relevant button and the system will automatically add the required character to the text.

Warning. No additions to cornered parenthesis are allowed. Otherwise, the system will not be able to show the special characters.

3.2.5 Abstract The summary of the article should be entered to Abstract window at this level. There must be an English summary for all articles and the quantity of words must be not more than 200. If special characters such as alpha, beta, pi or gamma are used in summary, they can be added by Abstract window. You may add the character by clicking the relevant button and the system will automatically add the required character to the text. The abstract of the articles is accessible for arbitrators; so, you should not add any information related to the institutions and authors in this summary part. Otherwise, the article will be returned without evaluation. Authors will be required to comply with the rules.

Warning. No additions to cornered parenthesis are allowed. Otherwise, the system will not be able to show the special characters.

3.2.6 Keywords There must be five words to define the article at the keywords window, which will be diverged with commas. Authors should pay attention to use words, which are appropriate for "Medical Subjects Headings" list by National Library of Medicine (NLM).

3.2.7 Cover Letter If the submitting article was published as thesis and/or presented in a congress or elsewhere, all information of thesis, presented congress or elsewhere should be delivered to the editor and must be mentioned by the "Cover Letter" field.

3.3.1 Adding Article This process consists of four different steps beginning with the loading of the article in to system. Browse button is used to reach the article file, under the Choose a file to upload tab. After finding the article you may click to Choose File and file will be attached.

Second step is to select the file category. Options are: Main Document, Black and White Figure, Color Figure and Video.

The explanation of the files (e.g., Figure 1, Full Text Word File, supplements etc.) should be added on third step and the last step is submitting the prepared article into the system. Therefore, Download button under the Send your file by clicking on download button tab is clicked.

Reminder. If the prepared article includes more than one file (such as main document, black and white figure, video), the transaction will be continued by starting from the first step. The image files must be in previously defined format. After all required files were added, Continue button should be clicked.

All details and features of the article might be reached from the Article Information page.

This page is the last step of the transaction which ensures that entered information is controlled.

3.3.2 Your Files After adding the article you may find all information related to article under Your Files window.

File Information This window includes file names, sizes, forming dates, categories, order numbers and explanations of files. The details about the files can be reached by clicking on Information button.

If you click on Name of File, the file download window will be opened to reach the copy of the file in system.

File Download This window submits two alternatives, one of them is to ensure the file to be opened in valid site and the second one is to ensure to download submitted file into the computer.

Opening the Category part on fourth column can change the category of the file.

Opening the Order column on fifth column can change the order of file.

The file can be deleted by clicking on Delete button on the last column. Before deleting, system will ask the user again if it is appropriate or not.

3.3.3. Sending Article Last level is submitting the article and the files into the system. Before continuing the transaction, Article Information window must be controlled where it is possible to return back; by using Previous button and required corrections can be made. If not, clicking the Send the Article button completes transaction.

3.3.4 Page to Follow the Article The Main Page of Author ensures possibility to follow the article. This page consists of three different parts; some information and bridges related to the sent articles, revision required articles and the articles that are not completed to be sent.

3.3.4.1 Articles Not Completed to be Sent After the sending transaction was started, if article is not able to continue until the ninth step or could not be sent due to technical problems shown at this part. Here you can find the information such as the article's number which is assigned by system, title and formation date. You may delete the articles by using Delete button on the right column, if the article is not considered to send into the system.

3.3.4.2 Articles that Require Revision Articles, which were evaluated by the referee and accepted by the editor with revision, continues to Waiting for Revision table.

The required revisions can be seen in "Notes" part by clicking the articles title.

In order to send any revision, Submit Revision button on the last column should be clicked. This connection will take the author to the first level of Adding Article and the author can complete the revision transaction by carrying out the steps one by one. All changes must be made in the registered file, and this changed file must be resent. Author's most efficacious replies relating to the changes must be typed in "Cover Letter" part.

If the is transaction is discontinued, the system move the revised article to Submitted Manuscripts part and the transaction can be continued from here.

After the transaction was completed, the system moves the revised article to "Submitted Manuscripts" part.

3.3.5 Submitted Manuscripts Information related to articles can be followed through the Submitted Manuscripts line. Here you can find the information such as the article's number assigned by system, title, sending date and transaction situation. The Manuscript Details and summary files can be reached by clicking the title of the article and the Processing Status part makes it possible to follow the evaluation process of the article.

Article Review Process

Articles uploaded to the Manuscript submission system are checked by the journal administration for format consistency and similarity rate which is required to be less than 20%. Then sent to the chief editor if found appropriate.

Articles that are not suitable are sent back to the author for correction and resubmit (sent back to the author). Studies that have not been prepared using the draft for submitting to Acta Pharmaceutica Sciencia "acta_msc_tmp" and that have not been adapted in terms of format, will be directed to the editor-in-chief, after the 3rd time, by giving the information that "the consistency requirements have not been met".

The manuscripts sent to the chief editor will be evaluated and sent to the "language and statistics editor" if deemed appropriate.

Studies found appropriate after language-statistics editor will be sent to field editors. If the field editor does not deem it appropriate after evaluating the article scientifically, he/she will inform the editor-in-chief of its negative com-

ments, otherwise, at least two independent referee comments will be asked.

Authors should consider that this time may take time because of the reviewer assignments and acceptance for review may take time for some cases.

Our review system is double-blind. The editor, who evaluates according to the comments of the referees, submits his/her comment and suggestion to the editor-in-chief. In this way, the article takes one of the acceptance, rejection, or revision decisions. In the case of revision, after the author revises, the editor submits his/her final opinion to the editor-in-chief. The editor-in-chief conveys his or her final decision to the author. After the accepted articles are subjected to the final control by the journal and the corresponding author, the article starts to be included in the "accepted papers" section by giving the inactive DOI number. When the article is placed in one of the following issues, the DOI number will be activated and displayed in the "current issue" section on the journal homepage.

EDITORIAL

Exosomes as drug delivery system

Editorial Article

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There are many factors that need to be taken into consideration when converting an active substance into a drug, such as the properties of the molecule, biological barriers, pathological condition, and technical problems.

However, the most important among these factors is the transportation of the molecule with pharmacological activity to the required region with an appropriate carrier system. The carriers developed for the active substance to date have different problems and cause difficulties in treatment. These include those that pose serious problems in drug administration, such as the blood-brain barrier, in reaching the active substance to the brain.

The main purpose of drug delivery systems is the entry of the therapeutic molecule into the cell. Today new dosage forms such as liposomes and polymeric nanoparticles are the most preferred drug delivery systems. Nevertheless, the circulatory capacity and stability of the liposomal system as well as its ability to invade the host immune system without toxicity remain elusive. Although polymeric nanoparticles solve the stability problem, their toxicity and biocompatibility remain a significant problem, especially when non-biodegradable polymers are used. Secreted membrane vesicles, which are essentially nature-derived liposomes, can potentially overcome some of the limitations of synthetic liposomes, such as the toxicity of lipid membranes. Among the different secreted membrane vesicles, exosomes are the most clearly defined and are most amenable to development as drug delivery vehicles.

Extracellular vesicles are cell-derived nanoparticles that are important mediators in intercellular communication. This function makes them auspicious candidates for therapeutic and drug-delivery applications and this function makes them convenient candidates for therapeutic and drug delivery applications.

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Among the most highly researched extracellular vesicles are exosome. Recent studies show that exosomes derived from cells have different roles and targets.

Exosomes, have intercellular material derived from endosomes of parent cells. Exosomes have a wide variety of components, such as, heat shock proteins surface proteins, lysosomal proteins, tumor-responsive gene, fusion proteins, and nucleic acids, each with specific functions. Proteins exhibit a distinct function in biogenesis and transport mechanisms in exosomes. These are phospholipid bilayer microparticles with a size of 50-100 nm.

Exosomes have many characteristics of an ideal drug delivery vehicle. First, the presence of proteins and genetic materials in exosomes means that such biological materials can be loaded into exosomes. Secondly, exosomes are well tolerated in the body and their distribution in biological fluids such as blood, urine and breast milk has also been demonstrated.

Therefore, exosome-derived drug delivery systems will likely be better tolerated, resulting in longer circulating half-life and increased efficacy. Third, exosomes have been shown to cross the plasma membrane to deliver their cargo to target cells. Fourth, exosomes have an inherent ability to target tissues. Much circumstantial evidence suggests that exosomes have preferential homing targets depending on their cellular source. Finally, exosomes are suitable for membrane modifications that enhance cell type-specific targeting.

Although most cell types produce exosomes, but the amount of exosomes produced by each cell type is variable. Exosomes express cell recognition molecules on their surface that facilitate selective targeting and uptake by recipient cells. The process of exosomes entering cells occurs by transferring their signals to the cell through a 3-step mechanism; receptor interaction, membrane fusion and endocytosis/phagocytosis. Currently, different methods are applied for exosome isolation; differential centrifugation, filtration, size-exclusion chromatography, and polymer precipitation.

An exosome-based delivery system has specific benefits such as specificity, stability, and safety, they can deliver their cargo to specific targets over long distances. Exosomes can also be used to deliver small and large molecules such as proteins and peptides. Exosomes naturally transport nucleic acids such as DNA, RNA, and siRNA to targeted cells and cause genetic modifications in both biological and pathogenic processes. Researchers have shown that doxorubicin loaded exosomes were readily up taken by cells and re-distributed. In another study, exosomal system evaluated for delivery of paclitaxel. Exosome-based carrier systems have been used for gemcitabine in the treatment of pan-

creatic cancer and for dopamine in the treatment of Parkinson's disease. For exosomes to be used effectively as drug delivery systems, drugs must be efficiently loaded into exosomes. They are introduced into exosomes via two ways: active or passive loading/encapsulation.

On the contrary traditional nanoparticulate system, exosomes can possibly avoid the endosomal pathway and lysosomal degradation and deliver cargos directly to the cytoplasm.

If exosomes are to be used as drug delivery carriers on a large scale, large-scale isolation, and separation of exosomes with high purity is important. However, it is not yet possible to isolate and separate exosomes in high purity on a large scale. This is an important challenge. Exosomes role in disease must be investigated in detail to enable clinical translation. Few clinical studies using exosomes as drug delivery systems are ongoing.

Recent literature shows continued exploration and promise of exosomes as drug delivery carriers for various diseases including solid tumors, bone regeneration, cardiac diseases, Parkinson's amongst others.

Briefly, it can be said that exosomes as a drug delivery system with minimal toxicity, biocompatibility, tissue and tumor targeting, and long circulating half-life is appearing as a superior choice, overcoming the shortcomings of liposomes or polymeric nanoparticles.

Keywords: exosome, delivery vehicle, nanoparticles, extracellular vesicles, drug

REFERENCES

1. Sun D, Zhuang X, Zhang S, Deng ZB, Grizzle W, Miller D, et al. Exosomes are endogenous nanoparticles that can deliver biological information between cells. Adv Drug Deliv Rev, 2013;65(3):342-347. Doi: 10.1016/j.addr.2012.07.002

2. Ratajczak MZ, Ratajczak J. Extracellular microvesicles/exosomes: discovery, disbelief, acceptance, and the future? Leukemia, 2020;34(12):3126-3135. Doi: 10.1038/s41375-020-01041-z

3. Richter M, Vader P, Fuhrmann G. Approaches to surface engineering of extracellular vesicles. Adv Drug Deliv Rev, 2021;173:416-426. Doi: 10.1016/j.addr.2021.03.0204

4. Kooijmans SA, de Jong OG, Schiffelers RM. Exploring interactions between extracellular vesicles and cells for innovative drug delivery system design. Adv Drug Deliv Rev, 2021;173:252-278. Doi: 10.1016/j.addr.2021.03.017

5. Gutierrez-Millan C, Calvo Díaz C, Lanao JM, Colino CI. Advances in exosomes-based drug delivery systems. Macromol Biosci, 2021;21(1):2000269. Doi: 10.1002/mabi.202000269

REVIEW ARTICLES

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Historical eponyms of vitamin A (1863–1954)

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ABSTRACT

The discovery of vitamin A by Socin in 1891 and its isolation in crystalline form by Holmes and Corbet in 1937 paved the way for further discoveries involving its role in storage, photoreceptors in the eye, the cornea, epithelium, and normal immune function. Various symptoms and physical findings have been recognized and eponymously named a syndrome in honor of the person(s) who first reported those clinical entities about vitamin A. Seeking to fill the historical gap in the literature, the focus of this paper is to describe the eponymic cells, diseases, observations, syndromes, or tests ascribed to hypovitaminosis and hypervitaminosis A. In a chronological sequence based on the initial publication year of related reference, we presented concise biographical data concerning the scientist(s) responsible for recording the eponym, along with the presentation of the original depiction of the sign. We identified 12 eponyms related to vitamin A that were described between 1863 and 1954. These eponyms were named after 17 scientists from nine countries. Among them, Marie and Sée described the phenomena occurring in infants with hypervitaminosis A. The detailed and comprehensive description of the cornea or retina in vitamin A deficiency by Bitot, Lobo, Petzetakis-Tzakos, and Uemura remains relevant today.

Keywords: avitaminosis, biography, deficiency disease, eponyms, vitamins

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INTRODUCTION

The discovery of vitamin A by Carl A. Socin in 1891, its isolation in crystalline form by Harry N. Holmes and Ruth E. Corbet in 1937, and synthesis by Otto Isler and colleagues in 1947 paved the way for further discoveries involving its role in storage, photoreceptors in the eye, the cornea, epithelium, and normal immune function[1-3]. Additionally, various symptoms and physical findings have been recognized and, in some cases, eponymously named a syndrome in honor of the person(s) who first reported those clinical entities about vitamin A. Seeking to fill the historical gap in the literature, the focus of this paper is to describe the eponymic cells, diseases, observations, syndromes, or tests ascribed to hypovitaminosis and hypervitaminosis A. Included is a brief overview of the discovery of vitamin A and the refinement of the nomenclature surrounding the vitamins in general.

Historical background

Physicians have long recognized the relationship between diseases, including night blindness and scurvy, and their treatment by consuming raw beef liver by Hippocrates (460-370 BC) and lemon juice by the Dutch and Portuguese in the 16th century[4,5]. Little was known about vitamin A and other vitamins before the early twentieth century. Carl Andreas Socin (1966–1933) in 1891 and Wilhelm Otto Stepp (1882–1964) in 1911 identified an unknown substance or fat soluble factor necessary for growth[1,6]. Sir Frederick Gowland Hopkins (1861–1947) recognized the deficiency of a "dietetic factor" and the onset of diseases including scurvy and rickets and presented those findings at the meeting of the Society of Public Analysts in 1906[7]. Hopkins in 1912 also demonstrated that rats fed a mixture of pure protein, fats, carbohydrates, and salts did not grow[8]. Growth occurred when the rats were fed small quantities of raw milk, suggesting that a substance or "accessory factor" in the milk promoted growth[8]. Casmir Funk (1884–1967) introduced the general term "vitamine" to define a substance deficient in scurvy and beriberi and preventing the onset of disease[9]. Elmer Verner McCollum (1879–1967) and Marguerite Davis (1887–1967) in 1913 found that laboratory rats grew when ether-soluble egg yolk or butter extracts were added to a diet containing purified proteins, carbohydrates, fats, and salts contained accessory factors that are essential for promoting normal growth and development[10].

McCollum and Cornelia Kennedy (1881–1969) in 1916 challenged the use of the term "vitamine" by Funk based on their view that it did not accurately describe the underlying substance[11]. Their opinion was based on the lack of evidence that these substances contained an amino group and that the prefix "vita" refers to that the substance is essential and thus confers a mistaken impression

that they supersede other essential and relevant biological processes[11]. In lieu of the word "vitamin" they suggested that the term fat-soluble and water soluble be used in reference to those unknown substances necessary for growth. Furthermore, based on this classification, alphabetical letters can be assigned and added as fat-soluble and water-soluble are recognized in each class[11]. In 1920, Drummond recommended a revision of the nomenclature to address the discrepancy within the literature regarding the naming of these substances. He recommended dropping the "e" in "vitamine" such that the term "vitamin" now be recognized as the standard nomenclature used by the Chemical Society with the letter designation (A, B, C) to follow[12].

METHODOLOGY

We conducted word searches in PubMed, Medline, Internet search engines, medical dictionaries, and bibliographies from textbooks, using specific Medical Subject Headings (MeSH) related to the names of eponyms and keywords associated with vitamins. Our inclusion criteria focused on eponyms associated solely with vitamin A, including studies on the cell, observations, syndromes, and tests, narrowing the focus of the content of this paper. In a chronological sequence based on the initial publication year of related reference, we presented concise biographical data concerning the scientist(s) responsible for recording the eponym, along with the presentation of the original depiction of the sign.

RESULTS and DISCUSSION

We identified 12 eponyms related to vitamin A named after 17 scientists from nine countries: Brasilia, Canada, Denmark, England, France, Greece, Japan, Spain, and the United States between 1863 and 1954 (Table 1). Only two eponyms (Bitot spot and Brazilian ophthalmia) were identified before the 20th century. Dorothy Hansine Andersen (1901-1963), an American pediatrician and pathologist, is the only female eponym namesake.

Bitot spots

Pierre Alain Bitôt (1822-1888) was born in Podensac, France, completed medical school in Bordeaux, France, and received his medical degree from the Faculty of Paris, France, in 1848[13]. He served as head of anatomy at the Bordeaux School of Medicine in 1848 and professor of anatomy the following year[14]. He was appointed doctor at the Charité in 1849, an assistant surgeon in 1852, professor and chair of anatomy at Bordeaux School of Medicine in 1854, titular surgeon of hospitals in 1856, chief of the surgical services at the Military Hospital in 1865, medical inspector of the department services for the mentally ill, head of the children's surgical clinic in 1878, and honorary professor of

the faculty of medicine, Bordeaux in 1879[13,14]. He was named to the Medical Society of Bordeaux in 1850. He co-founded the Medical and Surgical Society of Bordeaux in 1866[13].

While professor of anatomy at the Bordeaux School of Medicine and honorary surgeon of the Hospital of Bordeaux, Bitôt identified a collection of bright white dots producing a pearly or silvery spot on the epithelial layer of the conjunctiva adjacent to the cornea in patients with night blindness[15]. Bitôt in 1863 provided a detailed description of the characteristics of these spots. Included are some of the following excerpts:

> While standing in front of the patient, we can distinguish them quite well when the eyes are converged. (...) They appear as an aggregate of small dots or thin, short lineaments resembling a half-frozen white foam patch. When the spots are observed, their color varies slightly and is apparent in each person. As they disappear, their whiteness fades. (...) The spots are generally triangular, with an external vertex; the base close to the cornea is slightly concave but may be circular, oval, or linear. The particles composing it are often agglomerated to constitute a punctuated, rough surface. They are frequently arranged in series or flexuous, parallel lines, making them appear wavy or wrinkled. These various forms can be modified by applying pressure using one or two fingers on the eyelids. These changes in form appear to give the impression that the spots are not conjoined but juxtaposed and displaceable by reducing a spot to a simple line or a vertical or horizontal bundle, then reforming it immediately by flattening this beam by moving the eyelids in the opposite direction (p. 287)[15].

Bitôt is credited for providing the first comprehensive clinical description of the ocular findings in nyctalopia (night blindness), the condition later known to be caused by vitamin A deficiency (Table 1).

Brazilian ophthalmia

Manoel da Gama Lobo (1831-1883) was born in the province of Monte Alegre, Pará, Brazil, and received his medical degree from the Faculty of Medicine in Rio de Janeiro, Brazil, in 1858[16,17]. He was a physician in the War Arsenal of the Court in Rio de Janeiro and pursued ophthalmology training in Germany. He returned to Germany in 1872, seeking additional training in ophthalmology and histology[16]. He served as head of the eye service at Santa Casa da Misericórdia, Brazil, and was a member of the Imperial Academy of Medicine in 1863[17].

Gama Lobo, in 1865, described the ocular findings of enslaved children aged two to seven, which he referred to as “Brazilian ophthalmia”:

> In my opinion, is one of the manifestations of a general condition and is only present when the person is already in an extremely deteriorated state. In addition to chronic bronchitis, liver congestion, and large voluminous diarrhea, the patient presents in a cachectic state, succumbing to marasmus. (...) It is a chronic type of disease that begins to manifest itself in the conjunctiva extending to the choroid and retina (p. 16)[18].

He described three stages of the disease. Salient features reported during the second stage of the disease are as follows:

> The palpebral conjunctiva is of a deep reddish-purple color and covered with small elevations. The ocular conjunctiva is grayish-white due to the movements made by the eye. It is covered with rugosities, giving the appearance of small ripples on the surface like water driven by the wind. From the sclerocorneal insertion until the palpebral reflection, the conjunctiva is devoid of vessels, and the one or two observed, exist on the surface of the sclera. From the beginning of the oculo-palpebral reflection, the vessels resemble the deep purple-reddish soil color. The papilla is normal and well-defined during ophthalmoscopy, and the vessels can be followed to their last branches. However, its color is a whitish red. In this period, secretions of tears pass over the eye but fail to moisten it, and thus it appears smeared with very fine globes of fat (p. 17)[18].

During the third stage, the conjunctiva is dry and grayish, with wrinkles on the bulbar conjunctiva occurring during eye movement. The cornea contains a round ulcer with a hypopyon in the eye's anterior chamber[18]. Gama Lobo was unaware of the etiology of the ocular finding but keenly observed that the diet provided to enslaves people in different Brazilian provinces accounted for disparities in their overall health and mortality, corneal ulceration, and night blindness (nyctalopia) (Table 1). Thus, Brazilian ophthalmia, also known as xeropthalmia, occurs secondary to vitamin A deficiency or hypovitaminosis A.

Carr-Price reaction

Francis Howard Carr (1874-1969) was born in Croydon, England, and received his education at Finsbury Technical College from 1889 to 1892 and Guilds College, London[19]. He served as a research assistant to Wyndham Rowland Dunstan (1861-1949) at the Pharmaceutical Society between 1892 and 1896, then at the Imperial Institute[20]. Carr received the Salter's Research Fellowship from 1894 to 1898[19,20]. He was chief manufacturing chemist at Burroughs, Wellcome & Company from 1898 to 1914 and Director and Chief Chemist at Boots Pure Drug Company from 1914 to 1919. He assisted in establishing the Associa-

tion of British Manufactures in 1916[20]. Carr was awarded the Commander of the Order of the British Empire in 1920. He was a Fellow of Imperial College, Chairman of the British Drug Houses, and President of the Society of Chemical Industry and Association of British Chemical Manufacturers in 1926[19]. He received a D.Sc. degree from Manchester University in 1929[20].

There is limited information on Ernest Arthur Price (1882-1956)[21]. He was born in Oxford, England, and at the time of his joint paper with Carr titled "Colour reactions attributed to vitamin A," served at the Laboratories of The British Drug House, London[22].

Carr and Price, in 1926, described a quantitative method for measuring vitamin A using a colorimetric technique:

> We were naturally led, therefore, to make experiments with a view to improving the technique of the colour tests in order to find conditions whereby quantitative and strictly comparable readings may be obtained. This work has resulted in our utilizing for the purpose yet another reagent of a similar character, namely, a solution of antimony trichloride in chloroform. By its use we have been enabled to determine standard conditions whereby direct readings of the intensity of the colour may be taken with the help of tintometer (p. 497)[22]. (...) [w]e found a 30% solution of antimony trichloride in chloroform (weight in volume), decidedly the most suitable and convenient. Its advantages are: 1) the oil and solvent do not need to be perfectly dry or entirely free from alcohol. (In many of the tests either water or alcohol interferes with the colour). 2) the colour is an intense blue, slightly more intense and more permanent than that produced by arsenic trichloride. It is very much better in this respect than the colour produced by any other reagent we have tried. 3) it may be depended on to produce the same intensity of colour with the same oil on very occasion. 4) as compared with arsenic trichloride the reagent is innocuous; it is, however, somewhat corrosive to the skin (p. 499)[22].

Thus, the Carr-Price reaction was a quantitative method for measuring retinol or vitamin A (Table 1). Current analysis of vitamin A includes measurement of serum retinol alone or in combination with B-carotene[23].

Uemura disease

Misao Uemura (Uyemura) (1900-1997) received his medical degree from the Faculty of Medicine, Keio University, Japan, in 1925 and his MD degree in 1929[24]. At Keio University, Tokyo, he served as an assistant professor from 1931 to 1941 and as professor and chair of the department of ophthalmolo-

gy from 1941 to 1961[24]. He was director of the University Hospital from 1957 to 1959, dean of the School of Medicine from 1959 to 1961, director of Tokyo Second National Hospital from 1961 to 1971, and director of Ryuky University Hospital, Nishihara, Okinawa, from 1971 to 1975[24]. He also served as executive director of the Japanese Ophthalmology Society[24].

Uemura was president of the Japanese Association of Illumination from 1961 to 1965, a member of the International Council of Ophthalmology from 1958 to 1970, the Medical Ethic Council of the Ministry of Health and Welfare from 1967 to 1969, and the Council for Medical College Evaluation of the Ministry of Education and Culture from 1961 to 1965[24]. He received the Ichikawa Award from the Japanese Ophthalmological Society and the Second Order of the Rising Sun for distinguished government service[24].

Uemura, in 1928 described the fundoscopic findings in two patients with hemeralopia[25]. One patient had Bitot spots on the bulbar conjunctiva while in both, as fully described in one case during ophthalmoscopic examination:

> The delicate gray-white clouded background is densely dotted with innumerable yellowish-white dots on both sides except for the macular regions and in the vicinity of the papilla. These dots are most dense in the equatorial region and look somewhat elongated, as if short rods had been driven obliquely into the retina. Toward the periphery, their number gradually decreases, and at the same time, they appear more prominent and polygonal (p. 472)[25].

Uemura disease refers to reversible white spots in the deep retinal layer, occurring in patients with avitaminosis caused by vitamin A deficiency (Table 1).

Friderichsen test

Carl Friderichsen (1886-1979) was born in Copenhagen, Denmark, and received his medical degree from the University of Copenhagen in 1912[26]. He served as a physician at the Children's Department of the Rigshospitalet and was a superintendent of the children's department at Sundby Hospital, Copenhagen, Denmark[26]. Friderichsen was chair of the Danish Pediatric Society from 1828-1934[27]. His name is best recognized eponymously along with Rupert Waterhouse (1873-1958), who described the findings of bilateral adrenal hemorrhages and degeneration of the adrenal glands associated with cutaneous hemorrhages (Waterhouse-Friderichsen syndrome)[28-30].

Friderichsen, in 1937, devised a bedside clinical test to determine whether there are adequate stores of vitamin A in children less than two years of age[31]. The method measures the reflex irritability of the eyes to light and involves:

> Flashing a light in a child's eyes after it has been in the dark for at least half an hour, the "minimum relexibile" (m.r.) being the smallest light irritation capable of provoking certain reflex movements through the child's eyes, wrinkling of the forehead, and an upward movement of the eyebrows when the light comes from above. Or the movements may be prompted by an oculomotor reflex, the eyes moving very quickly in the direction of the source of light. Or there may be other movements such as rotation of the head in the direction of the light or snatching at the light[31].

Friedrichsen and Edmund in 1937 found that a low minimum relexibile may be associated with either a low absorption or dietary insufficiency of vitamin A (Table 1). Moreover, through this test, they were able to quantitate the international units of various preparations of vitamin A[32].

Andersen syndrome

Dorothy Hansine Andersen (1901-1963) was born in Asheville, North Carolina, and received her medical degree from Johns Hopkins University School of Medicine in 1926[33]. She was an assistant in anatomy at Rochester School of Medicine from 1926 to 1927 and completed a surgical internship at Strong Memorial Hospital, Rochester, New York, from 1927-1928[34,35]. Andersen was a faculty member of Columbia University College of Physicians and Surgeons (currently Columbia University Vagelos College of Physicians and Surgeons) as an instructor in pathology in 1929[33,35]. She received a doctorate of medical science degree in endocrinology. She was appointed assistant pathologist at the Babies Hospital Columbia-Presbyterian Medical Center in 1935, ascending to the rank of chief of pathology in 1952 and professor of pathology at Columbia University College of Physicians and Surgeons in 1958[34-37]. Her active medical and research interest was in the area of cystic fibrosis[34].

Andersen was a member of several national organizations, including the American Board of Pathology, the American Association of Pathologists and Bacteriologists, the American Academy of Pediatrics, the American Society of Experimental Pathology, and the College of American Pathologists[33]. She served as honorary chair of the National Cystic Fibrosis Research Foundation on the general medical and scientific advisory council[33,36]. She was the recipient of several awards and accolades, including the Edward Mead Johnson Award of Pediatrics in recognition of her discovery of cystic fibrosis, and Borden Award for research in nutrition in 1948, and a citation from Mount Holyoke College in 1952, Elizabeth Blackwell for Women in Medicine, New York Infirmary in 1954, and a posthumous distinguished service award from Columbia-Presbyterian Medical Center in 1963[33,35-37].

Cecil Clarke (1886-1925) and Geoffrey Hadfield (1899-1968) were the first to report the postmortem findings in a four-year-old with steatorrhea and atrophy of the pancreas in 1924:

> The gland was represented by fat, and a fraction of normal gland tissue estimated at one-twentieth. The surviving pancreatic tissue was active but appeared to be undergoing slow replacement fibrosis; it contained islet tissue in more than the normal amount; there was no clear evidence of pancreatitis (p. 364)[38].

Andersen, in 1938, identified in 44 cases of cystic fibrosis of the pancreas that 23% had severe vitamin A deficiency, and more mild degrees may have been present in the remainder[39]. She found that:

> The frequent occurrence of vitamin A deficiency was probably due to the failure of absorption of this vitamin. It is suggested that the pulmonary infection was possibly secondary to vitamin A deficiency (p. 382)[39]. (...) There is a good deal of evidence to show that, at least in the majority of cases, the pancreatic lesion comes first. (...) The evidence at hand suggests that the pancreatic lesion prevents the normal digestion and absorption of fats, the poor absorption of fats results in poor absorption of the fat-soluble vitamin A, and epithelial metaplasia, bronchiectasis, and bronchopneumonia are consequences of vitamin A deficiency (p. 399)[39].

Andersen not only identified and named the disease cystic fibrosis but recognized the deficiency of vitamin A in this disease secondary to malabsorption and the essential role of vitamin A in epithelial development and the immune response (Table 1)[39].

Vilanova-Cañadell syndrome

Xavier Vilanova i Montiu (1902-1965) was born in Barcelona, Spain, and received his medical degree from the Central University of Barcelona in 1923 and doctorate in 1928[40]. He continued his training at St. Louis Hospital in Paris; Pasteur Institute and Curie Institute in Strasbourg; and Milan, and finally, Aguas de Dios leprosy clinic in Colombia in 1936[40,41]. He was appointed professor and first chair of dermatology and venereology at the University of Valladolid, Spain, in 1942, chair at the University of Valencia, Spain, in 1942, and chair of dermatology at the Faculty of Medicine at the University of Barcelona, Spain in 1947[40-42].

He was president of the Spanish Academy of Dermatology and Venereology, a member of Valencia and Catalonia Royal Academies of Medicine in 1944 and 1950, an officer of the Order of Public Health, and a corresponding member of

the French National Academy of Medicine[41,42]. He founded the Ibero-Latino-American College of Dermatology, serving as president in 1962, and the Spanish Academy of Dermatology in 1963[43]. He received from the Brazilian Society of Dermatology the International Dermatologic Merit Medal[44].

Josep Maria Cañadell (1915-1997) was born in Reus, Spain, and enrolled at the University of Barcelona Medical School from 1933 to 1934, which was interrupted because of the Spanish Civil War (1936-1939)[42]. During that time, he was a lieutenant in the Military Health Corps in Husca and Manresa in Spain. He completed his medical studies graduating from the University of Barcelona in 1941[42]. He continued his studies in Boston, Massachusetts, and Rochester, New York, from 1947 to 1948 and in London, Oxford, and Paris the following year[42]. He served as a professor of medicine in the Faculty of Medicine as director of the endocrinology department in Clínica Médica B at the University of Barcelona. He taught postgraduate courses in endocrinology beginning in 1948[42].

Cañadell was a corresponding academic member of the Spanish Academy of Medicine, an honorary member of the Academy of Medicine, Turin, and an elected member of the Royal Academy of Medicine, Balearic Islands[42]. He was an Officer of the Order of Public Health, France. He co-founded with Mário Cardia and was co-Editor-in-Chief of the journal *Acta Endocrinologica et Gynecologica Hispano-Lusitana* which later became *Acta Endocrinologica Iberia*[42]. He also served on the editorial board as its editor from 1943 to 1956. He was the recipient of the Order of the Star of Italian Solidarity[42].

Vilanova and Cañadell, in 1949, reported on the relationship between hypothyroidism, vitamin A deficiency, and dermopathy:

> Among our cases of severe and untreated thyroid insufficiency, especially in infantile and juvenile hypothyroidism, we have frequently observed cutaneous findings corresponding to those caused by avitaminosis A. Clinically, these alterations manifest in the form of xeroderma, starting with dermatosis due to dryness and roughness of the skin, which is better felt than seen during its early stage. Furthermore, an eruption of perfectly delimited elements, with a horny appearance, the size of pinheads, sits on the opening points of the pilosebaceous follicles corresponding dermatologically to keratosis pilaris. It is typically located on the back of the forearms and the anterior and lateral aspects of the legs. Only in the most severe cases do the lesions extend to the arms, shoulders, back, thighs, and the remainder of the body. The lesions are almost always absent on the face, neck, and scalp. When looking at

> these patients, it could be difficult to determine if the dermopathy is due to the thyroid or a vitamin A deficiency. The majority of subjects with these skin lesions heal slowly with thyroid opotherapy or even faster by administering vitamin A, even when the corresponding provitamin is completely ineffective[45].

Thus, they identified the permissive effect of thyroid hormone on converting provitamin A to the active form of vitamin A (Table 1).

Bassen-Kornzweig syndrome

Frank Albert Bassen (1903-2003) was born in St. George, Nova Scotia, Canada, and received his medical degree from McGill University in 1928[46]. He interned at Jersey City Medical Center, New Jersey, from 1928 to 1930 and was a medical resident at Sinai Hospital, Maryland, Baltimore, from 1930 to 1933. He served as an adjunct professor in hematology and was appointed clinical assistant in medicine at Mt. Sinai Hospital, New York[47]. During World War II, he served as Captain and Lt. Colonel Marine Corps, returning to Mount Sinai Hospital in 1946[46,47].

Abraham Leon Kornzweig (1900-1982) was born in New York City, New York, and received his medical degree from New York University (NYU) Medical School in 1925[48]. He completed an internship at Mount Sinai Hospital from 1925 to 1928 and returned for additional residency training in ophthalmology[48]. He served at NYU-Bellevue Postgraduate Medical School, where he taught embryology of the eye, achieving the rank of associate clinical professor, followed by appointment as a clinical and emeritus professor of ophthalmology at Mount Sinai School of Medicine, New York[48]. He was chief of ophthalmology and director of research at the Jewish Home and Hospital for the Aged in New York. His research interests were ocular problems in older adults. He was instrumental in founding The Society of Geriatric Ophthalmology[48].

Bassen and Kornzweig in 1950 described the case of an 18-year-old female with atypical pigmentary degeneration of the retina with macula involvement, oscillating nystagmus, ataxia, sensory neuropathy, high arch palate, epicanthal fold and male-type of pubic escutcheon[49]. An unusual finding which had not been previously reported was identified on a peripheral blood smear in the patient and her brother:

> The count, in general, was quite normal except for a slight anemia. Great numbers of the stained red cells, however, revealed unusual abnormalities in shape and, to some extent, in size. (...) No two cells looked exactly alike. In general, they presented a crenated appearance but of such

> that they took on bizarre shapes, stimulating small beetles, crabs, and turtles. Others were star shaped. The variations depended on the number and length of what appeared to be appendages growing out of the cells. Some of the cells appeared small and deeply stained. They resembled spherocytes from which buds or pseudopods were protruding, and these cells in particular varied from ordinary crenation (p. 385)[49].

The unusual shape of the erythrocytes is referred to as acanthocytosis. The red cell membrane appeared rough or prickly; hence it was called a "burr cell." The syndrome is also referred to as abetalipoproteinemia in recognition of the lack of serum B lipoprotein resulting in defective intestinal absorption and transport of fat and fat-soluble vitamins, including vitamin A. The principal clinical manifestations of this syndrome include ocular and central nervous system defects and steatorrhea (Table 1).

Petzetakis-Tzakos syndrome

Limited historical information is identified on Michel Petzetakis (1899-1975). He was an associate professor of pathology at the University of Athens, Greece[50]. At the time of his publication, he was chief physician at the General Hospital in Athens[51].

Konstantinos Tzakos (1903-1984) was born in Pogoni, Greece, and relocated to Istanbul with his family in 1908. He completed his education at the Phanar (Fener) Greek Orthodox College in Istanbul before pursuing a medical degree at Athens University[52]. Under the supervision of Benediktos Adamantiadis (1875–1962), he gained practical experience at the trauma clinic of Polygonos and the Hippocrates Hospital. As a recipient of a national scholarship, Tzakos studied at the Military Medical School in Lyon, France, with the understanding that he would later serve as a military doctor in the Greek army. While in Lyon, he specialized in ophthalmology and completed his dissertation[52]. From 1929 to 1930, he served as an assistant at the Ophthalmology Clinic of the University of Lyon. Subsequently, he underwent further training at the Ophthalmology Clinic of the Val-de-Grâce Military Hospital in Paris. Following his return to Greece, he fulfilled his mandatory service obligations[52].

In 1941, Tzakos established and organized the Ophthalmology Clinic at the General State Hospital of Athens, where he served as Director until his retirement in 1968[52]. In 1947, he was appointed a professor of ophthalmology at the University of Athens. His considerable research on "Ocular changes during pellagra" is widely regarded as a seminal work[52]. His studies entitled "Ocular changes due to edema and pellagra" as "Traumatic lachrymalitis" gained inter-

national recognition and are regarded as classic texts in the field of ophthalmology. However, his most significant contribution to Greek ophthalmology was his four-volume publication titled *Ophthalmology* (1954–1962), which included his illustrations[52].

Referring to Tzakos's previous work, Petzetakis, in 1950 described in patients, ocular and systemic symptoms following the famine during the Nazi occupation of Greece from 1941 to 1944:

> These ocular disorders, which we noted from the beginning of the famine, prompted us to study them more methodically with M. Tzakos, and it is in this way that we have described a particular form of keratitis, which I studied first from an anatomo-pathological point of view, under the name of superficial trophopenic keratitis. It was found in 85% of cases with generalized edema and 15% in dry forms (p. 1082)[51].

He identified in superficial tropopenic keratitis (epithelial keratopathy), the following symptoms caused by vitamin deficiencies:

> 1) Palpebral edema, 2) Edema of the bulbar conjunctiva, 3) Hypoaesthesia of the cornea, 4) Decreased iris reflexes, 5) Decreased tear secretion, 6) Alterations of the precorneal layer of Rollet; 7) Corneal disorders. These findings, studied using different instruments, are as follows: i. frequent edema of the anterior layers of corneal epithelium; ii. very fine granulation, which are of different shapes and sizes and sometimes raised (bulged) from the surface cornea; iii. small ulcerations of different shapes and sizes which are predominately superficial and polymorphic. The combination of several lesions sometimes gives a meandering or geographical map of these superficial ulcerations (p. 1082)[51].

In addition to vitamin A, B, and C deficiencies, he observed hypoproteinemia, hypolipidemia, hypoglycemia, and alterations in the central and peripheral nervous system (Table 1)[51].

Jacobs syndrome

Eugene Coryell Jacobs (1905-2000) was born in Schenectady, New York, and received his medical degree from the University of Michigan Medical School in 1929[53,54]. He served in the Army Medical Reserve Corps in 1934 at Walter Reed Hospital as Chief of the Gastrointestinal Section[55,56]. During World War II, he was Captain and Commanding Officer of Camp John Hay, Philippines[54]. He was the chief of medical service in the Japanese Prisoner of War (POW) Camp No. 1 Hospital in Cabanatuan province of Nueva Ecija, Philippines, from

1942 to 1944[56]. He was a prisoner of war at Moji Military Hospital in Fukuoka, Japan, and later at Camp Hoten in Mukden, Manchuria prison camps in 1945 and liberated from the camp that same year[53]. He achieved the rank of Colonel, Medical Corps, US Army, Washington, DC, by 1965[55].

Jacobs was the recipient of the Medical Combat Badge, the Distinguished Unit Citation, the Legion of Merit, the Bronze Star, the Army Commendation Pendant, the Purple Heart, the Philippine Presidential Unit Citation, the George Washington Honor Medal, and US Army and Medical Service Medallion[55]. For his research, he was recognized and received the Henry Wellcome Medal and Prize for "the most useful original investigation in the field of military medicine"[54]. He also published *Blood Brothers: A Medic's Sketch Book* in 1985[57].

Jacobs described the constellation of symptoms among prisoners of war in the Cabanatuan Prison Camp in 1942. He coined the term oculo-genital syndrome to define this entity which consisted of:

> 1) A deficiency disease comprised of an exfoliating dermatitis of the scrotum, stomatitis, and conjunctivitis insidiously appeared in more than 75 percent of 8,000 American prisoners-of-war after six months of an inadequate rice diet.
>
> 2) The syndrome was quickly and markedly improved by two months of an adequate diet.
>
> 3) On return to an inadequate rice diet, the syndrome was far less prevalent, indicating some adaption of the body to lowered caloric and vitamin intake.
>
> 4) The vitamin requirements of the body appeared to be less after a loss of considerable body weight.
>
> 5) The syndrome did not develop on a minimal diet composed of corn and soybeans.
>
> 6) The syndrome is thought to be closely associated with pellagra but not pellagra per se (p. 1053)[58].

In addition to the symptoms mentioned earlier, some prisoners also developed amblyopia (optic atrophy) and burning of the feet (sensory peripheral neuropathy). He attributed the amblyopia to vitamins A and B_1 deficiency (thiamine) and sensory peripheral neuropathy to vitamin B_1 deficiency[58]. Even though he recognized scrotal dermatitis, angular cheilitis, and stomatitis were caused by deficiencies involving the vitamin B complex, Jacob was unable to determine the specific deficiencies involved[58]. This may be attributed to multiple vitamin

deficiencies caused by malnutrition and overlapping symptom presentations. The oculo-oro-urogenital syndrome has since been recognized to be caused by a polyhypovitamininosis due to deficiencies involving B_2 or riboflavin and B_6 or pyridoxine deficiencies and manifesting as angular cheilitis, stomatitis, conjunctivitis, and scrotal dermatitis[59]. Amblyopia from optic atrophy can be caused by vitamin A and B_1 deficiencies[59].

Ito cells

Toshio Ito (1904-1991) was born in Aichi, Japan[60]. He completed postgraduate studies in anatomy at Keio University Faculty of Medicine and received his doctorate in 1936[61]. He was an assistant from 1930 to 1932, an instructor from 1932 to 1941, and an assistant professor from 1934 to 1941 at Keio Medical School[62]. Ito was a professor of anatomy at Tokyo Women's Medical College from 1941 to 1947 and at Gunma University School of Medicine, Mayebashi, from 1954 to 1970, serving as dean from 1961 to 1963[60-62].

Ito and Nemoto in 1952 identified cells in the blood capillary wall in the human liver, which was later recognized to be the fat storage cell containing vitamin A (Table 1):

> In 1950, while studying stellate cells, one of us accidentally discovered hitherto unknown cells in the capillary wall, and due to the fact that they mostly contained small fat globules in varying numbers, he named them "fat-storing cells" and published about it in 1950 in the 55th Assembly of the Japanese Anatomical Society. Through further investigation of these cells, we have come to the conclusion that morphologically they represent a quite distinct type of cell from the stellate cells (p. 243–4)[63].

As to the morphological location of these cells, they are located within the latticework of the capillary wall on the surface facing away from the liver in a depression between the surface of the adjacent neighboring liver cells toward the capillary lumen or in a shallow indentation on the surface of the liver cell. They are primarily spindle-shaped and contain small fat globules. As to the properties of these cells, the authors believed that:

> The fat-storage cells probably represent functionally important cells in the liver, which secrete lipids from the blood and store them as neutral fat to release them back into the blood when needed. The fat in fat storage cells is nothing other than a reserve nutrient. In this way, the fat storage cells most likely participate in the lipid metabolism of the liver (p. 256)[63].

Thus, their primary function involves the storage of vitamin A (Table 1). In pathological conditions, Ito cells are also involved in collagen deposition and fibrosis[64].

Marie-Sée syndrome

There is limited historical information on Julien Marie (1899-1987) and Georges Sée (1904-2000). Marie was a professor of infant medical clinic and social pediatrics in the Faculty of Medicine, Paris, and a physician in the Hôpital des Enfants Malades (Hospital for Sick Children), Paris[65].

Marie and Sée first reported the cases and published a series of three cases of acute hypervitaminosis A in infants presented to the Society of Pediatrics, Paris, on February 20, 1951:

> We aim to draw attention to the acute events that may follow the administration of vast quantities of vitamin A in infants. These incidents are mainly characterized by acute hydrocephalus, spontaneous and intensive fontanel bulging, frequent vomiting, agitation, or insomnia, without any meningeal signs or other general disorders. The disorder begins 12 hours after ingestion of the drug and ends 24 to 48 hours later, either spontaneously or after a lumbar puncture (p. 731)[65].

They identified that an increased cerebrospinal fluid and pressure was associated with the increased vitamin A concentration (Table 1).

In conclusion, eponyms related to vitamin A have been named for syndromes, cells, diseases, or qualitative or quantitative tests. Syndromes have been primarily described concerning conditions leading to inadequate intake or those interfering with vitamin A's absorption or metabolism. In contrast, Marie and Sée described the phenomena occurring in infants with hypervitaminosis A[65]. The detailed and comprehensive description of the cornea or retina in vitamin A deficiency by Bitôt, Lobo, Petzetakis-Tzakos, and Uemerua remains relevant today. It is important to recall that many of these physicians recognized, through their astute insights and observations, the relationship between the deficiency of certain substances contained within certain foodstuff and the emergence of disease (e.g., Gama Lobo and Jacobs)[18,58].

A deficiency of vitamin A causes primarily ocular and cutaneous manifestations. Xeropthalmia is the term used to describe the ocular spectrum of signs and symptoms found in vitamin A deficiency, including Bitot spots, nyctalopia (night blindness), conjunctivitis, and keratitis[66]. Although the specific cause was unknown at that time, these ocular findings were first recognized by Pierre

Alain Bitôt and Manoel de Gama Lobo in the mid-19th century, prior to the isolation and synthesis of vitamin A in the early twentieth century[15,18].

Eponyms have been ascribed to physicians and scientists who made substantial contributions recognizing and describing the constellation of clinical manifestations of vitamin A deficiency occurring in isolation (Anderson, Vilanova-Cañdell, Bassen-Kornzweign, Petzetakis-Tzakos), a hypervitaminosis A (Marie-Sée), a general hypovitaminosis (Petzetakis-Tazkos, Jacob) or as a method to measure and quantitate its level (Friedrichsen). Deficiencies of vitamin A were caused by conditions fostering malnutrition (Jacob) or mechanisms interfering with absorption (Andersen) or absorption and transportation (Bassen-Kornzweig) and storage (Ito). With further discoveries, investigators identified that the synthetic and natural analogous of vitamin A or retinoids are useful in treating conditions including acne and psoriasis and in the cosmetic industry being promoted because of their anti-aging process[67,68].

Table 1. Eponyms related to vitamin A, described between 1863 and 1954

Year described	Eponym	Related person(s) or namesake(s)	Definition	Category
1863	Bitot spots (patches)[15]	Pierre Alain Bitôt♂ (1822-1888), French anatomist, physician, and surgeon	Small, circumscribed, triangular shiny gray deposits on the bulbar conjunctiva. Seen in vitamin A deficiency and other conditions.	observation
1865	Brazilian ophthalmia[18]	Manoel da Gama Lobo♂ (1831-1883), Brazilian physician	Degeneration of cornea, secondary to vitamin A deficiency (syn: xeropthalmia)	observation
1926	Carr-Price reaction[22]	Francis Howard Carr♂ (1874-1969), English chemist; Ernest Arthur Price♂ (1882-1956), English biochemist	A quantitative method for measuring retinol or determination of vitamin A using a colorimetric technique.	test
1928	Uemura (Uyemura) disease[25]	Misao Uemura (Uyemura)♂ (1900-1997), Japanese ophthalmologist	Reversible white spots in the deep retinal layer occur in patients with vitamin A deficiency. (syn: night blindness syndrome)	disease
1937	Friderichsen test[31]	Carl Friderichsen♂ (1886-1979), Danish pediatrician	Indicator of vitamin A deficiency. The smallest light causes oculomotor reflex, in which the eyes or head move in the direction of the light source. Other findings include wrinkling of the forehead and upward movement of the eyebrows.	test

1938	Andersen syndrome (triad)[39]	Dorothy Hansine Andersen♀ (1901-1963), American pediatrician and pathologist	A triad of cystic fibrosis of the pancreas, vitamin A deficiency, and steatorrhea. (syn: pancreatic infantilism)	syndrome
1949	Vilanova-Cañadell syndrome[45]	Xavier Vilanova i Montiu♂ (1902-1965), Spanish dermatologist; Josep Maria Cañadell♂ (1915-1997), Spanish endocrinologist	A combination of phrynoderma, hypothyroidism, and vitamin A deficiency. (syn: hypothyroid phrynoderma)	syndrome
1950	Bassen-Kornzweig syndrome (disease)[49]	Frank Albert Bassen♂ (1903-2003), Canadian hematologist and internist; Abraham Leon Kornzweig♂ (1900-1982), American ophthalmologist	An autosomal recessive condition, with onset at 6-16 years of age. Marked by neuromuscular abnormalities, retinitis pigmentosa, defective intestinal absorption and transport of fat and fat-soluble vitamins, including vitamin A, and burr shaped red blood cells (syn: abetalipoproteinemia or acanthocytosis).	syndrome
1950	Petzetakis-Tzakos syndrome[51]	Michel Petzetakis ♂ (1899-1975), Greek pathologist; Konstantinos Tzakos ♂ (1903-1984), Greek ophthalmologist	Keratitis, eyelid edema, and other eye ailments caused by severe malnutrition, including insufficient vitamin A intake, and poor hygiene. (syn: trophopenic superficial keratitis)	syndrome
1951	Jacobs syndrome[58]	Eugene Coryell Jacobs♂ (1905-2000), American military physician	A deficiency disease observed in American prisoners of war who were fed a rice diet. Characterized by the ocular findings (e.g., keratitis and conjunctivitis), oral (stomatitis and angular cheilitis and cutaneous (scrotal dermatitis) findings. Manifested with deficiency of riboflavin B_2 and vitamin B_6. Amblyopia (optic atrophy) caused by vitamin A and vitamin B_1 (thiamine) deficiencies.	syndrome
1952	Ito cells[63]	Toshio Ito♂ (1904-1991), Japanese anatomist and physician	Fat storage cells containing vitamin A lining hepatic sinusoids.	cell
1954	Marie-Sée syndrome[65]	Julien Marie♂ (1899-1987), French pediatrician; Georges Sée♂ (1904-2000), French pediatrician	An increased cerebrospinal fluid and pressure associated with hypervitaminosis A. syn: hypervitaminosis hydrocephalus syndrome pseudotumor cerebri or idiopathic intracranial hypertension.	syndrome

STATEMENT OF ETHICS

This study, focusing solely on the analysis of historical materials, does not require ethical approval or consent, as it involves no human or animal participants, and all sources used are in the public domain or have been properly cited in accordance with academic standards.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest regarding the publication of this paper. This includes, but is not limited to, financial, personal, or professional affiliations that could be construed as influencing the objectivity, integrity, or interpretation of the research findings.

AUTHOR CONTRIBUTIONS

All authors have contributed equally to the conception, drafting, and critical revision of the manuscript, and approve of the final version to be published.

REFERENCES

1. Socin CA. In welcher Form wird das Eisen resorbiert? Z Physiol Chem, 1891;15:93-139.

2. Holmes HN, Corbet RE. The isolation of crystalline vitamin A. J Am Chem Soc, 1937;59(10):2042-2047. Doi: 10.1021/ja01289a075

3. Isler O, Huber W, Ronco A, Kofler M. Synthese des vitamin A. Helv Chim Acta, 1947;30(6):1911-1927. Doi: 10.1002/hlca.19470300666

4. Wolf G. Vitamin A: historical introduction. In: Nutrition and the Adult: Micronutrients, vol. 3B. Alfin-Slater RB, Kritchevsky D., Eds. New York: Plenum Press; 2012. p. 97-203.

5. Skinner HA. Origin of medical terms. Baltimore: Williams & Wilkins; 1949.

6. Stepp W. Experimentelle Untersuchungen über die Bedeutung der Lipoide für die Ernährung. Z Biol, 1911;57:135-170.

7. Hopkins FG. The analyst and the medical man. The Analyst, 1906;31(369):385-404.

8. Hopkins FG. Feeding experiments illustrating the importance of accessory factors in normal dietaries. J Physiol, 1912;44(5-6):425-460. Doi: 10.1113/jphysiol.1912.sp001524

9. Funk C. The etiology of the deficiency diseases: beri-beri, polyneuritis in birds, epidemic dropsy, scurvy, experimental scurvy in animals, infantile scurvy, ship beri-beri, pellagra. J State Med, 1912;20:341-368.

10. McCollum EV, Davis M. The necessity of certain lipins in the diet during growth. J Biol Chem, 1913;15(1):167-175. Doi: 10.1111/j.1753-4887.1973.tb07065.x

11. McCollum EV, Kennedy C. The dietary factors operating in the production of polyneuritis. J Biol Chem, 1916;24(4):491-502.

12. Drummond JC. The nomenclature of the so-called accessory food factors (vitamins). Biochem J, 1920;14(5):660. Doi: 10.1042/bj0140660

13. Anonymous. Nécrologie: Pierre Bitôt. In: Mémoires et Bulletins de la Société de Médecine et de Chirurgie de Bordeaux, Année 1889. Bordeaux: Impr. G. Gounouilhou; 1890. p. 10-12.

14. Féret E. Personnalités et notables girondins de l'Antiquité à la fin du XIXe siècle. Bordeaux: Féret et fils; 1889. p. 69-70.

15. Bitôt P. Mémoire sur une lésion conjonctivale non encore décrite, coïncidant avec l'hémeralopie. Gaz Hebd Méd Chir, 1863;10:284-288.

16. Anonymous. Manoel da Gama Lobo. União Médica, 1883;3(6):335-336. [Portuguese]

17. Vasconcelos FAG, Santos LMP. Tributo a Manoel da Gama Lobo (1835–1883), pioneiro na epidemiologia da deficiência de vitamina A no Brasil. Hist Cienc Saude Manguinhos. 2007;14(4):1341-1356.

18. Gama LM. Da ophthalmia Brasiliana. Annaes Brasilienses de Medicina, 1865;17(1):16-24.

19. Anonymous. Carr, Francis Howard (1874–1869), chemist. Imperial College Archives and Corporate Records Unit. https://discovery.nationalarchives.gov.uk/details/c/F62846. Accessed 11 May 2023.

20. Williams KJ. British Pharmaceutical Industry, Synthetic Drug Manufacture and the Clinical Testing of Novel Drugs, 1895-1939. [Doctoral thesis. University of Manchester Faculty of Life Sciences, England; 2005.]

21. Anonymous. Ernest Arthur Price. Geni. https://www.geni.com/people/Ernest-Price/6000000030590908495.Accessed 20 April 2023.

22. Carr FH, Price EA. Colour reactions attributed to vitamin A. Biochem J, 1926;20(3):497-501. Doi: 10.1042/bj0200497

23. Greaves RF, Woollard GA, Hoad KE, Walmsley TA, Johnson LA, Briscoe S, et al. Laboratory medicine best practice guideline: vitamins A, E and the carotenoids in blood. Clin Biochem Rev, 2014;35(2):81-113.

24. Wayenborgh JW, Keeler CR, Mishima S. Uemura M, (1900–1997). In: IBBO-International Biography and Bibliography of Ophthalmologists and Visual Scientists (A-Z). Amsterdam: Wayenborgh Publishing; 2018. p. 409.

25. Uyemura M. Ueber eine merkwürdige Augenhintergrundveränderung bei zwei Fällen von idiopathischer Hemeralopie. Klin Monatsbl f Augenh, 1928;81:471-473.

26. Bak-Jensen S. Carl Friderichsen og pædiatriens etablering. Ugeskrift for Læger. https://ugeskriftet.dk/videnskab/carl-friderichsen-og-paediatriens-etablering. Accessed 5 May 2023.

27. Ibsen KK. Børnelager i 100 År. Frederikshavn: Sansk Paediatrisk Selskab; 2008.

28. Friderichsen C. Nebennieren-Apoplexie bei kleinen Kindern. Jahrb Kinderheilkd, 1918;87:109-125.

29. Waterhouse R. A case of suprarenal apoplexy. Lancet, 1911;177(4566):577-578. Doi: 10.1016/S0140-6736(01)60988-7

30. Glanzmann E. Beitrag zur Klinik, Hämatologie und Pathologie des Syndroms von Waterhouse - Friderichsen (Nebennierenapoplexie bei kleinen Kindern). Jahrb Kinderheilkd, 1933;139(1-2):49-63.

31. Friderichsen C. Epitome of current medical literature: light reflex test for hypovitaminosis. Br Med J, 1937;1(3968):13. Doi: 10.1136/bmj.1.3968.E13

32. Friderichsen C, Edmund C. Studies of hypovitaminosis a: Clinical experiments in the vitamin A balance in children after various diets. Am J Dis Children, 1937;53(5):1179-1201. Doi: 10.1001/archpedi.1937.04140120003001

33. Anonymous. Andersen, Dorothy Hansine. JAMA, 1963;184(8):670.

34. Windsor LL. Andersen, Dorothy Hansine (1901–1963). In: Women in Medicine: An Encyclopedia. Santa Barbara: ABC-Clio; 2002. p. 7-8.

35. Machol L. Andersen, Dorothy Hansine. In: Notable American Women: The Modern Period, vol. 4. Sicherman B, Green CH., Eds. Massachusetts: Harvard University Press; 1980. p. 18-20.

36. Anonymous. Dr. Dorothy Hansine Andersen. Changing the Face of Medicine: Celebrating America's Women Physicians. https://cfmedicine.nlm.nih.gov/physicians/biography_8.html. Accessed 10 May 2023.

37. Andersen DH. Dorothy H. Andersen Papers, Archives & Special Collections. Columbia University Health Sciences Library. https://www.library-archives.cumc.columbia.edu/finding-aid/dorothy-h-andersen-papers-1930-1966. Accessed 12 May 2023.

38. Clarke C, Hadfield G. Congenital pancreatic disease with infantilism. Q J Med, 1924; 17(68):358-364. Doi: 10.1093/qjmed/os-17.68.358

39. Andersen DH. Cystic fibrosis of the pancreas and its relation to celiac disease: a clinical and pathological study. Am J Dis Child, 1938;56(2):344-399. Doi: 10.1001/archpedi.1938.01980140114013

40. Ferrando J. Breve historia de la dermatología Catalana. Med Cutan Iber Lat Am, 2003;31(1):57-70.

41. Corominas F. Vilanova i Montiu, Xavier. Reial Acadèmia de Medicina de Catalunya. http://ramc.cat/aw-team-member/vilanova-i-montiu-xavier/. Accessed 15 May 2023.

42. Baños JE, Guardiola E. Eponímia mèdica catalana: la frinodèrmia hipotiroïdal o síndrome de Vilanova-Cañadell. Ann Med, 2010;93(1):31-35.

43. Anonymous. Xavier Vilanova i Montiu. Galeria de Metges Catalans. https://www.galeriametges.cat/galeria-fitxa.php?icod=EIE. Accessed 15 May 2023.

44. Jadassohn W. Necrologia: Professeur Xavier Vilanova Montiu [Necrology: Professor Xavier Vilanova Montiu]. Dermatologica, 1965;130(6):479. Doi: 10.1159/000254562 [Spanish]

45. Vilanova X, Cañadell JM. Dermopatías, hipotiroidismo y avitaminosis. Actas Dermosifiliogr, 1949;40:689-695.

46. Anonymous. Bassen, Frank Albert. In: Directory of Medical Specialists Certified by American Boards. 16th ed. Chicago: Marquis Who's Who; 1974. p. 519.

47. Aufses AH, Niss B. This House of Noble Deeds: The Mount Sinai Hospital, 1852–2002. New York: New York University Press; 2003.

48. Podos SM. Abraham L. Kornzweig, MD. Ophthalmic Paediatr Genet, 1983;2(1):1. Doi: 10.3109/13816818309007144

49. Bassen FA, Kornzweig A L. Malformation of the erythrocytes in cases of atypical retinitis pigmentosa. Blood, 1950;5(4):381-387. Doi: 10.1182/blood.V5.4.381.381

50. Anonymous. Dr. M. Petzetakis, professeur agrégé de pathologie à l'Université d'Athènes. Rev Méd Hyg Trop, 1937;29-30:20.

51. Petzetakis M. Les troubles oculaires pendant la trophopénie (maladie œdémateuse) et l'épidémie de pellagre (1941–1944): la kératite superficielle trophopénique (kératopathie épithéliale). Presse Méd, 1950;58(61):1082-1084.

52. Anonymous. The history of the Hellenic Ophthalmological Society. Hellenic Ophthalmological Society. www.eyenet.gr/η-εταιρεία/ιστορία-της-ελληνικής-οφθαλμολογική/. Accessed 24 May 2023.

53. Anonymous. Jacobs, Eugene C. Social Networks and Archival Context. https://snaccooperative.org/ark:/99166/w6th98ks. Accessed 16 May 2023.

54. Anonymous. Col. Eugene Coryell Jacobs. Find a Grave. https://www.findagrave.com/memorial/28700258/eugene-coryell-jacobs. Accessed 15 May 2023.

55. Anonymous. Archive record: Dr. Eugene Jacobs. American Defenders of Bataan and Corregidor Museum. https://philippinedefenders.pastperfectonline.com/archive/506B9085-8653-4134-A380-962388558692. Accessed 10 May 2023.

56. Roland CG. Eugene Coryell Jacobs, MD: experiences as a prisoner of War, World War 2. Oral History Archives Hannah Chair of the History of Medicine. 24 March 1984. https://www.hkvca.ca/submissions/Charles_Roland_Interviews/Dr%20E%20C%20Jacobs.pdf. Accessed 25 May 2023.

57. Jacobs EC. Blood Brothers: A Medic's Sketch Book. Rohlfing S, ed. New York: Carlton Press; 1985.

58. Jacobs EC. Oculo-oro-genital syndrome: a deficiency disease. Ann Int Med, 1951; 35 (5):1049-1054. Doi: 10.7326/0003-4819-35-5-1049

59. Friedli A, Saurat JH. Oculo-orogenital syndrome: a deficiency of vitamins B_2 and B_6. NEJM, 2004;350(11):1130. Doi: 10.1056/NEJMicm980412

60. Haubrich W. Ito of Ito's cells. Gastroenterol, 2006;130(3):714. Doi: 10.1053/j.gastro.2006.01.066

61. Anonymous. Toshio Ito. Gastroenterología. http://articulos.sld.cu/gastroenterologia/archives/3895. Accessed 5 June 2023.

62. Baguena MJ. Toshio Ito (1904–1991). Historia de la Medicina. https://www.historiadelamedicina.org/ito.html. Accessed 7 June 2023.

63. Ito T, Nemoto M. Über die Kupfferschen Sternzellen und die "Fettspeicherungszellen" (fat storing cells) in der Blutkapillarenwand der menschlichen Leber. Okajimas Folia Anat Jpn, 1952;24(4):243-258. Doi: 10.2535/ofaj1936.24.4_243

64. Jang KT, Jung YM, Lim IK, Lee YS, Myung NK, Kim MR, et al. Ito cell activity and hepatocyte proliferation activity according to collagen content in liver cirrhosis. Korean J Hepatol, 1998;4(3):254-263.

65. Marie J, Sée G. Acute hypervitaminosis A of the infant: its clinical manifestation with benign acute hydrocephalus and pronounced bulge of the fontanel; a clinical and biologic study. AMA Am J Dis Child, 1954;87(6):731-736. Doi: 10.1001/archpedi.1954.02050090719008

66. McLaren DS, Kraemer K. Xerophthalmia. In: Manual on Vitamin A Deficiency Disorders. Basel: Karger Publications. 2012. p. 65-75.

67. Zasada M, Budzisz E. Retinoids: active molecules influencing skin structure formation in cosmetic and dermatological treatments. Adv Dermatol Allergol, 2019;36(4):392-397. Doi: 10.5114/ada.2019.87443

68. Mukherjee S, Date A, Patravale V, Korting H C, Roeder A, Weindl G. Retinoids in the treatment of skin aging: an overview of clinical efficacy and safety. Clin Interv Aging, 2006;1(4):327-348. Doi: 10.2147/ciia.2006.1.4.327

ORIGINAL ARTICLES

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The effect of decolonization-decontamination prophylaxis versus traditional prophylaxis in orthopedic surgery in Kosovo

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ABSTRACT

This study aimed to compare empirical prophylactic treatment with decolonization-decontamination prophylaxis protocol in order to reduce surgical site infections. The study was conducted in Kosovo Ortomedica Orthopedic Hospital, the data from all patients admitted to the hospital between June 2018 and June 2019 was collected retrospectively, all the patients admitted to the hospital between November 2021 and January 2022 were followed prospectively. 127 patients were treated empirically, and 93 patients were prospectively treated with decolonization-decontamination prophylaxis protocol. The empirically treated patients were given cefazolin before surgery. However, the prospectively treated patients were first tested for MRSA infections and the observed infections were treated with decolonization-decontamination prophylaxis protocol. The infection status and the postoperative CRP values of the patients were compared and found to be significantly higher in the empirical group (4.7% versus 0, p=0.038 and 7.1% versus 0, p=0.006, for empirical and decolonization -decontamination groups respectively). In conclusion, the implementation of the decolonization-decontamination protocol has been shown to effectively decrease the incidence of infections in orthopedic surgical procedures. Nevertheless, it is imperative to conduct additional research utilizing

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a more extensive sample size and pharmacoeconomic studies in order to substantiate its viability as a prophylaxis measure.

Keywords: surgical site infection, empirical, decolonization, decontamination, orthopedic surgery

INTRODUCTION

Surgical site infection (SSI) is a major concern for both the patient and the operating surgeon. It is defined as an infection observed at or near the incision site within 30 days of surgery or one year after implant insertion and is thus responsible for healthcare costs, mortality, and patient injury[1,2]. These infections account for approximately 40% of nosocomial infections after surgical intervention[3]. During the first eight weeks after hospital discharge, the cost of care for patients with SSI is approximately three times that of surgical patients without infection[4,5]. In addition to impairing the patient's quality of life, and increased morbidity and mortality[4], these infections are also responsible for increased hospitalization and treatment cost related to surgical operation[6,7].

Surgical antibiotic prophylaxis (SAP) is the universal protocol used to reduce postoperative SSIs. It is initiated closely prior to the operative procedures[8]. Since the inception of SAP in the 1960s, antibiotic administration has reduced mortality, and the time taken for patients to return to normal life, thereby lowering the cost of treatment and the length of hospital stay[9].

The primary reference for SAP is found in the guidelines of the American Society of Health-System Pharmacists (ASHP)[10], however, in local recommendations, the uncertainty of the indications, antibiotic selection, preoperative timing, and duration of administration may change the surgeon's approach towards SSI. Moreover, these personal preferences have a significant impact on global antibiotic consumption, which saw an increase of more than 60% over the last decade[11]. The barriers to guideline adherence include the logistical insufficiency of surgical wards as well as a lack of awareness of the appropriate guidelines and compliance with their recommendations[12]. As a result, the personal preference for antibiotics and their duration of administration have led to inappropriate antibiotic use in half of all general elective surgeries[13] and the emergence of bacterial resistance[14].This study aimed to compare the empirical prophylactic treatment with decolonization-decontamination prophylaxis protocol effectivness in the reduction of surgical site infections.

METHODOLOGY

This is an observational study that is both retrospective and prospective in nature. This study included all orthopedic surgery patients who were admitted to Kosova Ortomedica Orthopedic Hospital between June 2018 and June 2019 as group 1, and those admitted between November 2021 and January 2022 as group 2. The demographics variables (age, sex, socioeconomic status, educational status, history of antibiotic use), duration of hospital stay, laboratory tests, antibiotics, and other drug dosing frequency, type of surgery, and comorbidities of all patients admitted to Kosovo Ortomedica Orthopedic Hospital and who underwent surgery between June 2018 and June 2019 were retrospectively collected. These data had already been thoroughly documented on the patient profile, and a double check had been made during the data collecting procedure; additionally, the data had been compared with hospital pharmacy records to guarantee its accuracy. The treatment protocol for these patients (Group 1) was empirical based on ASHP therapeutic guidelines[10]. In brief, all of the patients were given cefazolin 1g one hour before surgery. We couldn't conduct the prospective study immediately after the retrospective one because of the COVID-19 pandemic, so we had to wait until the condition improved. For Group 2, between 15 November 2021 and 10 January 2022, nasal/throat/skin swab samples were collected from all patients before any surgical operation and then the patients followed prospectively. Five days before surgery, these samples were tested for the presence of methicillin-resistant *Staphylococcus aureus* (MRSA). If MRSA was not detected, no further action was taken. If the results were positive, nasal decolonization was performed for patients via administration of 2% mupirocin cream twice a day for the next five days up to the date of surgery. Decontamination of the skin was carried out by showering using chlorhexidine gluconate soap the night before and the morning of the procedure. Cefazolin was given as prophylaxis the day before and during the surgery. Patients were followed prospectively for any signs of a new infection, such as an elevated white bold cell (WBC), C-reactive protein (CRP), fever, or other signs of infection at the surgery site daily for 3 weeks. The prospective (Group 2) study protocol was adapted from a previous study that followed a similar protocol, and these studies were included in the ASHP report[10, 15, 16]. Additionally, intranasal mupirocin has been approved by the FDA for the treatment of MRSA nasal colonization in adult patients and healthcare workers[17].

All the data collected for (Group 1), was also collected for (Group 2). The researchers then examined the efficacy of the empirical and decolonization-decontamination prophylaxis protocol. The primary outcome measures were the percent of postsurgical infection, the C-reactive protein level three weeks post-

surgery, and hospitalization days.

All procedures were carried out following the ethical guidelines of the Chamber of Pharmacists of Kosovo Non-invasive Ethical Committee (Decision Number: 378, 12.11.2021). Individual written informed permission was obtained from all participants in the study. The STROBE Checklist combined was used to evaluate the quality of both retrospective and prospective data.

The data obtained in the research were analyzed using the SPSS for Windows (Version 22.0). Numbers, percentage, and mean and standard deviation were used for descriptive statistical analysis. The Kolmogorov-Smirnov test was utilized to assess the normality of the data, and the findings indicated that the data follows a normal distribution. The t-test was applied to compare quantitative, normally distributed, continuous data between two independent groups and the Chi-square test was used to compare independent variable groups. Results were considered statistically significant at $p \leq 0.05$.

RESULTS and DISCUSSION

In this study, we compared the empirical prophylactic treatment protocol to the decolonization-decontamination prophylaxis protocol to determine the best regimen to use at Kosovo Ortomedica Orthopedic Hospital. All patients in the empirical treatment group received empirical prophylaxis before surgery, whereas in the decolonization-decontamination prophylaxis group, the antibiotic was directed by the culture results. Both Groups 1 and 2 have comparable demographic variables and comorbidities ($p>0.05$). Despite some differences in some laboratory data ($p<0.05$), these data are still within the normal healthy range.

Among the 127 patients analyzed retrospectively in Group 1, 68 were female, and 59 were male. The mean age was 41.9 ±20.6. There were 93 patients evaluated prospectively in Group 2. Among them, 54 were female and 39 were male. The mean age was 54.9 ±21.9. The most common reasons for 127 patients in Group 1 to be admitted to the hospital were knee surgery in 53 cases (42%), hip replacement in 30 cases (24%), and spine surgery in 27 cases (21%). In contrast, among the 93 patients inGroup 2, 37 patients (40%) underwent knee surgery, 17(18%) underwent hip replacement surgery, 11 (12%) underwent spine surgery, and 13 (13) underwent bone surgery (14%). Those in Group 1 received antibiotic prophylaxis 1 hour before surgery, and those in Group 2 received it the day before and 1 hour before surgery.

Table 1 presents the demographic characteristics, laboratory results for both Group 1 (empirical prophylaxis) and Group 2 (decolonization-decontamination prophylaxis).

Table 1. Demographic characteristics and laboratory results of Group 1 (empirical prophylaxis) and Group 2 (decolonization-decontamination prophylaxis)

Variables	Group 1 Patients (n = 127) n (%)	Group 2 Patients (n = 93) n (%)	p value
Sex	Female 68 (53.5) Male 59 (46.5)	Female 54 (58) Male 39 (42)	p=0.5
Age (years)	41.9 ±20.6	54.9 ±21.9	p=0.24
Average WBC count	8.07±11.2	7.4±3.78	p=0.05
ALT U/L	26.2 ±27.3	24.25±40.8	p=0.460
AST U/L	25.2± 11.4	21.59±10.1	p=0.01*
Urea mmol/L	4.68 ±2.17	5.48±4.65	p=0.002*
Creatinine mmol/L	70.3±37.9	71.5±12.1	p=0.765
Comorbidities	41 (32.3)	32 (34.4)	p=0.425
Hypertension	32 (25.2)	27 (29)	p=0.315
Hyperlipidemia	3 (2.4)	3 (3.2)	p=0.503
Lower Respiratory Tract Infection	0 (0)	1 (1.1)	p= 0.423
Medication Use in Chronic Disease	42 (33.1)	31 (31.3)	p=0.540

*$p<0.05$ considered significant[1]

The infection status revealed that infected patients and 3-week postoperative CRP levels were found to be higher in Group 1 (4.7% versus 0, p=0.038 and 7.1% versus 0, p=0.006, for empirical and decolonization -decontamination groups respectively). Table 2 shows the differences between the two groups in terms of the primary outcome measures like infection status, CRP and hospitalization days.

Table 2. Comparison of empirically treated (Group 1) versus decolonization-decontamination prophylaxis (Group 2) patients in terms of primary outcome measures

Variables		Group 1		Group 2		p
		N	%	N	%	
		42	33.1	31	33.3	
Infection	No	121	95.3	93	100	p=0.038*
	Yes	6	4.7	0	0	
3-Week Post-Surgery CRP	No	118	92.9	93	100	p=0.006*
	Yes	9	7.1	0	0	
Hospitalization days	Mean ±SD	3 ±4.9		3.2 ±4.7		p=0.827

*p<0.05 considered significant

During the hospitalization time, 14 patients in Group 1 required additional antibiotic treatment 5 female (36%) and 9 male (64%), the average age was 45.9 ±23.4 years, the average hospital stay was 3.62 ±0.4 days, and 4/14 patients had an infection three weeks following surgery (28.6 %). Of the 113 patients who did not receive further antibiotics therapy, 63 (56%) were female and 50 (44%) males, with a mean age of 41.4 ±20.2, a mean hospital stay of 3 ±5.1 days, 36/113 chronic diseases, and with 2/113 (1%) who acquired an infection three weeks after surgery. The number of patients with infection in the first three weeks post-surgery was higher if the patient had been administered further antibiotic treatment ($p<0.05$). In Group 2, nose, throat, and skin swab samples taken from the patients before surgery revealed *Staphylococcus aureus* (*S. aureus*)-based infection in 13 of the 93 patients. Of these 13 patients (average age 54.9 ±21.9 years), 10 (18%) female and 3 (8%) male, received prophylactic therapy, and three weeks following surgery none had developed an infection. Similarly, 80 patients 44 (82%) female and 36 (92%) male did not have an *S. aureus* infection and hence did not require prophylactic treatment.

Table 3 and Table 4 summarize the characteristics of the patients who received antibiotics in Group 1 (empirically treated) and Group 2 (decolonization-decontamination prophylaxis) respectively.

Table 3. Characteristics of patients who received antibiotics in Group 1 (empirically treated)

Variables	Sex	Infection in the First 3 Weeks Post-Surgery	Hospitalization (days)	Age (years)
Patients Receiving Further Antibiotics Treatment n=14	Female 5 Male 9	4 (28.6%)	3.6 ±2.4	45.9 ±23.4
Patients Receiving No Further Antibiotic Treatment n=113	Female 63 Male 50	2 (1%)	3 ±5.1	41.4 ±20.2
p value	>0.05	<0.05*	>0.05	>0.05

*p<0.05 considered significant

Table 4. Characteristics of patients who received antibiotics in Group 2 (decolonization-decontamination prophylaxis)

Variables	Sex	Infection in the First 3 Weeks Post-Surgery	Hospitalization (days)	Age (years)
Infection in the First 3 Weeks Post-surgery n=0	Female Not applicable Male Not applicable	0 (0%)	Not applicable	Not applicable
Patients Receiving No Prophylactic Treatment n=80	Female 44 Male 36	0 (0%)	Not applicable	43.6 ±19.7
p-value	>0.05	<0.001*	>0.05	>0.05

* p<0.05 considered significant

In this study, we compared the empirical prophylactic treatment protocol to the decolonization-decontamination prophylaxis protocol to determine the best regimen to use at our hospital. All patients in the empirical treatment group received empirical prophylaxis before surgery, whereas in the decolonization-decontamination prophylaxis group, the antibiotic was directed by the culture results.

In our study, six of the 127 (4.7%) patients who followed the empirical prophylaxis plan, which included antibiotic medication given 1 h before surgery and up to 4 h during surgery, developed infections. Vargas et al. studied the effect of a short-term antimicrobial prophylaxis regimen on the prevalence of postoperative infection in elective orthopedics and traumatology. In the group that received the empirical antibiotic treatment, the prevalence of infection was 3/69 (4.3%), which is virtually identical to our findings[18]. Antibiotic prophylaxis before surgery is critical to ensure adequate antibiotic concentrations. We administered the antibiotic 1 h before the surgery. In the medical literature, the administration timing is still up for debate, and in different studies, ranges between 15 and 120 min before the skin incision[19-21]. According to Yeap et al., antibiotics should be given 30–60 min before surgery, during anesthesia induction, or at least 10 min before the tourniquet is inflated[19]. Most antibiotics should be given 30 min before skin incision, according to Stefánsdóttir et al., and administration more than 60 min before surgery or incision is linked to a greater risk of surgical infection[22]. Several investigations have found a link between *S. aureus* colonization and the development of surgical site infection in cardiothoracic, gastrointestinal, and orthopedic surgeries[23]. In 2017, the American College of Surgeons (Chicago, Illinois) and the Surgical Infection Society (East Northport, New York) published guidelines that addressed this issue, stating that screening and decolonization should be based on baseline surgical site infection and methicillin-resistant *S. aureus* rates[24]. Before total joint replacement and cardiac surgeries, the American Society of Health-System Pharmacists (Bethesda, Maryland) recommends screening and decolonization for all patients colonized with *S. Aureus*[10]. Methicillin-resistant *S. aureus* bundles (screening, decolonization, contact precautions, and hand hygiene) are extremely successful when all of the components are used together. The guidelines also state that in the literature, no single decolonization technique has been proven effective. Nasal mupirocin has been used alone and in combination with chlorhexidine gluconate bathing. The anterior nares have also been decolonized with povidone-iodine solutions[25]. This guideline was used in our prospective group (n = 93), and we noticed that there was no infection in this group.

In our study, we started nasal mupirocin five days before surgery and informed patients who had a positive MRSA culture result to shower the night before and in the morning of the procedure. We had remarkable success with this strategy because the infection rate was zero and no elevation in CRP noted. Nasal decolonization exhibited a significant prophylaxis effect against surgical site infections caused by *S. aureus*, according to a meta-analysis of 17 stud-

ies. Essentially, seven studies looked at a protocol that included decolonization and glycopeptide prophylaxis only for MRSA-colonized patients, as we did in our study, and found that it had a significant prophylaxis effect against Gram-positive surgical site infections[26]. For the outcome to be effective, Murphy et al. advocated the use of these techniques within three months of surgery[27].

Schweizer et al., by comparing the empirical antibiotic prophylaxis with the decolonization-decontamination method, concluded that *S. aureus* screening, decontamination, and targeted prophylaxis as part of a bundle were linked to a small but statistically significant reduction in complex *S. aureus* SSIs[26].

A study conducted in an orthopedic hospital in Spain and published in 2019 revealed findings that are comparable to those of the present study. The study encompassed a control group consisting of 400 patients who underwent surgical procedures from January 2009 to December 2013. Additionally, a second intervention group of 403 patients was included, who were exposed to a screening and decontamination strategy for nasal carriers of *S. aureus* between January 2014 and December 2016. Upon doing a comparative analysis of surgical-site infection (SSI) rates, it was observed that the intervention group exhibited a statistically significant decrease in both overall SSI ($p<0.009$) and *S. aureus*-specific SSI ($p<0.02$)[28].

The study covered all patients who underwent orthopedic surgery during a specific time period, and because the hospital has a limited capacity and the restricted condition of COVID-19, only a small number of patients were included, which is a constraint that prevents the study's findings from being generalized. Additionally, the economic burden of the decolonization-decontamination implementation approach was not measured in our study, which is another limitation.

According to our findings, using the decolonization-decontamination method reduces the rate of infection in orthopedic surgeries, and we can advocate it as a preventive strategy. However, more pharmaco-economic research with a larger sample size is needed to determine the cost-effectiveness and practical utilization of the method.

STATEMENT OF ETHICS

All procedures performed were in accordance with the ethical guidelines of the Chamber of

Pharmacists of Kosovo Non-invasive Ethical Committee (Decision Number: 12.11.2021/378).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

DA, NA, and BB conceived and designed the study; DA obtained ethics approval and collected patient's data; DA and NA wrote the article. NA and BB performed the statistical analysis. BB supervised the overall study and revised the article.

REFERENCES

1. Horan TC, Gaynes RP, Martone WJ, Jarvis WR, Emori TG. CDC definitions of nosocomial surgical site infections, 1992: a modification of CDC definitions of surgical wound infections. Infect Control Hosp Epidemiol, 1992;13:606-608.

2. Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR. Guideline for Prevention of Surgical Site Infection, 1999. Centers for Disease Control and Prevention (CDC) Hospital Infection Control Practices Advisory Committee. Am J Infect Control, 1999;27(2):97-96. Doi: 10.1086/501620

3. Singh R, Singla P, Chaudhary U. Surgical site infections: classification, risk factors, pathogenesis and preventive management: review article. Int J Pharma Research Health Sci, 2014;2:203-214.

4. Perencevich EN, Sands KE, Cosgrove SE, Guadagnoli E, Meara E, Platt R. Health and economic impact of surgical site infections diagnosed after hospital discharge. Emerg Infect Dis, 2003;9:196-203. Doi: 10.3201/eid0902.020232

5. Badia J, Casey A, Petrosillo N, Hudson P, Mitchell S, Crosby C. Impact of surgical site infection on healthcare costs and patient outcomes: a systematic review in six European countries. J Hosp Infect, 2017;96:1-15. Doi: D1016/j.jhin.2017.03.004oi:10.

6. Monahan M, Jowett S, Pinkney T, Brocklehurst P, Morton DG, Abdali Z, et al. Surgical site infection and costs in low- and middle-income countries: a systematic review of the economic burden. PLoS One, 2020;15(6):e0232960. Doi: 10.1371/journal.pone.0232960

7. Cecchini M, Langer J, Slawomirski L. Antimicrobial resistance in G7 countries and beyond: economic issues, policies and options for action. OECD better policies for better lives, 2015; 1-75.

8. Waddell TK, Rotstein OD. Antimicrobial prophylaxis in surgery. Committee on Antimicrobial Agents, Canadian Infectious Disease Society. CMAJ, 1994;151:925-931.

9. Talbot TR. Surgical site infections and antimicrobial prophylaxis. In: Mandell GL, Bennett JE, Dolin R., Eds. Principle and practice of infectious diseases. 7th edition. Philadelphia: Churchill Livingstone, 2010; 3890-3905.

10. Bratzler DW, Dellinger EP, Olsen KM, Perl TM, Auwaerter, Bolon MK, et al. Clinical practice guidelines for antimicrobial prophylaxis in surgery. Am J Health Syst Pharm, 2013;70:195-283. Doi: 10.1089/sur.2013.9999

11. Klein EY, Van Boeckel TP, Martinez EM, Pant S, Gandra S, Levin SA, et al. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. Proc Natl Acad Sci, 2018; 115(15):E3463-E3470. Doi: 10.1073/pnas.1717295115

12. Van Kasteren MEE, Kullberg BJ, De Boer AS, Mintjes-de Groot J, Gyssens IC. Adherence to local hospital guidelines for surgical antimicrobial prophylaxis: a multicentre audit in Dutch hospitals. *J* Antimicrob Chemother*,* 2003;51:1389-1396. Doi: 10.1093/jac/dkg264

13. Bedouch P, Labarère J, Chirpaz E, Allenet B, Lepape A, Fourny M, et al. Compliance with guidelines on antibiotic prophylaxis in total hip replacement surgery: results of a retrospective study of 416 patients in a teaching hospital. Infect Control Hosp Epidemiol, 2004;25:302-307. Doi: 10.1086/502396

14. Butt SZ, Ahmad M, Saeed H, Saleem Z, Javaid Z. Post-surgical antibiotic prophylaxis: Impact of pharmacist's educational intervention on appropriate use of antibiotics. J Infect Public Health, 2009; 12:854-860. Doi: 10.1016/j.jiph.2019.05.015

15. Bode LG, Kluytmans JA, Wertheim HF, Bogaers D, Vandenbroucke-Grauls CM, Troelstra A, et al. Preventing surgical-site infections in nasal carriers of Staphylococcus aureus. N Engl J Med, 2010;362: 9-17. Doi: 10.1056/NEJMoa0808939

16. Hebert C, Robicsek A. Decolonization therapy in infection control. Curr Opin Infect Dis, 2010;23:340-345. Doi: 10.1097/QCO.0b013e32833ae214

17. Kaur DC, Narayan PA. Mupirocin resistance in nasal carriage of Staphylococcus aureus among healthcare workers of a tertiary care rural hospital. Indian J Crit Care Med, 2014;18(11):716-721. Doi: 10.4103/0972-5229.144013

18. Vargas-Mena R, Arredondo-Gómez E, Pavía-Carrillo EF. Efecto de un esquema corto de profilaxis antimicrobiana sobre la prevalencia de infecciones postoperatorias en cirugía electiva de traumatología y ortopedia Effect of a short antimicrobial prophylaxis regimen on the prevalence of postoperative infection in elective orthopedics and traumatology surgery. Acta Ortop Mex, 2012;26:369-374.

19. Yeap JS, Lim JW, Vergis M, Au Yeung PS, Chiu CK, Singh H. Prophylactic antibiotics in orthopaedic surgery: Guidelines and practice. Med J Malaysia, 2006;61:181-188.

20. Niimi R, Hasegawa M, Kawamura G, Sudo A. One-day antibiotic infusion for the prevention of postoperative infection following arthroplasty: A case control study. ISRN Orthop, 2011;839641. Doi: 10.5402/2011/839641

21. Andersson AE, Bergh I, Karlsson J, Eriksson BI, Nilsson K. The application of evidence-based measures to reduce surgical site infections during orthopedic surgery-Report of a single-center experience in Sweden. Patient Saf Surg, 2012;6:1-11. Doi: 10.1186/1754-9493-6-11

22. Stefánsdóttir A, Robertsson O, W-Dahl A, Kiernan S, Gustafson P, Lidgren L. Inadequate timing of prophylactic antibiotics in orthopedic surgery. We can do better. Acta Orthop, 2009;80:633-638. Doi: 10.3109/17453670903316868

23. Singla A, Qureshi R, Chen DQ, Nourbakhsh A, Hassanzadeh H, Shimer Al, et al. Risk of surgical site infection and mortality following lumbar fusion surgery in patients with chronic steroid usage and chronic methicillin-resistant Staphylococcus aureus infection. Spine, 2019;44:408-413. Doi: 10.1097/BRS.0000000000002864

24. Ban KA, Minei JP, Laronga C, et al. American College of Surgeons and Surgical Infection Society: Surgical Site Infection Guidelines, 2016 Update. J Am Coll Surg, 2017;224:59-74. Doi: 10.1016/j.jamcollsurg.2016.10.029

25. Anderson MJ, David ML, Scholz M, Bull SJ, Morse D, Hulse-Stevens M, et al. Efficacy of skin and nasal povidone-iodine preparation against mupirocin-resistant methicillin-resistant Staphylococcus aureus and S. aureus within the anterior nares. Antimicrob Agents Chemother, 2015;59:2765-2773. Doi: 10.1128/AAC.04624-14

26. Schweizer M, Perencevich E, McDanel J, Carson J, Formanek M, Hafner J, et al. Effectiveness of a bundled intervention of decolonization and prophylaxis to decrease Gram positive surgical site infections after cardiac or orthopedic surgery: systematic review and meta-analysis. BMJ, 2013;346:f2743. Doi: 10.1136/bmj.f2743

27. Murphy E, Spencer S J, Young D, Jones B, Blyth MJ. MRSA colonisation and subsequent risk of infection despite effective eradication in orthopaedic elective surgery. J Bone Joint Surg Br, 2011;93: 548-551. Doi: 10.1302/0301-620X.93B4.24969

28. Pelfort X, Romero A, Brugués M, García A, Gil S, Marrón A. Reduction of periprosthetic Staphylococcus aureus infection by preoperative screening and decolonization of nasal carriers undergoing total knee arthroplasty. Acta Orthop Traumatol Turc, 2019;426-431. Doi: 10.1016/j.aott.2019.08.014

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Levels of adiponectin, malondialdehyde and lipid profile in women with polycystic ovary syndrome

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a heterogeneous disease affects about 4-18% of women of reproductive age worldwide, with associated increased risk of endocrine, metabolic, and reproductive defects. Adiponectin (ADP), the most abundantly secreted adipokine, is a homeostatic regulating factor for insulin, lipid, and glucose through its antioxidant, anti-fibrotic, and anti-inflammatory effects. Serum levels of adiponectin, malondialdehyde (MDA), and lipid profile were evaluated in the fasting sample in 30 healthy underweight women as a control group, and 30 females with PCOS, age, and body mass index (BMI) matched with the healthy control. Compared with the healthy control, serum levels of adiponectin were significantly lower in females with PCOS. Additionally, total cholesterol (TC) levels were significantly higher in concomitant women compared to the control group. Interestingly, no significant variations were observed in the serum levels of MDA, LDL, TG, VLDL, and HDL. However, no significant correlations were found between the study groups. In conclusion, findings of our study revealed that low adiponectin and high total cholesterol levels could serve as predictive markers of PCOS risk in lean women with a family history of PCOS, or women with fewer symptoms.

Keywords: PCOS, lipids, malondialdehyde, adiponectin, BMI

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex and heterogeneous endocrine disease affecting women of reproductive age, with a prevalence rates ranged from 4-20% using the applied criterion[1]. The presence of two out of the following three criteria is required for the diagnosis of PCOS: polycystic ovaries, ovulatory dysfunction, and hyperandrogenism[2]. PCOS is not only a reproductive disorder but also a metabolic disorder, with affected women being at an increased risk of developing insulin resistance, dyslipidemia, obesity, type 2 diabetes, and cardiovascular disorder[3-6]. Adiponectin is an adipose tissue-derived adipokine and a hormone that influences multiple metabolic processes through its anti-atherogenic, anti-inflammatory, and insulin-sensitizing properties[7]. Adiponectin has been shown to decrease in females with PCOS, and this decrease has been attributed to the insulin resistance which is a characteristic of PCOS[8,9]. Insulin resistance leads to decreased production of adiponectin, which in turn aggravates insulin resistance and contributes to the metabolic abnormalities associated with PCOS[7]. Moreover, adiponectin has a direct effect on ovarian function. Adiponectin receptors are expressed in the ovary, and studies have shown that adiponectin can stimulate steroidogenesis and follicular growth[10-12]. Adiponectin also reduces the production of androgens in the ovary, which is a key feature of PCOS[13]. The decrease in adiponectin levels in PCOS contributes to the hyperandrogenism and ovulatory dysfunction associated with this disorder[14].

Oxidative stress play a significant role in the pathophysiology of several diseases, including PCOS[15]. The underlying mechanisms linking PCOS with oxidative stress are not fully understood. It is believed that oxidative stress may lead to the development of insulin resistance by impairing insulin signaling pathways and promoting inflammation[16]. Furthermore, oxidative stress may lead to the hyperandrogenism seen in PCOS by promoting the production of androgens by the ovaries and adrenal glands[17]. Moreover, it has been shown that adiponectin has antioxidant properties and protects against oxidative stress, and the decline in adiponectin levels may be a factor in PCOS patients' increased oxidative stress[18]. Malondialdehyde (MDA) is a byproduct of lipid peroxidation that is often used as a marker for the presence of oxidative stress in the body[19,20]. Several studies have reported increased levels of MDA in females with PCOS and is related with insulin resistance and dyslipidemia[15, 16, 21]. The elevated levels of MDA in females with PCOS suggest the presence of oxidative stress in this population.

Dyslipidemia is a common metabolic abnormality PCOS, and females with PCOS are more likely to have dyslipidemia including, decreased high-density lipoprotein (HDL) levels and increased triglyceride, low-density lipoprotein

(LDL) levels, compared to women without PCOS[22,23]. The mechanisms underlying the relationship between dyslipidemia and PCOS are thought to be related to insulin resistance, androgen excess, and obesity[22]. Dyslipidemia is a significant risk factor for the development of cardiovascular disease (CVD) in women with PCOS.

Previous studies have shown that adiponectin, MDA, and lipid profile are altered in women with PCOS[9,15,16,21]. However, the relationship between these biomarkers and the metabolic disturbances associated with PCOS is not well understood. Therefore, this study aimed to assess the serum levels of adiponectin, MDA, and lipid profile in females with PCOS and compare them with those of healthy controls. We hypothesize that women with PCOS will have lower levels of adiponectin, higher levels of MDA, and dyslipidemia compared to healthy controls.

METHODOLOGY

Subjects

This cross-sectional study involved 60 females with an age range between (16-35) years, from August 2022 to September 2022. Thirty women with polycystic ovary syndrome and thirty healthy underweight women as a control group. For all participants, the body mass index (BMI) was calculated from the measured weight and height.

Biochemical measurements

All blood samples were obtained from women after overnight fasting and incubated for 10 mins at 37°C in a water bath, and then centrifuged at 3500 rpm for 12 mins. Sera were obtained and stored at -20°C for estimation of adiponectin, malondialdehyde, TC, LDL, TG, VLDL and HDL.

ELISA was applied to determine the concentration of adiponectin using a kit provided by USBIOLOGICAL (USA)[24]. The modified method, in which MDA and thiobarbituric acid (TBA) react to form a pink compound detectable at 532 nm, was used to determine the serum malondialdehyde concentration[25].

An enzymatic colorimetric method was used to measure fasting serum TG[26], TC, and HDL using BIOLABO kit while, VLDL and LDL levels were determined using Friedewald's equation[27].

Data analysis

All values are set as mean ± standard deviation (SD). Unpaired t-tests were used for comparisons between PCOS and control groups, using GraphPad Prism software version 8.0.2, California, USA.

RESULTS and DISCUSSION

The demographic characteristics of the control and PCOS groups are described in Table 1. Sixty women included in this study, of which 30 were healthy women and 30 had PCOS. No significant variations in mean age were observed between the control and the PCOS group (p-value 0.29). However, there was a significant difference in body mass index (BMI) between the women with PCOS and control groups (p-value 0.0012). PCOS women had significantly higher BMI (mean difference 1.81) (Figure 1).

Table 1. Demographic characteristics of the control and PCOS groups

Parameters	Control (n=30)	PCOS (n=30)
Age (years)	23.67 ± 6.126	21.97 ± 4.478
BMI (Kg/m^2)	17.74 ± 0.7032	19.55 ± 1.956*

PCOS: polycystic ovary syndrome. BMI: body mass index. Values set as mean ± standard deviation (SD). Unpaired t-test was used, where *p < 0.05 sets as statistically significant

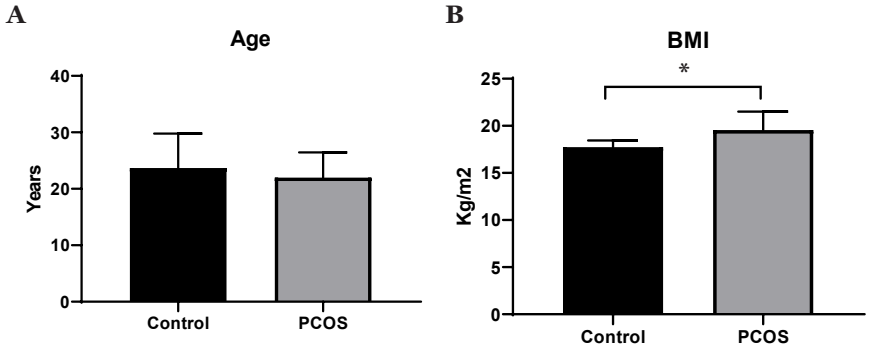

Figure 1. Demographic characteristics of the control and PCOS groups. (A) Age, (B) BMI. PCOS: polycystic ovary syndrome. BMI: body mass index. Values set as mean ± standard deviation (SD). Unpaired t-test was used, where *p < 0.05 sets as statistically significant.

Validation of serum levels of adiponectin, malondialdehyde and lipid profile

Women with PCOS had significantly lower serum adiponectin levels (Figure 2), and significantly higher total cholesterol levels than the control group (Figure 3). However, no significant variations in the levels of MDA (Figure 4), LDL, VLDL, TG, and HDL (Figure 3) were observed between the study groups Table 2.

Table 2. Serum levels of adiponectin, malondialdehyde and lipid profile

Parameters	Control (n=30)	PCOS (n=30)
Adiponectin (µg/ml)	11.24 ± 1.140	10.12 ± 1.403*
Malondialdehyde (µmol/L)	0.3953 ± 0.1802	0.6647 ± 0.5970
TC (mmol/L)	120.8 ± 16.56	144.6 ± 37.08*
LDL (mmol/L)	52.13 ± 22.39	66.01 ± 35.66
TG (mmol/L)	89.33 ± 17.45	106.4 ± 34.00
VLDL (mmol/L)	17.89 ± 3.500	21.31 ± 6.778
HDL (mmol/L)	49.67 ± 10.95	53.90 ± 11.42

TC: total cholesterol. LDL: low-density lipoprotein. TG: triglyceride. VLDL: very low-density lipoprotein. HDL: high-density lipoprotein. Values set as mean ± standard deviation (SD). *p < 0.05 represents statistically significant differences, as set by unpaired t-test

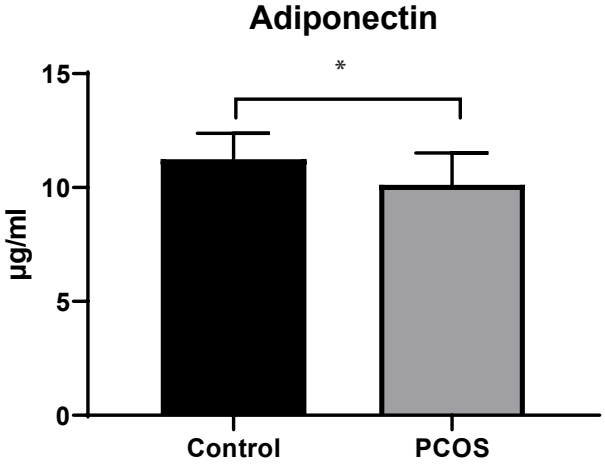

Figure 2. Serum levels of adiponectin. Values set as mean ± standard deviation (SD). *p < 0.05 represents statistically significant differences, as set by unpaired t-test.

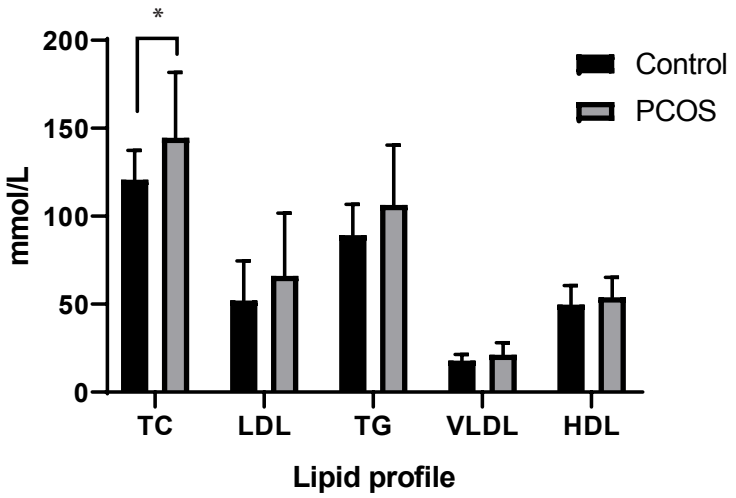

Figure 3. Lipid profile. TC: total cholesterol. LDL: low-density lipoprotein. TG: triglyceride. VLDL: very low-density lipoprotein. HDL: high-density lipoprotein. Values set as mean ± standard deviation (SD). *$p < 0.05$ represents statistically significant differences, as set by unpaired t-test.

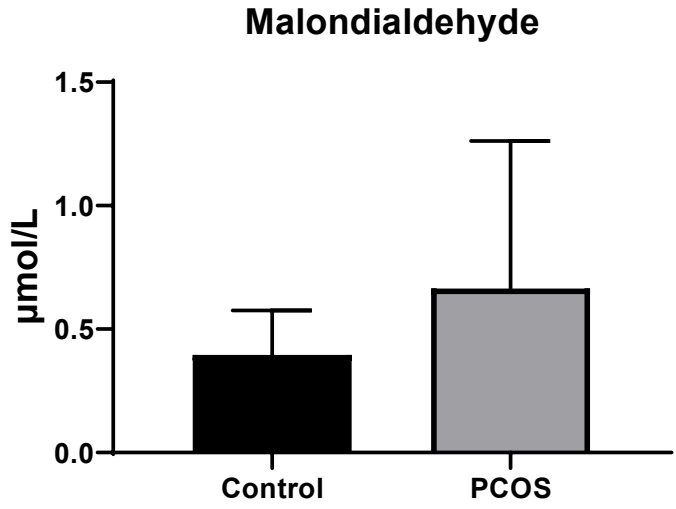

Figure 4. Serum levels of malondialdehyde. Values set as mean ± standard deviation (SD).

The complicated nature of PCOS makes it extremely challenging for physicians to trace the underlying cause and identify the key signs to aid in the diagnosis of illness and the associated metabolic repercussions[28]. One of the principal alterations in PCOS is dyslipidemia, which is primarily caused by insulin resist-

ance and glucose intolerance and has an impact on other potential biomarkers including adiponectin[29,30]. Thus, reduced adiponectin level could indicate the persistence of PCOS[31]. The current study focused on lean women with PCOS, indicated by BMI, to reveal an average reduced level of adiponectin. Physiologically, decreased adiponectin level is a feature in obese individuals which reversed with reduced body weight[32,33]. Although women involved in the current study are not apparently obese, they indicated significant increase in BMI compared to their corresponding control. It is known that adiponectin level is negatively correlated with BMI[32]. Consequently, the present results may reflect normal findings as the adiponectin level in tested individuals with PCOS were inversely related to BMI. This was in agreement with certain studies that indicated reduced adiponectin production with increased obesity, and this is well correlated also with the dyslipideamia associated with PCOS[34-36]. However, the fact that these women are considered underweight, indicated by BMI, may highlight the metabolic abnormality arises from PCOS's pathophysiological changes spotted by lower adiponectin and increased cholesterol levels. This is an important characteristic of PCOS where there are changes in the circulating levels of adipokines, including adiponectin, causing disturbed lipid metabolism which may even be observed in non-obese women[37]. This was consistent with Mirza S. et al. whom conducted a case control study to explore the level of adiponectin in PCOS non-obese females[38]. The study indicated lower levels of adiponectin in PCOS women compared to their healthy controls of the same age and weight range. The study also claimed the usefulness of adiponectin as a biomarker for PCOS in lean young women. Beyazit et al. investigated the levels of adipokines, including adiponectin, and their correlation with obesity in PCOS women[39]. The study reveals reduced levels of adiponectin in PCOS women with averaged BMI below than 25 and concluded that adiponectin may serve as a significant biomarker in the diagnosis of PCOS which may support the findings from the current study. However, in a study by Arikan et al. to evaluate the variations in the resistin and adiponectin levels in non-obese PCOS young women, the results where contrary with that of the current study[40]. It reveals significantly elevated adiponectin levels in PCOS women compared to their controls. Geloneze B et al. raised another claim demanding that the level of adiponectin is positively correlated with that of HDL independently from obesity or BMI[34]. The latter may partially correlate with the present results in highlighting decreased levels of adiponectin in PCOS and dyslipidaemia changes. However, the current study disagreed with the results from Geloneze B et al. in revealing no significant variations between HDL levels in PCOS women compared to their controls[41]. In fact, only total cholesterol in the current results, out of other

lipid profile markers, was significantly elevated in PCOS women. This is possibly because of the younger age and below averaged weight of women included in the study which could limit the chances of showing advanced pathological changes normally observed in older and obese PCOS patients. This could be the same reason why malonaldehyde level was not significant compared to controls showing no indication of altered oxidative stress. Chen et al. evaluated the levels of adiponectin and leptin and their association with lipid profile in obese and lean women with PCOS[37]. the study results revealed no significant changes in the level of adiponectin in lean PCOS women compared to controls which disagreed with the results from the current study. The results also were not consistent with the present study in showing significant elevation in serum levels of LDL, and triglyceride in lean women with PCOS. Uçkan et al. studied the relationship between oxidative stress markers and metabolic abnormalities in PCOS women, and indicated strong correlation between oxidative stress and metabolic changes in PCOS[42]. The study disagreed with the results of the current research as it revealed significant changes in MDA, LDL, and HDL levels in non-obese women with PCOS compared to controls.

Gözüküçük et al., also investigated the levels of adiponectin and leptin along with lipid profile in normal weight PCOS women[43]. The results were consistent with the results of the current study as it revealed no significant variations in lipid profile including serum triglycerides, LDL, and HDL between PCOS women and control subjects. Karadeniz et al. explored oxidative stress markers and lipid profile in young non-obese PCOS patients[41]. The study concluded no significant variations between PCOS patients with controls owing to the younger age of the patients and being non-obese. This was consistent with the results from the current work and may thus support the claims that adiponectin and total cholesterol levels could serve as useful markers in PCOS. This was evident as these indices showed significant difference in PCOS patients compared to other markers even in younger non-obese individuals.

Findings from the current study revealed statistical difference in the serum levels of both adiponectin and total cholesterol spotted in lean young women with PCOS. These markers may serve as early predictive markers of PCOS in these individuals even when other indicators are not evident or yet significant.

STATEMENT OF ETHICS

The approval was obtained from the University of Mosul/ Ethics Committee (25.04.2021-No. 5/5/7044).

CONFLICT OF INTEREST STATEMENT

No conflict of interest was declared by the authors.

AUTHOR CONTRIBUTIONS

Surgical and Medical Practices: Z.M.Y., Z.H.F. Concept: Z.M.Y., Z.H.F. Design: J.A.M., Z.H.F. Data Collection or Processing: S.M.M., Z.M.Y. Analysis or Interpretation: Z.H.F., Z.M.Y. Literature Search: J.A.M., S.M.M., Z.M.Y., Z.H.F. Writing: J.A.M., S.M.M., Z.M.Y., Z.H.F.

REFERENCES

1. Deswal R, Narwal V, Dang A, Pundir CS. The prevalence of polycystic ovary syndrome: a brief systematic review. J Hum Reprod Sci, 2020;13(4):261-271. Doi: 10.4103/jhrs.JHRS_95_18

2. Jayasena CN, Franks S. The management of patients with polycystic ovary syndrome. Nat Rev Endocrinol, 2014;10(10):624-636. Doi: 10.1038/nrendo.2014.102

3. Carvalho LML, Ferreira CN, Soter MO, Sales MF, Rodrigues KF, Martins SR, et al. Microparticles: inflammatory and haemostatic biomarkers in polycystic ovary syndrome. Mol Cell Endocrinol, 2017;443:155-162. Doi: 10.1016/j.mce.2017.01.017

4. Palomba S, Santagni S, Falbo A, La Sala GB. Complications and challenges associated with polycystic ovary syndrome: current perspectives. Int J Womens Health, 2015;7:745-763. Doi: 10.2147/IJWH.S70314

5. Papadakis G, Kandaraki E, Papalou O, Vryonidou A, Diamanti-Kandarakis E. Is cardiovascular risk in women with PCOS a real risk? Current insights. Minerva Endocrinol, 2017; 42(4):340-355. Doi: 10.23736/S0391-1977.17.02609-8

6. Pavaleanu I, Gafitanu D, Popovici D, Duceac LD, Pavaleanu M. Treatment of metabolic alterations in polycystic ovary syndrome. Rev Med Chir Soc Med Nat Iasi, 2016;120(2):258-263.

7. Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. Endocr Rev, 2005; 26(3):439-451. Doi: 10.1210/er.2005-0005

8. Ardawi MS, Rouzi AA. Plasma adiponectin and insulin resistance in women with polycystic ovary syndrome. Fertil Steril, 2005;83(6):1708-1716. Doi: 10.1016/j.fertnstert.2004.11.077.

9. Shin HY, Lee DC, Lee JW. Adiponectin in women with polycystic ovary syndrome. Korean J Fam Med, 2011;32(4):243-248. Doi: 10.4082/kjfm.2011.32.4.243

10. Chabrolle C, Tosca L, Crochet S, Tesseraud S, Dupont J. Expression of adiponectin and its receptors (AdipoR1 and AdipoR2) in chicken ovary: potential role in ovarian steroidogenesis. Domest Anim Endocrinol, 2007;33(4):480-487. Doi: 10.1016/j.domaniend.2006.08.002

11. Ramachandran R, Ocon-Grove O M, Metzger SL. Molecular cloning and tissue expression of chicken AdipoR1 and AdipoR2 complementary deoxyribonucleic acids. Domest Anim Endocrinol, 2007;33(1):19-31. Doi: 10.1016/j.domaniend.2006.04.004

12. Mohammed M, Mohammad J, Fathi Z, Al-Hamdany M, Alkazzaz N. Comparative evaluation of cystatin C and neutrophil gelatinase-associated lipocalin in patients with thalassemia major versus thalassemia intermedia. Pharmacia, 2021;68(4):741-746. Doi: 10.3897/pharmacia.68.e71475

13. Comim FV, Hardy K, Franks S. Adiponectin and its receptors in the ovary: further evidence for a link between obesity and hyperandrogenism in polycystic ovary syndrome. PLoS One, 2013;8(11):e80416. Doi: 10.1371/journal.pone.0080416

14. Delitala A P, Capobianco G, Delitala G, Cherchi P L, Dessole S. Polycystic ovary syndrome, adipose tissue and metabolic syndrome. Arch Gynecol Obstet, 2017;296(3):405-419. Doi: 10.1007/s00404-017-4429-2

15. Mohammadi M. Oxidative Stress and Polycystic Ovary syndrome: a brief review. Int J Prev Med, 2019;10(1):86. Doi: 10.4103/ijpvm.IJPVM_576_17

16. Murri M, Luque-Ramirez M, Insenser M, Ojeda-Ojeda M, Escobar-Morreale HF. Circulating markers of oxidative stress and polycystic ovary syndrome (PCOS): a systematic review and meta-analysis. Hum Reprod Update, 2013;19(3):268-288. Doi: 10.1093/humupd/dms059

17. Zuo T, Zhu M, Xu W. Roles of oxidative stress in polycystic ovary syndrome and cancers. Oxid Med Cell Longev, 2016;8589318. Doi: 10.1155/2016/8589318

18. Comim FV, Gutierrez K, Bridi A, Bochi G, Chemeris R, Rigo ML, et al. Effects of adiponectin including reduction of androstenedione secretion and ovarian oxidative stress parameters in vivo. PLoS One, 2016;11(5):e0154453. Doi: 10.1371/journal.pone.0154453

19. Abuja P M, Albertini R. Methods for monitoring oxidative stress, lipid peroxidation and oxidation resistance of lipoproteins. Clin Chim Acta, 2001;306(1-2):1-17. Doi: 10.1016/s0009-8981(01)00393-x

20. Fathi ZH, Mohammad JA, Mohammed MH. Levels of myeloperoxidase, malondialdehyde and lipid profile in type 2 diabetic patients on metformin versus glibenclamide therapy. Systematic Reviews in Pharmacy, 2020;11(11):1777-1782. Doi: 10.31838/srp.2020.11.248

21. Sabuncu T, Vural H, Harma M, Harma M. Oxidative stress in polycystic ovary syndrome and its contribution to the risk of cardiovascular disease. Clin Biochem, 2001;34(5):407-413. Doi: 10.1016/S0009-9120(01)00245-4

22. Diamanti-Kandarakis E, Papavassiliou A G, Kandarakis S A, Chrousos G P. Pathophysiology and types of dyslipidemia in PCOS. Trends Endocrinol Metab, 2007;18(7):280-285. Doi: 10.1016/j.tem.2007.07.004

23. Fathi ZH, Mohammad JA, Younus ZM, Mahmood SM. Hepcidin as a Potential Biomarker for the Diagnosis of Anemia. Turk J Pharm Sci, 2022;19(5):603-609. Doi: 10.4274/tjps.galenos.2021.29488

24. Ouchi N, Kobayashi H, Kihara S, Kumada M, Sato K, Inoue T, et al. Adiponectin stimulates angiogenesis by promoting cross-talk between AMP-activated protein kinase and Akt signaling in endothelial cells. J Biol Chem, 2004;279(2):1304-1309. Doi: 10.1074/jbc.M310389200

25. Guidet B, Shah SV. Enhanced in vivo H2O2 generation by rat kidney in glycerol-induced renal failure. Am J Physiol, 1989; 257(3): F440-F445. Doi: 10.1152/ajprenal.1989.257.3.F440

26. Reilly MP, Lehrke M, Wolfe ML, Rohatgi A, Lazar MA, Rader DJ. Resistin is an inflammatory marker of atherosclerosis in humans. Circulation, 2005;111(7):932-939. Doi: 10.1161/01.cir.0000155620.10387.43

27. Ruotolo G, Parlavecchia M, Taskinen M R, Galimberti G, Zoppo A, Le NA, et al. Normalization of lipoprotein composition by intraperitoneal insulin in IDDM. Role of increased hepatic lipase activity. Diabetes care, 1994;17(1):6-12. Doi: 10.2337/diacare.17.1.6

28. Zhao X, Feng X, Zhao X, Jiang Y, Li X, Niu J, et al. How to screen and prevent metabolic syndrome in patients of PCOS early: implications from metabolomics. Front Endocrinol, 2021; 12:659268. Doi: 10.3389/fendo.2021.659268

29. Hopkinson ZE, Sattar N, Fleming R, Greer IA. Polycystic ovarian syndrome: the metabolic syndrome comes to gynaecology. BMJ (Clinical research ed), 1998;317(7154):329-332. Doi: 10.1136/bmj.317.7154.329

30. Fathi ZH, Mohammad JA, Mohammed MH. Evaluation of the Vasoprotective Effects of Metformin versus Glibenclamide in Type 2 Diabetic Patients. Res J Pharm Technol, 2021; 14(12):6409-6412. Doi: 10.52711/0974-360X.2021.01108

31. Iqbal MN, Iqbal MA, Basit A. Association of Adiponectin Levels with Polycystic Ovarian Syndrome. Int J Curr Sci Res Rev, 2022;5(5):1705-1709. Doi: 10.47191/ijcsrr/V5-i5-39

32. Nigro E, Scudiero O, Monaco ML, Palmieri A, Mazzarella G, Costagliola C, et al. New insight into adiponectin role in obesity and obesity-related diseases. Biomed Res Int, 2014;2014:658913. Doi: 10.1155/2014/658913

33. Barber TM, Hanson P, Weickert MO, Franks S. Obesity and Polycystic Ovary Syndrome: Implications for Pathogenesis and Novel Management Strategies. Clin Med Insights Reprod Health, 2019;13:1179558119874042. Doi: 10.1177/1179558119874042

34. Geloneze B, Pereira JA, Pareja JC, Lima MM, Lazarin MA, Souza IC, et al. Overcoming metabolic syndrome in severe obesity: adiponectin as a marker of insulin sensitivity and HDL-cholesterol improvements after gastric bypass. Arch Endocrinol Metab, 2009;53(2):293-300. Doi: 10.1590/s0004-27302009000200022

35. Toulis KA, Goulis DG, Farmakiotis D, Georgopoulos NA, Katsikis I, Tarlatzis BC, et al. Adiponectin levels in women with polycystic ovary syndrome: a systematic review and a meta-analysis. Hum Reprod Update, 2009;15(3):297-307. Doi: 10.1093/humupd/dmp006

36. Michalakis KG, Segars JH. The role of adiponectin in reproduction: from polycystic ovary syndrome to assisted reproduction. Fertil Steril, 2010; 94(6):1949-1957. Doi: 10.1016/j.fertnstert.2010.05.010

37. Chen CI, Hsu MI, Lin SH, Chang YC, Hsu CS, Tzeng CR. Adiponectin and leptin in overweight/obese and lean women with polycystic ovary syndrome. Gynecol Endocrinol, 2015; 31(4):264-268. Doi: 10.3109/09513590.2014.984676

38. Mirza SS, Shafique K, Shaikh AR, Khan NA, Anwar Qureshi M. Association between circulating adiponectin levels and polycystic ovarian syndrome. J Ovarian Res, 2014;7:18. Doi: 10.1186/1757-2215-7-18

39. Beyazit F, Hiz MM, Turkon H, Unsal MA. Serum spexin, adiponectin and leptin levels in polycystic ovarian syndrome in association with FTO gene polymorphism. Ginekol Pol, 2021;92(10):682-688. Doi: 10.5603/GP.a2020.0176

40. Arikan S, Bahceci M, Tuzcu A, Kale E, Gökalp D. Serum resistin and adiponectin levels in young non-obese women with polycystic ovary syndrome. Gynecol Endocrinol, 2010;26(3):161-166. Doi: 10.3109/09513590903247816

41. Karadeniz M, Erdoğan M, Tamsel S, Zengi A, Alper GE, Cağlayan O, et al. Oxidative stress markers in young patients with polycystic ovary syndrome, the relationship between insulin resistances. Exp clin endocrinol, 2008;116(4):231-235. Doi: 10.1055/s-2007-992154

42. Uçkan K, Demir H, Turan K, Sarıkaya E, Demir C. Role of Oxidative Stress in Obese and Nonobese PCOS Patients. Int J Clin Pract, 2022;2022:4579831. Doi: 10.1155/2022/4579831.

43. Gözüküçük M, Yarcı Gürsoy A, Destegül E, Taşkın S, Şatıroğlu H. Adiponectin and leptin levels in normal weight women with polycystic ovary syndrome. Horm Mol Biol Clin Investig, 2020;41(4). Doi: 10.1515/hmbci-2020-0016

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Cladode and fruit anatomy of *Opuntia ficus-indica* (L.) Miller in Türkiye

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ABSTRACT

Opuntia ficus-indica (L.) Mill. belong to the Cactaceae family, since can grow in conditions where other plants cannot survive, it is distributed in many parts of the world due to its socioeconomic, nutraceutical, and ecological properties. Especially, its fruits and cladodes are used as herbal medicine for various health problems in different countries. Pharmacological effects such as antioxidant, antimicrobial, anticancer, antiulcer, hepatoprotective, wound healing, hypocholesterolaemia, anti-diabetic, and anti-obesity were demonstrated with conducted studies. In this study, the anatomy of cladode and fruit of *O. ficus-indica* grown in Türkiye has been studied in detail. Cyclocytic stomata were detected on both fruit and cladode surfaces. Abundant calcium oxalate crystals and mucilage cells were detected in both fruit and cladode. The importance of anatomical study was emphasized for plants to be used for food and medicinal purposes.

Keywords: *Opuntia ficus-indica*, cladode, fruit, anatomy

INTRODUCTION

Opuntia ficus-indica (L.) Mill. is a perennial drought-resistant plant in the Cactaceae family, adapted to arid and semi-arid areas. The plant, which is generally known as "prickly pear" in the world, is known by names such as "Frenk

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inciri, Hint inciri, Kaynana dili" in Türkiye. Although its origin is Mexico, it is grown in many parts of the world today such as Mediterranean countries, South Africa, and North and South America[1-3].

Shrub or small tree with the succulent trunk, which is 2-4 m high, are spread. As the trunk ages, woody branches form in the lower part. The trunk has undergone metamorphosis by taking the shape of a leaf and is called a cladode. Its trunk has an articulated structure, and the joints are 10-40 × 7-20 cm in size, ranging from oblong-elliptical to obovate and flattened. Caducous leaves are about 3 mm long and subulate. As with other plants, they also develop spines rather than leaves, these spines are white in colour. They may develop spiny structures called glochids are weak, thin, small brush-like structures, alongside or in place of these spines and yellow colour. Glochids are found in small meristematic eyes called areoles. Glochids are found in the areole space in clusters of 7-12. Areoles do not have spines and their number varies between 1-6. The surfaces of spines are rough, however, glochids have smooth ones. The flowers are hermaphrodites and bright yellow. The berry is oval, 5-10 cm long, red, yellow, orange, or purplish. There are areoles on the berry, the berry usually does not bear spines. The seed has bony testa. It is whitish, and numerous[3-5].

Food products made from its fruit and cladodes are plentiful. Its fruits are utilized to produce a variety of items such as jams, juices, alcoholic beverages, natural sweeteners, body lotions, shampoos, and creams. Its cladodes are consumed as vegetables. Their principal usage is flour, which can be used in place of maize or wheat flour in baked goods including bread, cookies, and cakes[6]. In Italy, cladodes are used directly for skin diseases, viral infections (Herpes), and joint pain, while in South Africa it is recommended for haemorrhoids and toothache[7,8]. In Ethiopia, the cladodes are set on fire for anthrax in livestock and applied to the affected area when hot, and crushed and rubbed into their skin for lice or flea infestations[9]. In the Philippines, the decoction of cladodes is recommended for diarrhoea[10]. In Bolivia, it is recommended as moxibustion for angina, headache, and oedema, while its sap is consumed for cough. The sap obtained by crushing it is used for burns in the form of direct application, for hair such as shampoo, for kidney pain directly or by heating, and its decoction for body cleansing[11,12]. While the jam of the fruit is consumed for cough and cold in Italy, the fruit is eaten directly for gastritis in Ethiopia[7,13].

The plant grows naturally in the Mediterranean and Aegean regions of Turkey and also is cultured in these regions[14]. The decoction of cladodes is used for bronchitis by drinking a glass three times a day, and its mash is used by making compresses for rheumatism in Turkey[15]. Its cladodes and fruits are applied

with salt once a day for 1-2 weeks for dislocation and tonsillitis by heating, boiling, or in the form of cataplasm[16]. The fruit is eaten as fruit after peeling the prickly skin in the Mediterranean Region of Turkey (Fig. 1). This fruit is also eaten fresh for stomach-ache, 10 times a day on an empty stomach[17]. After peeling, it is consumed for diabetes, and anaemia, as well as a laxative and aphrodisiac. Peeled fruits are also used for moistening in the form of mash[18, 19].

Figure 1. *Opuntia ficus-indica* fruits and peeling off the prickly skin of the fruit (Photo. A. Köroğlu)

It is reported that especially, flavonoids, phenolic acids, betalains, carotenoids, and sterols in the fruit[20-30]. The fruit also is rich in Vitamin C and E [20, 21, 25, 31]. Its seeds are regarded as a good source of unsaturated fatty acids[25,32]. It has been indicated that the cladodes contain especially flavonoids, phenolic acids, tannins, and carotenoids[33-39].

Antioxidant, antimicrobial, antileishmanial, and anticancer activities of the fruit have been demonstrated by *in vitro* studies[20,24,25,28,30,39-42]. Its neuroprotective, sedative, hypocholesterolemic, antiulcer, and hepatoprotective effects have been approved by *in vivo* studies[20,29,43,44]. Especially, antioxidant and antimicrobial activities studies have been carried out on the cladodes *in vitro*

[34,35,37,45,46]. The pharmacological effects of cladodes such as antiulcer, wound healing, anti-inflammatory, hepatoprotective, antidiabetic, antispasmodic, hypocholesterolemic, and anti-obesity have also been demonstrated by *in vivo* studies[34,35,47-52]. The number of anatomy studies on the cladode is limited[53-55]. In addition, no clear study was found about the anatomy of the fruit, except for the structure of the pericarp of the seed[56].

This study aims to determine the anatomical features of the fruit and cladode of *Opuntia ficus-indica*, which is of great importance in terms of both medicinal and food use.

METHODOLOGY

The fruits and cladodes samples were collected in Antalya-Olympos (Türkiye) on August 22, 2017 (Figure 1-2) and preserved in 70% alcohol. The specimens were identified by Professor Ayşegül Köroğlu. At least 5 different samples were examined, and cross and surface sections were taken. The cross and surface sections taken from these samples were examined under the light microscope (Leica CME) with Sartur reagent [57] and their images were obtained with the Leica DFC280 camera.

Figure 2. *Opuntia ficus-indica* (Photo. A. Köroğlu)

RESULTS and DISCUSSION

Anatomy of the cladode blade

The morphological view of the cross-section of the cladode blade is shown in Figure 3. In the cross-section, it is surrounded on the outside by a thick waxy cuticle layer. The epidermis cells are rectangular and single-row. The hypodermis is usually 4-rowed and the walls of its cells are thickened. Big druses are observed in this part. The chlorenchyma consists of multi-row, thin-walled, and long rectangular-shaped cells with plastids. It contains a large number of druses and starches. Mucilage cells are observed in the chlorenchyma. Below the chlorenchyma is the part where the vascular bundles are located. The vascular bundles are of the closed collateral bundle type, with a xylem on the inside and phloem on the exterior. The core is usually composed of round and colourless cells. It carries abundant druses and starches (Figure 4).

Figure 3. The morphological view of the cross-section of the cladode blade

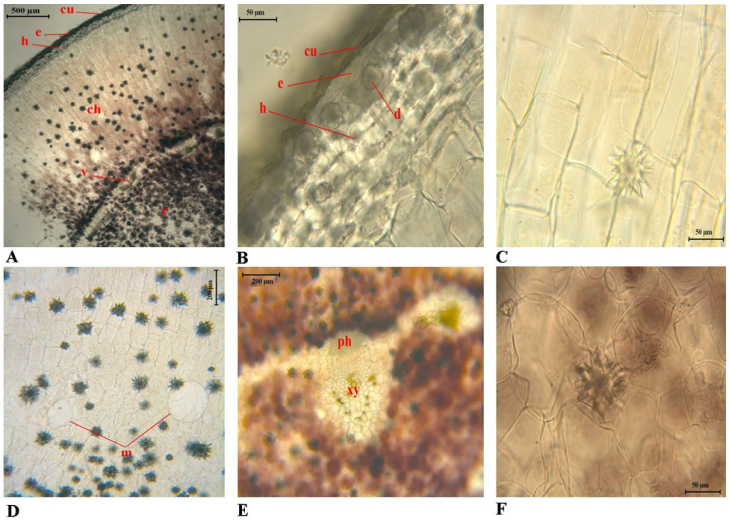

Figure 4. The microscopic image of the cross-section of the cladode blade; **A., B.:** General view, **C.** Druse in chlorenchyma, **D.** Mucilage cells in the chlorenchyma, **E.** Vascular bundle, **F.** Druse in the core. c: core, ch: chlorenchyma, cu: cuticula, d: druse, e: epidermis, h: hypodermis, m: mucilage cells, ph: phloem, v: vascular bundle, xy: xylem.

In the surface section, the epidermis cells are polygonal and slightly wavy-walled. Cyclocytic stomata were observed, the subsidiary cells of which were not very clear. The presence of druses and mucilage cells was observed (Figure 5).

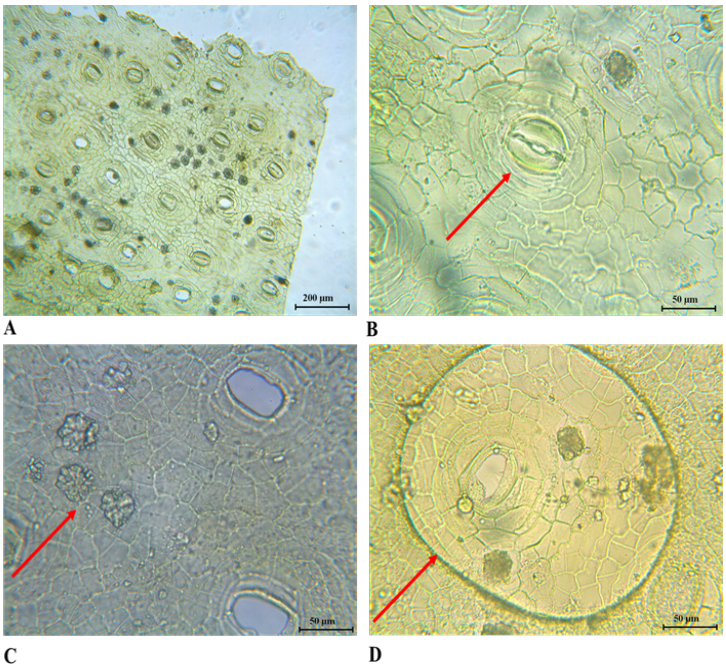

Figure 5. The microscopic image of the surface section of the cladode; **A.** General view, **B.** Stomata, **C.** Druses, **D.** Mucilage cell.

Anatomy of the fruit

The morphological view of the fruit and morphological view of the cross-section of the fruit are shown in Figures 6 and 7, respectively. In the exocarp, the thick waxy cuticular layer is located. Usually, rectangular and single-row cells make up the epidermis. The cells of the hypodermis usually have four rows and it has thicker cell walls. In the hypodermis, abundantly big druses are seen. Multi-row colourless long cylindrical parenchyma cells are located under the hypodermis. The mesocarp consists of irregularly shaped paranchymatic colourless cells with plastids. The presence of mucilage cells was observed in the mesocarp. Druses are observed more intensely in the mesocarp than in the exocarp. The vascular bundles are of the closed collateral bundle type and scattered in the mesocarp (Figure 8).

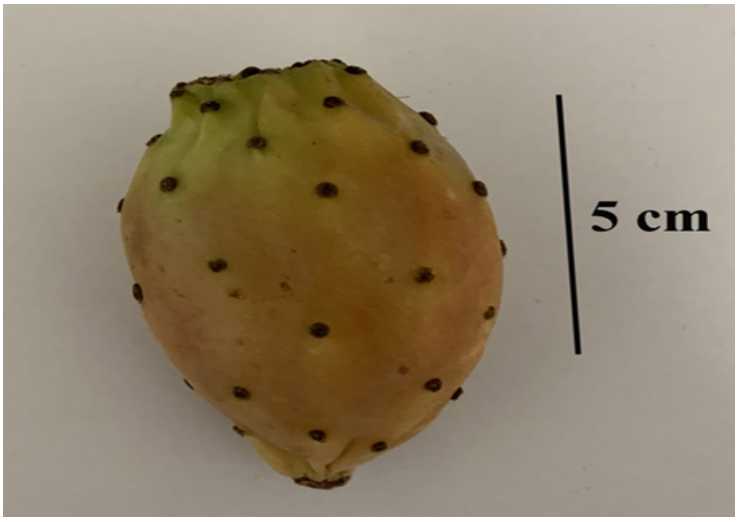

Figure 6. The morphological view of the fruit of *Opuntia ficus-indica*

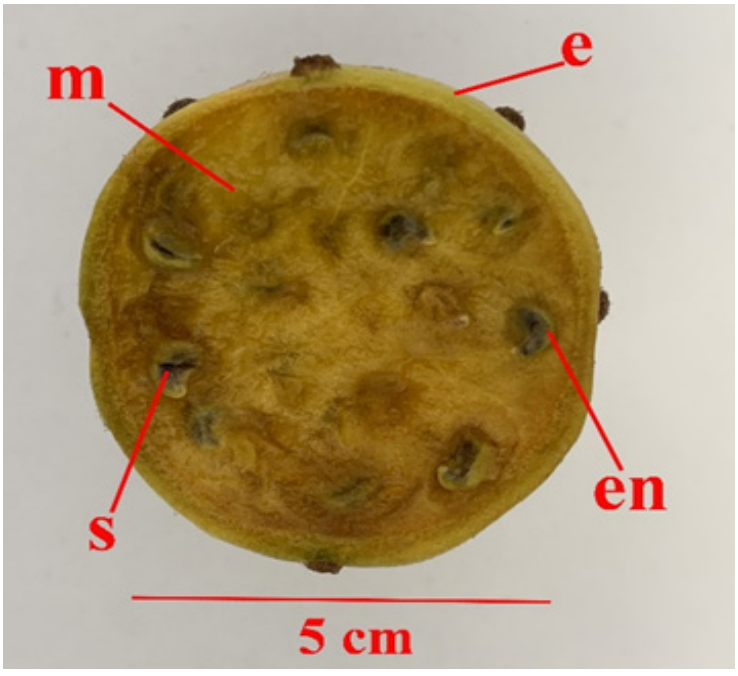

Figure 7. The morphological view of the cross-section of the *Opuntia ficus-indica* fruit; e: exocarp, m: mesocarp, en: endocarp, s: seed

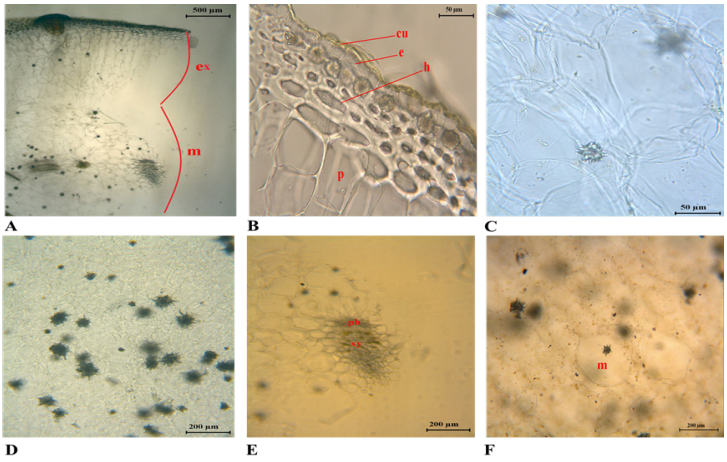

Figure 8. The microscopic image of the cross-section of the *Opuntia ficus-indica* fruit; A., B.: General view, C. Druse in exocarp parenchyma, D. Druses in the mesocarp, E. Vascular bundle, F. Mucilage cells in the mesocarp. e: epidermis, ex: exocarp, cu: cuticula, h: hypodermis, m: mesocarp, m: mucilage cell,.p: parenchyma, ph: phloem, xy: xylem.

In the surface section of the fruit of *Opuntia ficus-indica*, the epidermis cells are irregularly shaped and have slightly wavy walls. Cyclocytic stomata are present and the cells adjacent to the stoma are not clear. Abundant large druses are observed on the surface of the fruit (Figure 9).

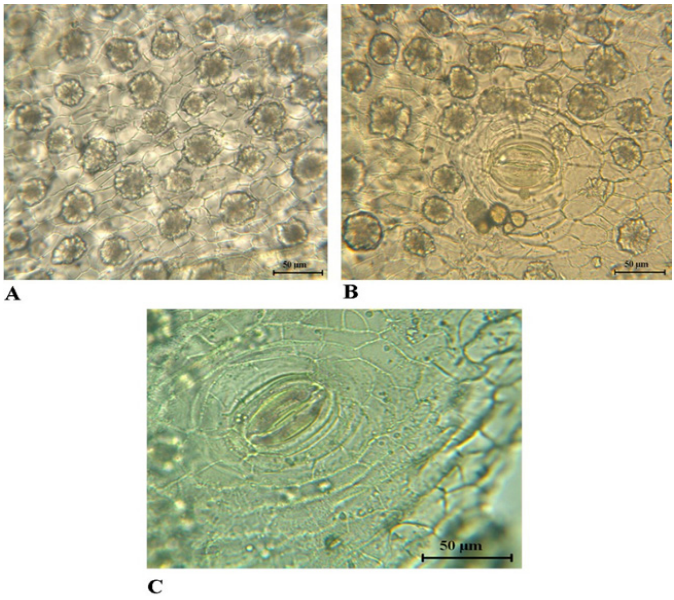

Figure 9. The microscopic image of the surface-section of the fruit; A. General view, B. Stomata and druses, C. Stomata

Different parts of *O. ficus-indica* are used in various fields such as health, nutrition, and cosmetics. Fruits and cladodes contain high amounts of important nutrients. The fruits and cladodes have been used in traditional folk medicine for many years in different parts of the world for different purposes. There are many studies about the chemical properties and biological activities of fruits and cladodes. However, the number of anatomy studies on the fruit and cladode is limited. Anatomical studies are very important for the identification of microcharacters of medicinal plants part (drugs).

As a result of the anatomical examinations; cyclocytic stomata with unclear adjacent cells in both cladode and fruit surface sections; on the other hand, abundant calcium oxalate crystals were detected in the cross and surface sections of two different parts of *Opuntia ficus-indica* (Figures 4, 5, 8, 9). It was observed that the hypodermis layer was collenchymatic in the cross-section of the cladode and fruit (Figures 4A, 4B, 8B and 9B). Mucilage cells were characteristically determined on the cladode surface and among the parenchyma cells in the cross-section (Figures 4D and 5D). In addition, mucilage cells were determined in the mesocarp part of the fruit (Figure 8F). The anatomical findings we obtained are generally combined with the source data found compatible[53-56]. The presence of pseudohypoderm in the cladode and that this structure has a collenchymatic and mucilage structure were stated by Metcalfe and Chalk (1965) [58], but this structure was not observed in our study and the presence of mucilage was found in the chlorenchyma part.

The main purposes of calcium oxalate crystal production in plants are high-capacity calcium regulation and herbivore defence. The crystals consist of calcium that the plant takes from the outside and oxalate that it biologically synthesizes. Studies have shown that ascorbic acid is a precursor in the biosynthesis of oxalate. Since ascorbic acid is also an antioxidant substance, there may be a relationship between antioxidant activity and the presence of calcium oxalate crystals. Because it increases the amount of oxalate excreted in urine and leads to the development of kidney stones, oxalate content may be harmful to human health[59].

In this study, the anatomical structures of *Opuntia ficus-indica* fruit and cladode grown in Türkiye were examined using light microscopy for the first time. The anatomical structure of the fruit has been revealed in detail. Cladodes and fruits, which are consumed for their medicinal and nutritive properties, contain plenty of druses. For this reason, clinical studies should be conducted on whether excessive or long-term consumption carries risks for human health.

STATEMENT OF ETHICS

No needed.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

GK: Designed and performed the study, and wrote the first draft. AK: Collected the plant, and reviewed the manuscript.

REFERENCES

1. Baytop T. Türkiye'de bitkilerle tedavi (Geçmişte ve Bugün). Nobel Tıp Kitabevleri, İstanbul, 1999.

2. de Wit M, Nel P, Osthoff G, Labuschagne MT. The effect of variety and location on cactus pear (*Opuntia ficus-indica*) fruit quality. Plant Foods Hum Nutr, 2010;65:136-145. Doi: 10.1007/s11130-010-0163-7

3. Abbas EY, Ezzat MI, El Hefnawy HM, Abdel-Sattar E. An overview and update on the chemical composition and potential health benefits of *Opuntia ficus-indica* (L.) Miller. J. Food Biochem, 2022;46:e14310. Doi: 10.1111/jfbc.14310

4. Matthews VA. Flora of Turkey and the East Aegean Islands. vol 4. Edinburgh University Press: Davis PH; 1973. p. 209.

5. WFO 2022. World Flora Online. http://www.worldfloraonline.org. Accessed 2 December 2022.

6. Barba FJ, Garcia C, Fessard A, Munekata PE, Lorenzo JM, Aboudia A, Ouadia A, Remize F. *Opuntia ficus indica* edible parts: a food and nutritional security perspective. Food Rev Int, 2022;38(5):930-952. Doi: 10.1080/87559129.2020.1756844

7. Ballero M, Poli F, Sacchetti G, Loi MC. Ethnobotanical research on the territory of Fluminimaggiore (southwestern Sardinia). Fitoterapia, 2001;72:788-801. Doi:10.1016/S0367-326X(01)00334-3

8. Kose LS, Moteetee A, Van Vuuren S. Ethnobotanical survey of medicinal plants used in the Maseru district of Lesotho. J Ethnopharmacol, 2015;170:184-200. Doi: 10.1016/j.jep.2015.04.047

9. Teklay A, Balcha A, Mirutse G. An ethnobotanical study of medicinal plants used in Kilte Awulaelo District, Tigray Region of Ethiopia. J Ethnobiol Ethnomed, 2013;9,65. Doi: 10.1186/1746-4269-9-65

10. Tantengco OAG, Condes MLC, Estadilla HHT, Ragragio EM. Ethnobotanical survey of medicinal plants used by Ayta Communities in Dinalupihan, Bataan, Philippines. Pharmacogn J, 2018;10(5):859-870. Doi: 10.5530/pj.2018.5.145

11. Fernandez EC, Sandi YE, Kokoska L, Ethnobotanical inventory of medicinal plants used in the Bustillo Province of the Potosi Department, Bolivia. Fitoterapia, 2003;74:407-416. Doi: 10.1016/S0367-326X(03)00053-4

12. Macia MJ, Garcia E, Vidaurreb PJ. An ethnobotanical survey of medicinal plants commercialized in the markets of La Paz and El Alto, Bolivia. J Ethnopharmacol, 2005;97(2):337-350. Doi: 10.1016/j.jep.2004.11.022

13. Wondimu T, Asfaw Z, Kelbessa E. Ethnobotanical study of medicinal plants around 'Dheeraa' town, Arsi Zone, Ethiopia. J Ethnopharmacol, 2007;112(1):152-161. Doi: 10.1016/j.jep.2007.02.014

14. Kabas O, Ozmerzi A, Akinci I. Physical properties of cactus pear (*Opuntia ficus-indica* L.) grown wild in Turkey. J Food Eng, 2006;73(2):198-202. Doi: 10.1016/j.jfoodeng.2005.01.016

15. Güneş S, Savran A, Paksoy MY, Koşar M, Çakılcıoğlu U. Ethnopharmacological survey of medicinal plants in Karaisalı and its surrounding (Adana-Turkey). J Herb Med, 2017;8:68-75. Doi: 10.1016/j.hermed.2017.04.002

16. Sargin SA. Ethnobotanical survey of medicinal plants in Bozyazı district of Mersin, Turkey. *J Ethnopharmacol*, 2015;173:105-126. Doi: 10.1016/j.jep.2015.07.009

17. Akaydın G, Şimşek I, Arıtuluk Z C, Yeşilada E. An ethnobotanical survey in selected towns of the Mediterranean subregion (Turkey). Turk J Biol, 2013;37:230-247. Doi:10.3906/biy-1010-139

18. Gürdal B, Kültür Ş. An ethnobotanical study of medicinal plants in Marmaris (Muğla, Turkey). J Ethnopharmacol, 2013;146:113-126. Doi: 10.1016/j.jep.2012.12.012

19. Güzel Y, Güzelşemme M, Miski M. Ethnobotany of medicinal plants used in Antakya: A multicultural district in Hatay Province of Turkey. J Ethnopharmacol, 2015;174:118-152. Doi: 10.1016/j.jep.2015.07.042

20. Galati EM, Mondello MR, Giuffrida D, Dugo G, Miceli N, et al. Chemical characterization and biological effects of Sicilian *Opuntia ficus indica* (L.) Mill. fruit juice: Antioxidant and antiulcerogenic activity. J Agric Food Chem, 2003;51(17):4903-4908. Doi: 10.1021/jf030123d

21. Ramadan MF, Mörsel J. Recovered lipids from prickly pear [*Opuntia ficus-indica* (L.) Mill] peel: a good source of polyunsaturated fatty acids, natural antioxidant vitamins and sterols. Food Chem, 2003;83(3):447-456. Doi: 10.1016/S0308-8146(03)00128-6

22. Cano MP, Gómez-Maqueo A, García-Cayuela T, Welti-Chanes J. Characterization of carotenoid profile of Spanish *Sanguinos* and *Verdal* prickly pear (*Opuntia ficus-indica*, spp.) tissues. Food Chem, 2017;237:612-622. Doi: 10.1016/j.foodchem.2017.05.135

23. Slimen IB, Mabrouk M, Hanène C, Najar T, Abderrabba M. LC-MS analysis of phenolic acids, flavonoids and betanin from spineless *Opuntia ficus-indica* fruits. Cell Biology, 2017;5(2):17-28. Doi: 10.11648/j.cb.20170502.12

24. Aruwa CE, Amoo S, Kudanga T, Phenolic compound profile and biological activities of Southern African *Opuntia ficus-indica* fruit pulp and peels. LWT-Food Sci Technol, 2019;111:337-344.

25. Belviranlı B, Al-Juhaimi F, Özcan MM, Ghafoor K, Babiker EE, Alsawmahi ON. Effect of location on some physico-chemical properties of prickly pear (*Opuntia ficus-indica* L.) fruit and seeds. J Food Process Preserv, 2019;43(3). Doi: 10.1111/jfpp.13896

26. García-Cayuela T, Gómez-Maqueo A, Guajardo-Flores D, Welti-Chanes J, Cano MP. Characterization and quantification of individual betalain and phenolic compounds in Mexican and Spanish prickly pear (*Opuntia ficus-indica* L. Mill) tissues: A comparative study. J Food Compos Anal, 2019;76: 1-13. Doi: 10.1016/j.jfca.2018.11.002

27. Smeriglio A, Bonasera S, Germanò MP, D'Angelo V, Barreca D, et al. *Opuntia ficus-indica* (L.) Mill. fruit as source of betalains with antioxidant, cytoprotective, and anti-angiogenic properties. Phyther Res, 2019;33(5):1526-1537. Doi: 10.1002/ptr.6345

28. Zeghad N, Ahmed E, Belkhiri A, Heyden YV, Demeyer K. Antioxidant activity of *Vitis vinifera*, *Punica granatum*, *Citrus aurantium* and *Opuntia ficus indica* fruits cultivated in Algeria. Heliyon, 2019;5(4). Doi: 10.1016/j.heliyon.2019.e01575

29. Küpeli Akkol E, Ilhan M, Karpuz B, Genç Y, Sobarzo-Sánchez E. Sedative and anxiolytic activities of *Opuntia ficus indica* (L.) Mill.: An experimental assessment in mice. Molecules, 2020;25(8):1844. Doi: 10.3390/molecules25081844

30. Ali SK, Mahmoud SM, El-Masry SS, Alkhalifah DHM, Hozzein WN, Aboel-Ainin MA. Phytochemical screening and characterization of the antioxidant, antiproliferative and antibacterial effects of different extracts of *Opuntia ficus-indica* peel. J King Saud Univ Sci, 2022;34(7):102216. Doi: 10.1016/j.jksus.2022.102216

31. Gouws CA, Georgouopoulou E, Mellor DD, Naumovski N. The effect of juicing methods on the phytochemical and antioxidant characteristics of the purple prickly pear (*Opuntia ficus*

indica)-preliminary findings on juice and pomace. Beverages, 2019;5(2):28. Doi: 10.3390/beverages5020028

32. de Wit M, Hugo A, Shongwe N. South African cactus pear seed oil: a comprehensive study on 42 spineless burbank *Opuntia ficus-indica* and *Opuntia robusta* cultivars. Eur J Lipid Sci Technol, 2018;120:1700343. Doi: 10.1002/ejlt.201700343

33. González-Cruz L, Filardo-Kerstupp S, Bello-Pérez LA, Güemes-Vera N, Bernardino-Nicanor A. Carotenoid content, antioxidant activity and sensory evaluation of low-calorie nopal (*Opuntia ficus-indica*) marmalade. J Food Process Preserv, 2012;36(3),267-275. Doi: 10.1111/j.1745-4549.2011.00589.x

34. Ben Saad A, Dalel B, Rjeibi I, Smida A, Ncib S, et al. Phytochemical, antioxidant and protective effect of cactus cladodes extract against lithium-induced liver injury in rats. Pharm Biol, 2017;55(1):516-525. Doi: 10.1080/13880209.2016.1255976

35. Lanuzza F, Occhiuto F, Monforte MT, Tripodo MM, D'Angelo V, Galati EM. Antioxidant phytochemicals of *Opuntia ficus-indica* (L.) Mill. cladodes with potential anti-spasmodic activity. Pharmacogn Mag, 2017;13(Suppl 3):S424-429. Doi: 10.4103/pm.pm_495_16

36. Figueroa-Pérez MG, Pérez-Ramírez IF, Paredes-López O, Mondragón-Jacobo C, Reynoso-Camacho R. Phytochemical composition and *in vitro* analysis of Nopal (*O. ficus-indica*) cladodes at different stages of maturity. Int J Food Prop, 2018;21(1):1728-1742. Doi: 10.1080/10942912.2016.1206126

37. Aruwa CE, Amoo SO, Kudanga T. Extractable and macromolecular antioxidants of *Opuntia ficus-indica* cladodes: Phytochemical profiling, antioxidant and antibacterial activities. S Afr J Bot, 2019b; 125:402-410. Doi: 10.1016/j.sajb.2019.08.007

38. De Santiago E, Juániz I, Cid C, De Peña MP, Extraction of (poly)phenolic compounds of cactus (*Opuntia ficus-indica* (L.) Mill.) cladodes. Food Anal Methods, 2021;14(6):1167-1175. Doi: 10.1007/s12161-020-01946-6

39. Önem E, Kendir G, Akkoç S, Erzurumlu Y, Muhammed MT, Özaydın AG. Biochemical contents and antiquorum sensing, antiproliferative activities of *Opuntia ficus-indica* (L.) Mill. peel extract. S Afr J Bot, 2022;150:296-304. Doi: 10.1016/j.sajb.2022.07.024

40. Bargougui A, Champy P, Triki S, Bories C, Le Pape P, Loiseau PM. Antileishmanial activity of *Opuntia ficus-indica* fractions. Biomed Prev Nutr, 2014;4(2):101-104. Doi: 10.1016/j.bionut.2013.09.001

41. El-Beltagi HS, Mohamed HI, Elmelegy AA, Eldesoky SE, Safwat G. Phytochemical screening, antimicrobial, antioxidant, anticancer activities and nutritional values of cactus (*Opuntia ficus-indica*) pulp and peel. Fresenius Environ Bull, 2019;28(2A):1534-1551.

42. Smeriglio A, De Francesco C, Denaro M, Trombetta D. Prickly pear betalain-rich extracts as new promising strategy for intestinal inflammation: plant complex vs. main isolated bioactive compounds. Front Pharmacol, 2021;12:722398. Doi: 10.3389/fphar.2021.722398

43. Kim JH, Park SM, Ha HJ, Moon CJ, Shin TK, Kim JM, et al. *Opuntia ficus-indica* attenuates neuronal injury in *in vitro* and *in vivo* models of cerebral ischemia. J Ethnopharmacol, 2006;104:257-262. Doi: 10.1016/j.jep.2005.09.017

44. Kim JW, Kim TB, Kim HW, Park SW, Kim HP, Sung SH. Hepatoprotective flavonoids in *Opuntia ficus-indica* fruits by reducing oxidative stress in primary rat hepatocytes. Pharmacogn Mag, 2017;13(51):472-476. Doi: 2010.4103/pm.pm_232_16

45. Du Toit A, de Wit M, Osthoff G, Hugo A. Antioxidant properties of fresh and processed cactus pear cladodes from selected *Opuntia ficus-indica* and *O. robusta* cultivars. South Afri-

can J Bot, 2018;118: 44-51. Doi: 10.1016/j.sajb.2018.06.014

46. Blando F, Russo R, Negro C, De Bellis L, Frassinetti S. Antimicrobial and antibiofilm activity against *Staphylococcus aureus* of *Opuntia ficus-indica* (L.) Mill. cladode polyphenolic extracts. Antioxidants, 2019;8(5):117. Doi: 10.3390/antiox8050117

47. Galati EM, Monforte MT, Tripodo MM, d'Aquino A, Mondello MR. Antiulcer activity of *Opuntia ficus indica* (L.) Mill. (Cactaceae): ultrastructural study. J Ethnopharmacol, 2001;76:1-9. Doi: 10.1016/S0378-8741(01)00196-9

48. Park E-H, Chun M-J. Wound healing activity of *Opuntia ficus-indica*. Fitoterapia, 2001;72(2):165-167. Doi: 10.1016/S0367-326X(00)00265-3

49. Antunes-Ricardo M, Gutiérrez-Uribe JA, López-Pacheco F, Alvarez MM, Serna-Saldívar SO. *In vivo* anti-inflammatory effects of isorhamnetin glycosides isolated from *Opuntia ficus-indica* (L.) Mill cladodes. Ind Crops Prod, 2015;76:803-808. Doi: 10.1016/j.indcrop.2015.05.089

50. Padilla-Camberos E, Flores-Fernández, JM, Fernandez-Flores O, Gutierrez-Mercado Y, Carmona-de la Luz J, et al. Hypocholesterolemic effect and *in vitro* pancreatic lipase inhibitory activity of an *Opuntia ficus-indica* extract. BioMed Res Int, 2015;837452. Doi: 10.1155/2015/837452

51. Hwang SH, Kang I-J, Lim SS. Antidiabetic effect of fresh nopal (*Opuntia ficus-indica*) in low-dose streptozotocin-induced diabetic rats fed a high-fat diet. Evid Based Complement Altern Med, 2017. Doi: 10.1155/2017/4380721

52. Héliès-Toussaint C, Fouché E, Naud N, Blas-Y-Estrada F, del Socorro Santos-Diaz M, et al. F. *Opuntia* cladode powders inhibit adipogenesis in 3 T3-F442A adipocytes and a high-fat-diet rat model by modifying metabolic parameters and favouring faecal fat excretion. BMC Complement Med Ther, 2020;20:33. Doi: 10.1186/s12906-020-2824-x

53. Malainine ME, Dufresne A, Dupeyre D, Mahrouz M, Vuong R, Vignon MR. Structure and morphology of cladodes and spines of *Opuntia ficus-indica*. Cellulose extraction and characterisation. Carbohydr Polym, 2003;51:77-83. Doi: 10.1016/S0144-8617(02)00157-1

54. Ginestra G, Parker ML, Bennett RN, Robertson J, Mandalari G, et al. Anatomical, chemical, and biochemical characterization of cladodes from prickly pear [*Opuntia ficus-indica* (L.) Mill.]. J Agric Food Chem, 2009;57:10323-10330. Doi: 10.1021/jf9022096

55. Ben Salem-Fnayou A, Zemni H, Nefzaoui A, Ghorbel A. Micromorphology of cactus-pear (*Opuntia ficus-indica* (L.) Mill) cladodes based on scanning microscopies. Micron, 2014;56:68-72. Doi: 10.1016/j.micron.2013.10.010

56. Habibi Y, Heux L, Mahrouz M, Vignon MR. Morphological and structural study of seed pericarp of *Opuntia ficus-indica* prickly pear fruits. Carbohydr Polym, 2008;72(1):102-112. Doi: 10.1016/j.carbpol.2007.07.032

57. Turkish Pharmacopoeia. General Monographs II. 1st edition. Publication No: 1098. Ankara: T.C. Ministry of Health; 2018.

58. Metcalfe CR, Chalk, L. Anatomy of the dicotyledons. Clarendon Press: Oxford. 1957. vol. 2. p. 557.

59. Franceschi VR, Nakata PA. Calcium oxalate in plants: formation and function. Annu Rev Plant Biol, 2005;56:41-71. Doi:10.1146/annurev.arplant.56.032604.144106

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Metabolic profiling of *Brachychiton rupestris* (T.Mitch. ex Lindl.) K. Schum. leaves using UPLC-ESI-MS and their antimicrobial potential

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ABSTRACT

Globally, the number of multi-drug resistant microbes increases critically and the search for novel antimicrobial agents from medicinal plants becomes necessary to overcome such serious problem. The antimicrobial activity of the methanol extract of *Brachychiton rupestris* leaves and its fractions was assessed against six pathogens through two *in-vitro* assays: Agar well diffusion and broth microdilution. UPLC-ESI-MS analysis of these samples was also performed. *Mucor circinelloides* was resistant to the tested samples. The inhibition zone of the samples ranged between 10 and 23 mm while the minimum inhibitory values ranged between 31.25 and 1000 μg/mL. The samples produced significant antimicrobial potential. Based on the UPLC-ESI-MS analysis, the phytochemical profile of both the methanol extract and the ethyl acetate fraction comprise a variety of phenolic compounds and hydroxy fatty acids while that of the *n*-butanol fraction composed of phenolic compounds mainly

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flavonoids. *B. rupestris* could be considered a promising source of natural antimicrobials.

Keywords: *Brachychiton rupestris*, antimicrobial, UPLC-ESI-MS, phenolics, microdilution assay

INTRODUCTION

Infectious diseases represent one of the major causes of morbidity worldwide. Despite the existence of a variety of potent antibiotics in the market, the death rate due to microbial infections increases recently. This may be due to the ability of the pathogenic microbes to develop resistance against the available antimicrobial drugs as a way to survive. The excessive and improper use of the available commercial antibiotics participated in the emergence of antimicrobial resistance (AMR). The increased number of multidrug resistance microbes side by side with the multiplicity of the undesirable side effects of the existing antimicrobials and the appearance of new maladies for which no treatment yet exists creates a crucial need to find out novel and potent antimicrobial agents[1,]

For centuries, man depends on medicinal plants, in the treatment, control and prevention of various illnesses. Nowadays, people in both developed and developing countries are still relying on medicinal plants in providing their primary health care needs by the reason of their affordability, availability and safety due to their lesser toxicity and lesser side effects in comparison with conventional synthetic drugs[3]. Presently, medicinal plants are considered a valuable source for searching for new drug leads based on their richness with bioactive secondary metabolites such as flavonoids and phenolic acids[4].

Brachychiton rupestris (T.Mitch. ex Lindl.) K. Schum. is one of the medicinal plants that is characterized by its bottle shaped trunk and belongs to the family Malvaceae. Too little studies have been conducted on *B. rupestris*. *B. rupestris* was reported to possess significant antischistosomal potential on *Schistosoma mansoni* as well as potent hypoglycemic and hepatoprotective activity on experimental animals in addition to antioxidant, antimicrobial and cytotoxic activities [5- 8]. Based on the previously published papers, the phytochemical profile of *B. rupestris* comprises flavonoids, phenolics, sterols and volatile compounds[9-13].

Ultra-high performance liquid chromatography (UHPLC) hyphenated with mass spectrometer (MS) is a newly applied separation and identification technique which is characterized by its remarkable high resolution, speed, sensi-

tivity and time and resources saving. It has been widely used in the separation and tentative identification of secondary metabolites existed in plant extracts and fractions[14].

This study aimed to investigate the antimicrobial activity of the methanol extract *B. rupestris* leaves as well as its ethyl acetate and *n*-butanol derived fractions against six microbial strains through two *in-vitro* methods. Also, separation and tentative identification of their phytochemical profile through the application of UPLC-ESI-MS in the negative ionization mode.

METHODOLOGY

Plant material

Leaves of *Brachychiton rupestris* were collected in September 2017 from Orman Garden, Giza, Egypt. Both Mrs. Treaze Labib (a consultant of plant taxonomy at the Agriculture Ministry and the ex-director of Orman Garden) and Mrs. Rehab Mohamed Eid (a botanist at Orman Garden Herbarium) performed the identification process of the plant specimen and a voucher sample (No. 278 BC) was deposited in Orman Garden Herbarium. The collected plant leaves were shade dried at room temperature and were then blended to a coarse powder using an electric blender.

Extraction and fractionation processes

The plant leaves fine powder (200 g) was successively extracted with 85% aqueous methanol at room temperature. The resultant extract was then filtered and evaporated under a reduced vacuum using a rotary evaporator (BUCHI, Germany) till complete dryness affording crude methanol extract of 33.58 grams (16.79%). The crude extract was then fractionated using petroleum ether, methylene chloride, ethyl acetate and *n*- butanol successively and evaporated under reduced pressure till complete dryness giving approximately 1 g of petroleum ether, 2.70 g of methylene chloride, 0.90 g of ethyl acetate, 4.50 g of *n*- butanol and 11.10 g of aqueous fractions.

UPLC-ESI-MS profiling

Waters XEVO TQD UPLC-ESI-MS (MA01757, Milford, USA) equipped with ACQUITY UPLC- BEH C18 (1.7: 2.1 μm× 50 mm) column was used to separate and tentatively identify the major secondary metabolites in the *B. rupestris* methanol extract and its ethyl acetate and *n*-butanol derived fractions. A stock solution of the three test samples (100 μg/mL) was prepared through dissolution in HPLC analytical grade MeOH previously filtered with a 0.2 μm membrane disc filter. 10 μL of each sample was injected into the UPLC instru-

ment. The mobile phase elution was carried out at a flow rate of 0.2 mL/min using two eluents: eluent A is H_2O acidified with 0.1% formic acid and eluent B is MeOH acidified with 0.1% formic acid. The gradient elution was set as follows: 0–5 min, 10%–30% B; 5–15 min, 30%–70% B; 15–25 min, 70%–90% B, 25–32 min, 90%–100% B.

The negative ESI ionization ion mode was performed by applying the following parameters: source temperature 150 °C, cone voltage 60 eV, capillary voltage 3 kV, desolvation temperature 440 °C, cone gas flow 50 L/h, and desolvation gas flow 900 L/h. Mass spectra were detected in the ESI between *m/z* 100–1000. The peaks and spectra were processed using the Masslynx 4.1 software. The separated compounds were tentatively identified by comparing their retention time (R_t) and their mass spectra with the previously published literature.

Antimicrobial properties

Microbial strains and growth conditions

Four bacterial strains including two gram-positive bacteria; *Bacillus Subtilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 6538) and two gram-negative bacteria; *Escherichia coli* (ATCC 8739) and *Pseudomonas aeruginosa* (ATCC 90274) in addition to two pathogenic yeasts; *Candida albicans* (ATCC 10221) and *Mucor circinelloides* (AUMC 6696) were used to investigate the antimicrobial properties of the crude methanol extract of *B. rupestris* leaves and its ethyl acetate and *n*-butanol derived fractions. Non supplemented Mueller-Hinton agar or routine bacteriology laboratory Mueller-Hinton agar plates (pH, 7.2–7.4 after gelling) were used to culture the microbial strains. Gentamycin was used as a positive control.

Agar well diffusion assay

Briefly, Mueller-Hinton agar plates were inoculated with a suspension of the test microbes adjusted to 10^8 CFU/mL. Then, 6 mm diameter holes were made aseptically in the inoculated plates. In each hole, 100μL of the antimicrobial agent (gentamycin) or the plant test samples at a concentration of 10 mg/mL (methanol) were loaded. The agar plates were then incubated for 24 h at 37°C for bacteria or 48 h at 37°C for yeast. The antimicrobial agent as well as the test samples will diffuse in the agar medium and inhibit the growth of the microbial strains tested. After the incubation period, the zone of microbial growth inhibition was measured accurately in mm as an indication of antimicrobial activity[2]. The strength of the antimicrobial activity of the test samples against the test microorganisms was assigned based on the diameter of the growth inhibition zone as follows; potent antimicrobial potential when the growth inhibition

zone diameter is greater than 30 mm, strong when the growth inhibition zone diameter ranged between 30 and 21 mm, moderate when the growth inhibition zone diameter ranged between 20 and 16 mm, weak when the growth inhibition zone diameter ranged between 15 and 10 mm and little or no activity if the inhibition zone diameter is less than 10 mm[15].

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration is the lowest concentration of an antimicrobial agent that prevents the visible growth of a microorganism which can be determined via a broth microdilution susceptibility test. Briefly, pure cultures of the test microbes were grown overnight and diluted in Muller Hinton broth to a concentration between 10^5 CFU/mL to 10^6 CFU/mL. A stock solution of the test samples was then prepared by dissolving 10 mg of each sample in 10 mL of distilled water (1000 μg/mL). Furthermore, serial two fold dilutions of the test samples were prepared and distributed in 96-well microtiter plates ranging from 1000 to 0.06 μg/mL. Ten microliters of the prepared microbial suspension were added to the wells. Sterility and a growth control well were also included for every test microorganism. The microtiter plates were then incubated for 24 h at 37°C. The MICs were indicated via observing the turbidity which is the reflection of microbial growth. MIC is the lowest concentration where no visible growth is observed[1]fractions and isolated compounds from this plant. Bioassay guided fractionation was also undertaken to deeply evaluate the antibacterial activity of the water fraction of the leaves extract. This is to provide preliminary scientific evidence to the ethnopharmacology usage of this plant by investigating antibacterial properties of the plant and its isolated constituents. Methods: Bio-assay guided fractionation and subsequent isolation of compounds using open column chromatography. The antibacterial activity against gram positive and gram negative ATCC strain and resistant clinical strains were evaluated using microtiter broth dilution method to determine minimum inhibitory concentration (MIC.

RESULTS and DISCUSSION

Identification of the major chemical constituents of *B. rupestris* using (UPLC-ESI-(-ve)-MS)

The secondary metabolites existed in the crude methanol extract of *B. rupestris* leaves, the ethyl acetate and the *n*-butanol derived fractions were tentatively identified via UPLC-ESI-MS analysis. Their TICs were exhibited in Figure 1.

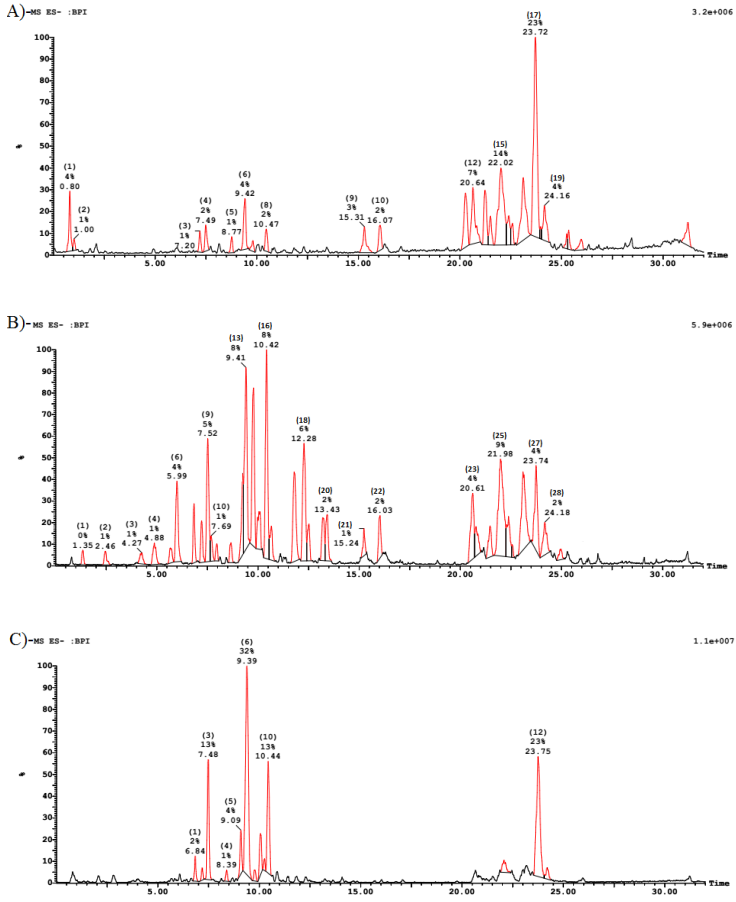

Figure 1. Total ion chromatograms (TIC) of A)-85% MeOH extract, B)- EtOAC fraction and C)-n-BuOH fraction of *B. rupestris* leaves, Numbering of peaks indicates compounds tentatively identified in the Tables (1-3).

Compounds tentatively identified in the methanol extract of *B. rupestris* leaves

Ten phenolics and five hydroxy fatty acids were identified in the methanol extract of *B. rupestris* leaves. The retention time, the molecular weight and the fragmentation data of these compounds were presented in **Table 1**. Peak 1, R_t 0.79 min, exhibited $[M-H]^-$ ion at m/*z* 341 and product ions at m/*z* 179 $[M-H-162]^-$ and 135 which are the characteristic fragments of caffeic acid produced due to loss of hexosyl residue (162 Da). So, this compound was identified as caffeoyl hexoside[16]. Peak 2, R_t 1.00 min, showed a molecular ion peak at m/*z* 191 with a fragment ion at m/*z* 127 $[M-H-CO-2H_2O]^-$ so that it was identified

as quinic acid[17]. Peak 3, R_t 7.20, had a $[M-H]^-$ ion at m/*z* 401 in addition to two base peaks at m/*z* 269 formed due to loss of pentosyl residue (132 Da) and 161 formed as a result of loss of both pentosyl and hexosyl residues (132 Da+162 Da). This compound was identified as benzyl alcohol hexose pentose[18]. Peak 4, R_t 7.50 min, exhibited the deprotonated molecule ion at m/*z* 593 and fragment ion at m/*z* 431 due to loss of hexose sugar in addition to two more fragment ions at m/*z* 163 and 119 which are indicative of coumaroyl residue thus this compound was identified as Kaempferol-3-*O*-coumaroylhexoside[19,20]. Peak 5, R_t 8.77 min, displayed a parent ion peak at m/*z* 739 and two main fragments at m/*z* 430 $[M-2H-146-162]^-$ (loss of hexose and deoxyhexose sugars) and 284 formed due to loss of deoxyhexosyl residue from the previous peak. This MS data suggested that this compound could be kaempferol deoxyhexoside hexoside deoxyhexoside [21]. Peak 6, R_t 9.41 min, had a parent ion at m/*z* 609 in addition to two major fragment ions at m/*z* 463 and 300 such MS data indicated that this compound could be rutin[22]. Peak 7, R_t 9.81 min, showed the $[M-H]^-$ ion at m/*z* 433 and two fragments ions at m/*z* 300 and 152 which are indicative of quercetin aglycone that was formed as a result of loss of pentose sugar (132 Da) so that this compound was identified as quercetin pentoside[23]. Peak 8, R_t 10.48 min, exhibited a molecule ion at m/*z* 593 and two fragments at m/*z* 447 (loss of rhamnosyl moiety) and 285 (loss of rutinosyl moiety). This MS data indicated that this compound could be kaempferol rutinoside[24]. Peak 9, R_t 15.31 min, exhibited a molecular ion peak at m/*z* 327 and fragment ions at m/*z* 239, 229, 211 and 171. This compound was identified as oxo-dihydroxy-octadecenoic acid isomer based on data published by[25]. Peak 10, R_t 16.08 min, showed a molecular ion peak at m/*z* 329 in addition to base peaks at m/*z* 229, 221 and 171. This compound was identified as trihydroxy-octadecenoic acid based on similar MS data reported by[25]. Peak 11, R_t 20.25 min, showed a molecule ion at m/*z* 293 in addition to a fragment ion at m/*z* 275. These MS fragments were consistent with MS data of hydroxy-octadecatrienoic acid that was reported by[26]. Peak 13, R_t 21.24 min, displayed a molecule ion at m/*z* 295 with three fragment ions at m/*z* 277, 195 and 171. Also, peak 14, R_t 21.15 min, displayed a molecule ion at m/*z* 309 with two fragment ions at m/*z* 295 and 180. These compounds were identified as hydroxy-octadecadienoic acid and dihydroxy-octadecadienoic acid, respectively depending on MS fragmentation patterns reported by[26]. Peak 15, R_t 22.02 min, exhibited a deprotonated molecule ion at m/*z* 577 and fragment ions at 397, 353 [M–H–104–120]; the loss of 104 Da indicated the partial fragmentation of *C*- deoxyhexosyl moiety ($^{0,2}X_o$)$^-$ ion while the loss of 120 Da is characteristic for *C*- hexosyl flavone and 311. According to the mentioned MS data, this compound was identified as Api-

genin 8-*C*-deoxyhexoside-6-*C*-hexoside[27]. Peak 16, R_t 23.13 min, exhibited a molecular ion peak at m/*z* 325 and a base peak at m/*z* 293 formed due to loss of O_2 molecule. This compound was identified as *p*-coumaroyl hexoside[20].

Table 1. Compounds tentatively identified in methanolic extract of *B. rupestris* leaves by UPLC-ESI-(-ve)-MS

Peak no.	RT (min)	MW	$[M-H]^-$ (m/z)	Fragments (m/z)	Tentative Identification
	0.79	342	341	179, 135	Caffeoyl-*O*-hexoside
	1.00	192	191	127	Quinic acid
	7.20	402	401	269, 161	Benzyl alcohol hexose pentose
	7.50	594	593	431, 163, 119	Kaempferol-3-O-coumaroylhexoside
	8.77	740	739	430, 284	Kaempferol deoxyhexoside hexoside deoxyhexoside
	9.41	610	609	463, 300	Rutin
	9.81	434	433	300, 152	Quercetin pentoside
	10.48	594	593	447, 285	Kaempferol rutinoside
	15.31	328	327	239, 229, 211, 171	Oxo-dihydroxy-octadecenoic acid isomer
	16.08	330	329	229, 211, 171	Trihydroxy-octadecenoic acid
	20.25	294	293	275	Hydroxy-octadecatrienoic acid
	20.64	298	297	265	Unknown
	21.24	296	295	277, 195, 171	Hydroxy-octadecadienoic acid
	21.51	310	309	295, 180	Dihydroxy-octadecadienoic acid
	22.02	578	577	397, 353, 311	Apigenin 8-*C*-deoxyhexoside-6-*C*-hexoside
	23.13	326	325	293	*p*-coumaroyl hexoside
	23.71	556	555	337	Unknown
	23.97	572	571	540, 483, 447, 339	Unknown
	24.17	582	581	483, 455, 339, 163	Unknown

Compounds tentatively identified in the ethyl acetate fraction derived from the methanol extract of *B. rupestris* leaves

Twenty-two phenolics and three hydroxy fatty acids were tentatively identified in the ethyl acetate fraction derived from the methanol extract of *B. rupestris*. The retention time, the molecular weight and the MS data of these compounds were presented in **Table 2**. Peak 1, R_t 1.35 min, exhibited a parent ion at m/*z* 169 and a daughter ion at m/*z* 125 which are indicative to gallic acid[28]. Peak 2, R_t 2.46 min, showed a precursor ion at m/*z* 153 that was fragmented by losing CO_2 molecule producing ion at m/*z* 109 and hence this compound was identified as protocatechuic acid[29]. Peak 3, R_t 4.27 min, showed a molecule ion at m/*z* 153 and a fragment ion at m/*z* 108 which was formed due to loss of carboxylic group (– COOH, 45 Da) so that it was identified as dihydroxybenzoic acid[30]. Peak 4, R_t 4.88 min, displayed $[M-H]^-$ ion at m/*z* 183 and a fragment ion at m/*z* 124 which are indicative of methyl ester of gallic acid[31]. Peak 5, R_t 5.67 min, showed a molecule ion at m/*z* 177 that was further fragmented by losing CO_2 molecule producing ion at m/*z* 133. So, this compound was identified as 6, 7-dihydroxycoumarin (esculetin)[32]. Peak 6, R_t 5.99 min, showed a parent peak at m/*z* 179 and a base peak at m/*z* 135 formed due to loss of 44 Da (CO_2) which suggested that this compound could be caffeic acid[25]. Peak 7, R_t 6.84, has a molecule ion at m/*z* 633 and three fragment ions at m/*z* 463, 301 and 166. This compound was identified as corilagin as its MS data was consistent with the reported MS data of corilagin[33]. Peak 8, R_t 7.21, showed a parent ion at m/*z* 327 $[2M-H]^-$ and fragment ions at 283 $[2M-H-44]^-$ formed due to loss of CO_2, 237 $[2M-H-2\times45]^-$ formed due to loss of two carboxylic groups (COOH) and 163 (*p*-coumaric acid). So, this compound was identified as a dimer of coumaric acid. Peak 9, R_t 7.52 min, exhibited a molecular ion peak at m/*z* 593 with fragment ion at 431 formed due to loss of hexose sugar in addition to two more fragments at m/*z* 163 and 119 which are indicative to coumaric acid thus this compound was identified as Kaempferol-3-*O*-coumaroylhexoside [34]. Peak 10, R_t 7.69 min, showed a molecule ion at m/*z* 305 and fragment ions at 273 and 247. This compound was identified as methyl brevifolin carboxylate[24]. Peak 11, R_t 7.97 min, presented a molecule ion at m/*z* 303 and fragments at m/*z* 285, 151, 125 and 119. Based on this MS data, this compound was identified as taxifolin[35]. Peak 12, R_t 8.67 min, showed a precursor ion at m/*z* 615 and daughter ions at 463 and 301. This fragmentation pattern was consistent with quercetin galloyl hexoside[28]. Peak 15, 16 and 19 possessed the same MS pattern; $[M-H]^-$ ion at m/*z* 593 and fragment ions at m/*z* 447 and 285 and so identified as kaempferol rutinoside isomers that was also identified in MeOH ext. of *B. rupestris*. Peak 13, 14, 21, 22, 24, 25 and 26 were identified as rutin,

quercetin hexoside, oxo-dihydroxyoctadecenoic acid, trihydroxyoctadecenoic acid, dihydroxy-octadecadienoic acid, apigenin 8-C-deoxyhexoside-6-C-hexoside and *p*-coumaroyl hexoside, respectively as they had already identified in the MeOH extract of *B. rupestris* based on the MS data of the same compounds reported in the literature. Peak 17, R_t 11.79 min, exhibited a molecular ion at m/*z* 301 and fragment ions at m/*z* 289, 179 and 151 and so was identified as quercetin[35]. Furthermore, peak 18, R_t 12.28 min, possessed a molecule ion at m/*z* 285 and a fragment at m/*z* 151 and hence was identified as luteolin or kaempferol[35]. Also, peak 20, R_t 13.43 min, had a molecule ion at m/*z* 269 and a fragment ion at m/*z* 151 and was identified as apigenin[36].

Table 2. Compounds tentatively identified in EtOAc fraction derived from methanolic extract of *B. rupestris* by UPLC-ESI-(-ve)-MS

Peak no.	RT (min)	MW	$[M-H]^-$ (m/z)	Fragments (m/z)	Tentative Identification
	1.35	170	169	125	Gallic acid
	2.46	154	153	109	Protocatechuic acid
	4.27	154	153	108	Dihydroxybenzoic acid
	4.88	184	183	124	Methyl gallate
	5.67	178	177	133	6,7-dihydroxycoumarin (Esculetin)
	5.99	180	179	135	Caffeic acid
	6.84	634	633	463, 301, 166	Corilagin
	7.21	328	327	283, 237, 163	Dimer of coumaric acid
	7.52	594	593	431, 163, 119	Kaempferol-3-*O*-coumaroylhexoside
	7.69	306	305	273, 247	Methyl brevifolin carboxylate
	7.97	304	303	285, 151, 125, 119	Taxifolin
	8.67	616	615	463, 301	Quercetin galloyl hexoside
	9.41	610	609	463, 301	Rutin
	9.77	434	433	301	Quercetin pentoside
	10.05	594	593	447, 285	Kaempferol rutinoside isomer
	10.42	594	593	447, 285	Kaempferol rutinoside isomer
	11.79	302	301	289, 179, 151	Quercetin

	12.28	286	285	151	Luteolin or Kaempferol
	12.52	594	593	447, 285, 227	Kaempferol rutinoside isomer
	13.43	270	269	151	Apigenin
	15.24	328	327	239, 229, 211, 171	Oxo-dihydroxyoctadecenoic acid
	16.03	330	329	229, 211, 171	Trihydroxyoctadecenoic acid
	20.61	298	297	265	Unknown
	21.47	310	309	295, 180	dihydroxy-octadecadienoic acid
	21.98	578	577	397, 353, 311	Apigenin 8-*C*-deoxyhexoside-6-*C*-hexoside
	23.10	326	325	293, 277	*p*-coumaroyl hexoside
	23.74	556	555	337	Unknown
	24.18	582	581	483, 455, 339, 279	Unknown

Compounds tentatively identified in the *n*-butanol fraction derived from the methanol extract of *B. rupestris* leaves

Eleven phenolic compounds were tentatively identified in the *n*-butanol fraction derived from the methanol extract of *B. rupestris*. The retention time, the molecular weight and the MS fragmentation data of these compounds were presented in **Table 3**. Peak 1, R_t 6.84 min, exhibited a molecular ion at m/*z* 633 with a fragment ion at m/*z* 301. This compound was identified as corilagin which was also identified in the ethyl acetate fraction of *B. rupestris*. Peak 2, R_t 7.19 min, showed a parent ion at m/*z* 401 and a fragment ion at m/*z* 269 resulting from loss of pentose sugar. So, this compound was identified as benzyl alcohol hexosyl pentoside[18]. Peak 3, 6 and 7 were identified as kaempferol-3-*O*-coumaroylhexoside, rutin and quercetin pentoside based on their MS fragmentation data that is consistent with previous reports. These compounds were also identified in both the *B. rupestris* MeOH ext. and its ethyl acetate derived fraction. Peak 8 and 10 had the same $[M-H]^-$ ion at m/*z* 593 as well as the fragment ions at m/*z* 447 and 285 and so they were identified as kaempferol rutinoside isomers that were also identified in the MeOH ext. and its EtOAc derived fraction. Peak 4, R_t 8.39 min, exhibited $[M-H]^-$ ion at m/*z* 755 and fragment ions at m/*z* 593 $[M-H-162]^-$, 445 $[M-2H-162-146]^-$ and 289. So, this compound was identified as Kaempferol hexosyl deoxyhexosyl hexoside[37]. Peak 5, R_t 9.09 min, showed a molecular ion peak at m/*z* 739 and fragment

ions at m/*z* 431 [M–H–308] $^-$ produced due to loss of rutinosyl moiety and 285[M–H–308–146] $^-$ formed as a result of losing of one more deoxyhexosyl moiety. This compound was identified as Kaempferol hexosyl di-deoxyhexoside[38]. Peak 9, R_t 10.26 min, showed [M–H] $^-$ ion at m/*z* 591 with two major base peaks at m/*z* 445 [M–H–146] $^-$ resulting from loss of deoxyhexosyl sugar and 269 [M–H–146–176] $^-$ formed as a result of breaking down of glucuronide moiety from the previous peak leading to formation of apigenin aglycone. So, this compound was identified as apigenin glucuronide deoxyhexoside[39]. Peak 11, R_t 21.94 min, showed a precursor ion at m/*z* 593 and fragment ions at m/*z* 577, 353 and 311. This compound was identified as vicenin-2[29].

Table 3. Compounds tentatively identified in n-BuOH fraction derived from methanolic extract of *B. rupestris* by UPLC-ESI-(-ve)-MS

Peak no.	RT (min)	MW	$[M-H]^-$ (m/z)	Fragments (m/z)	Tentative Identification
	6.84	634	633	301	Corilagin
	7.19	402	401	269	Benzyl alcohol hexosyl pentoside
	7.48	594	593	431, 163, 119	Kaempferol-3-*O*-coumaroylhexoside
	8.39	756	755	593, 445, 289	Kaempferol hexosyl deoxyhexosyl hexoside
	9.09	740	739	431, 285	Kaempferol hexosyl dideoxyhexoside
	9.39	610	609	463, 301	Rutin
	9.76	434	433	300	Quercetin pentoside
	10.06	594	593	447, 285	Kaempferol rutinoside isomer
	10.26	592	591	445, 269	Apigenin glucuronide deoxyhexoside
	10.44	594	593	447, 285	Kaempferol rutinoside isomer
	21.94	594	593	577, 353, 311	Vicenin -2 (Apigenin-6,8-di-C-hexoside)
	23.75	556	555	---	Unknown

Antimicrobial properties

Agar well diffusion assay

The antimicrobial potential of the crude methanol extract of *B. rupestris* leaves as well as its ethyl acetate and *n*-butanol derived fractions was assessed against six pathogenic microorganisms using agar well diffusion assay **(Table 4, Figure 2)**. In particular, the three test samples exerted no antimicrobial activity against the pathogenic yeast *Mucor circinelloides* (AUMC 6696). They also showed moderate antibacterial activity against *B. subtilis*. The *n*-butanol fraction exhibited strong antibacterial activity against *S. aureus* with an inhibition zone of 21 mm in diameter while the crude extract and the ethyl acetate fraction showed weak antibacterial activity with an inhibition zone of 15 mm and 13 mm, respectively. Also, the methanol extract exhibited strong antibacterial activity against *E. coli* with an inhibition zone of 23 mm diameter while the *n*-butanol fraction exhibited moderate antibacterial activity against *E. coli* with an inhibition zone of 18 mm diameter. The anti- *E. coli* response of the methanol extract and the *n*-butanol derived fraction was greater than the response produced by the used positive control (gentamycin) at the same concentration and conditions where its inhibition zone was 17 mm diameter. Both the ethyl acetate and the *n*-butanol fractions exerted moderate antimicrobial activity against *P. aeruginosa* and *C. albicans*, respectively with an inhibition zone of 16 mm in diameter. Moreover, the methanol extract and the *n*-butanol fraction had weak anti-*P. aeruginosa* with inhibition zone of 15 mm and 12 mm diameter, respectively. The same response was also observed for the crude extract and the ethyl acetate fraction against *C. albicans* whose inhibition zone diameter was 14 mm and 12 mm, respectively.

Table 4. Antimicrobial activity via well diffusion assay of the methanol extract, the ethyl acetate and the *n*-butanol derived fractions of *B. rupestris* leaves

Results are expressed as zone of growth inhibition (mm), NA: no growth inhibition

Sample / Pathogenic microorganism	Crude MeOH extract	EtOAc derived fraction	*n*-BuOH derived fraction	Gentamycin
Zone of growth inhibition (mm)				
Gram-positive bacteria				
Bacillus subtilis (ATCC 6633)	16	17	19	25
Staphylococcus aureus (ATCC 6538)	15	13	21	19
Gram-negative bacteria				
Escherichia coli (ATCC 8739)	23	10	18	17
Pseudomonas aeruginosa (ATCC 90274)	15	16	12	22
Fungal strains				
Candida albicans (ATCC 10221)	14	12	16	21
Mucor circinelloides (AUMC 6696)	NA	NA	NA	16

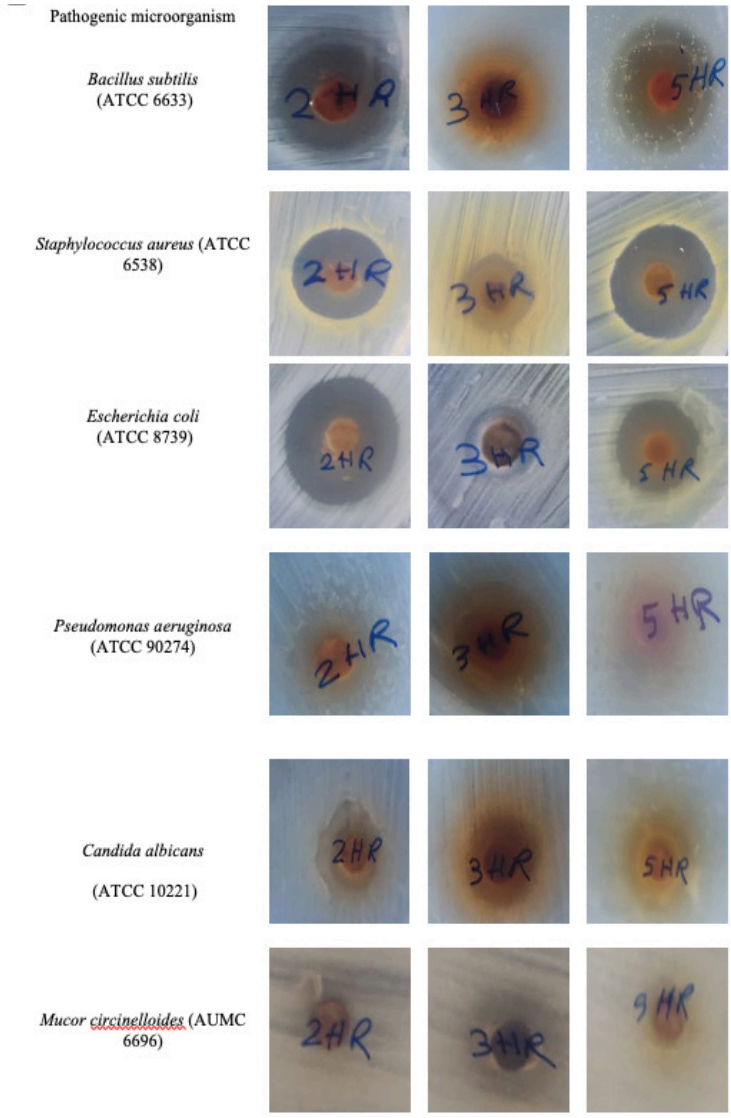

Figure 2. Antimicrobial activity via well diffusion assay of 2HR: 85% MeOH extract, 3HR: EtOAC derived fraction and 5HR: n-BuOH derived fraction of B. rupestris leaves (10 mg/mL) against tested microorganisms in MHA medium after incubation for 24 h at 37° for bacteria or 48 h at 37° for yeast.

Minimum inhibitory concentration (MIC)

The MIC values of the three samples under investigation against the five susceptible pathogens were demonstrated in **Table 5**. The test sample is considered a strong antimicrobial agent if its MIC value equals or lower than 500 μg/mL[1]. Accordingly, the methanol extract of *B. rupestris* leaves and its ethyl acetate fraction could be considered strong microbial inhibitors against the test samples where their MIC values ranged between 31.25 to 250 μg/mL and 100 to 500 μg/mL, respectively. Also, the *n*-butanol fraction exhibited strong antimicrobial activity against all the test microbes except for *P. aeruginosa* where its MIC values ranged between 62.5 and 1000 μg/mL. The most potent antimicrobial activities of the methanol extract, the ethyl acetate fraction and the *n*-butanol fraction were observed against E. coli, E. coli and B. subtilis, respectively where their MIC values were 31.25, 100 and 62.5 µg/mL, respectively.

Table 5. Minimal inhibitory concentrations of the methanol extract, the ethyl acetate and the n-butanol derived fractions of *B. rupestris* leaves against tested microorganisms

Samples / Pathogenic microorganisms	Crude MeOH extract	EtOAc derived fraction	*n*-BuOH derived fraction
Conc. (µg/mL)			
Bacillus subtilis (ATCC 6633)	125	125	62.5
Staphylococcus aureus (ATCC 6538)	125	500	250
Escherichia coli (ATCC 8739)	31.25	100	250
Pseudomonas aeruginosa (ATCC 90274)	250	125	1000
Candida albicans (ATCC 10221)	125	500	250

STATEMENTS OF ETHICS

Not applicable

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

EAE, MME and ESA conceived and supervised the study. HRM and MS performed experimental work. HRM wrote the first draft of the manuscript. All authors contributed to data analysis and interpretation, revising the article,

gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

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REFERENCES

1. Mogana R, Adhikari A, Tzar MN, Ramliza R, Wiart C. Antibacterial activities of the extracts, fractions and isolated compounds from *Canarium patentinervium* miq. Against bacterial clinical isolates. BMC Complement Med Ther, 2020;20(1):1-11. Doi: 10.1186/s12906-020-2837-5

2. Khalid M, Amayreh M, Sanduka S, Salah Z, Al-rimawi F, Al-mazaideh GM, et al. Assessment of antioxidant, antimicrobial, and anticancer activities of *Sisymbrium officinale* plant extract. Heliyon, 2022;8(9):e10477. Doi: Doi: 10.1016/j.heliyon.2022.e10477

3. Twilley D, Langhansová L, Palaniswamy D, Lall N. Evaluation of traditionally used medicinal plants for anticancer, antioxidant, anti-inflammatory and anti-viral (HPV-1) activity. S Afr J Bot, 2017; 112:494-500. Doi: 10.1016/j.sajb.2017.05.021

4. Shakya AK. Medicinal plants: future source of new drugs. Int J Herb Med, 2020;4(4):59-64. Doi: 10.13140/RG.2.1.1395.6085

5. El-Sherei MM, Ragheb AY, Kassem ME, Marzouk MM, Mosharrafa SA, Saleh NA. Phytochemistry, biological activities and economical uses of the genus *Sterculia* and the related genera: A review. Asian Pac J Trop Dis, 2016;6(6):492-501. Doi: 10.1016/S2222-1808(16)61075-7

6. Abou Zeid AH, Farag MA, Hamed MAA, Kandil ZAA, El-Akad RH, El-Rafie HM. Flavonoid chemical composition and antidiabetic potential of *Brachychiton acerifolius* leaves extract. Asian Pac J Trop Biomed, 2017;7(5):389-396. Doi: 10.1016/j.apjtb.2017.01.009

7. Thabet AA, Youssef FS, El-Shazly M, Singab AB. Anti-infective Properties of *Brachychiton rupestris* and *Brachychiton luridum* Leaves and their Qualitative Phytochemical Screening. Med Aromat Plants, 2017;6(4):1000299. Doi: 10.4172/2167-0412.1000299

8. Mohamed HR, Abdel-Hameed ES, El-Wakil EA, El-Hashash MM, Shemis M. Phytochemical Screening, *In-vitro* Antioxidant and Cytotoxic Potentials of *Brachychiton rupestris* Leaves. Res J Pharm Technol, 2021;14(6):3119-3127. Doi: 10.52711/0974-360X.2021.00544

9. Farag MA, Abou Zeid AH, Hamed MA, Kandeel Z, El-Rafie HM, El-Akad RH. Metabolomic fingerprint classification of *Brachychiton acerifolius* organs via UPLC-qTOF-PDA-MS analysis and chemometrics. Nat Prod Res, 2014;29(2):116-124. Doi: 10.1080/14786419.2014.964710

10. Thabet AA, Youssef FS, Korinek M, Chang F, Wu Y, Chen B, et al. Study of the anti-allergic and anti-inflammatory activity of *Brachychiton rupestris* and *Brachychiton discolor* leaves (Malvaceae) using *in vitro* models. *BMC Complement Altern Med*, 2018;18(1):299. Doi: 10.1186/s12906-018-2359-6

11. Thabet AA, Youssef FS, El-Shazly M, El-Beshbishy HA, Singab AB. Validation of the anti-hyperglycaemic and hepatoprotective activity of the flavonoid rich fraction of *Brachychiton rupestris* using *in vivo* experimental models and molecular modelling. *Food Chem Toxicol*, 2018;114:302-310. Doi: 10.1016/j.fct.2018.02.054

12. Ragheb AY, Kassem MES, El-Sherei MM, Marzouk MM, Mosharrafa SA, Saleh NAM. Morphological, phytochemical and anti-hyperglycemic evaluation of *Brachychiton populneus*. Rev Bras Farmacogn, 2019;29:559-569. Doi: 10.1016/j.bjp.2019.05.001

13. Rjeibi I, Ben Saad A, Ncib S, Souid S, Allagui MS, Hfaiedh N. *Brachychiton populneus* as a novel source of bioactive ingredients with therapeutic effects: antioxidant, enzyme inhibitory, anti-inflammatory properties and LC–ESI-MS profile. Inflammopharmacology, 2020;28(2):563-574. Doi: 10.1007/s10787-019-00672-8

14. Tailulu A, Li M, Ye B, Al-qudaimi R, Cao F, Liu W, et al. Antimicrobial and anticancer activities of Hainan dry noni fruit alcoholic extracts and their novel compounds identification using UPLC-Q-Exactive Obitrap-MS/MS. J Pharm Biomed Anal, 2022;220:114989. Doi:10.1016/j.jpba.2022.114989

15. Yang JY, Lee HS. Evaluation of antioxidant and antibacterial activities of morin isolated from mulberry fruits (*Morus alba* L.). J Korean Soc Appl Biol Chem, 2012;55(4):485-489. Doi: 10.1007/s13765-012-2110-9

16. Said RB, Hamed AI, Mahalel UA, Al-Ayed AS, Kowalczyk M, Moldoch J, et al. Tentative characterization of polyphenolic compounds in the male flowers of *Phoenix dactylifera* by liquid chromatography coupled with mass spectrometry and DFT. Int J Mol Sci, 2017;18:512. Doi: 10.3390/ijms18030512

17. Marzouk MM, Hussein SR, Elkhateeb A, El-Shabrawy M, Abdel-Hameed ES, Kawashty SA. Comparative study of *Mentha* species growing wild in Egypt: LC-ESI-MS analysis and chemosystematic significance. J Appl Pharm Sci, 2018;8(8):116-122. Doi:10.7324/JAPS.2018.8816

18. Djande CYH, Steenkamp PA, Piater LA, Madala NE, Dubery IA. Habituated *Moringa oleifera* callus retains metabolic responsiveness to external plant growth regulators. *Plant Cell Tissue Organ Cult*, 2019;137:249-264. Doi: 10.1007/s11240-019-01565-y

19. Felipe DF, Brambilla LZS, Porto C, Pilau EJ, Cortez DAG. Phytochemical analysis of *Pfaffia glomerata* inflorescences by LC-ESI-MS/MS. Molecules, 2014;19:15720-15734. Doi: 10.3390/molecules191015720

20. Vuković NL, Vukić MD, Đelić GT, Kacaniova MM, Cvijović M. The investigation of bioactive secondary metabolites of the methanol extract of *Eryngium amethystinum*. Kragujevac J Sci, 2018;40:113-129. Doi: 10.5937/KgJSci1840113V

21. Alberti Á, Riethmüller E, Béni S, Kéry Á. Evaluation of radical scavenging activity of *Sempervivum tectorum* and *Corylus avellana* extracts with different phenolic composition. Nat Prod Commun, 2016;11(4):469-474. Doi:10.1177/1934578x1601100412

22. Guo Y, Yang H, Wang Y, Chen X, Zhang K, Wang Y, et al. Determination of Flavonoids Compounds of Three Species and Different Harvesting Periods in *Crataegi folium* Based on LC-MS/MS. Molecules, 2021;26:1602. Doi:10.3390/molecules26061602

23. Guimarães R, Barros L, Dueñas M, Carvalho AM, Queiroz MJRP, Santos-Buelga C, et al. Characterisation of phenolic compounds in wild fruits from Northeastern Portugal. Food Chem, 2013;141(4):3721-3730. Doi: 10.1016/j.foodchem.2013.06.071

24. Kumar S, Singh A, Kumar B. Identification and characterization of phenolics and terpenoids from ethanolic extracts of *Phyllanthus* species by HPLC-ESI-QTOF-MS/MS. J Pharm Anal, 2017;7:214-222. Doi: 10.1016/j.jpha.2017.01.005

25. Ağalar HG, Çiftçi GA, Göger F, Kırımer N. Activity Guided Fractionation of *Arum italicum* Miller Tubers and the LC/MS-MS Profiles. Rec Nat Prod, 2018;12(1):64-75. Doi: 10.25135/rnp.06.17.05.089

26. Yang N, Yang Y, Li K. Analysis of Hydroxy Fatty Acids from the Pollen of *Brassica campestris* L. var. *oleifera* DC. By UPLC-MS/MS. J Pharm, 2013;2013:874875. Doi: 10.1155/2013/874875

27. Benayad Z, Gómez-Cordovés C, Es-Safi NE. Characterization of flavonoid glycosides from fenugreek (*Trigonella foenum-graecum*) crude seeds by HPLC-DAD-ESI/MS analysis. Int J Mol Sci, 2014;15:20668-20685. Doi: 10.3390/ijms151120668

28. Li C, Seeram NP. Ultra-fast liquid chromatography coupled with electrospray ionization time-of-flight mass spectrometry for the rapid phenolic profiling of red maple (*Acer rubrum*) leaves. J Sep Sci, 2018;41(11):2331-2346. Doi: 10.1002/jssc.201800037

29. Ahmed RF, Elkhrisy EA, EL-Kashak WA, El Raey MA, Nassar MI, Aboutabl EA. Structural Characterization of Polyphenolics in *Livistona chinensis* Using HPLC-PDA-MS. J Adv Pharm Res, 2019;3(1):23-29. Doi: 10.21608/aprh.2018.6527.1072

30. Beelders T, De Beer D, Stander MA, Joubert, E. Comprehensive phenolic profiling of *Cyclopia genistoides* (L.) Vent. by LC-DAD-MS and -MS/MS reveals novel xanthone and benzophenone constituents. Molecules, 2014;19:11760–11790. Doi: 10.3390/molecules190811760

31. Chernonosov AA, Karpova EA, Lyakh EM. Identification of phenolic compounds in *Myricaria bracteata* leaves by high-performance liquid chromatography with a diode array detector and liquid chromatography with tandem mass spectrometry. Rev Bras Farmacogn, 2017;27:576-579. Doi: 10.1016/j.bjp.2017.07.001

32. Yun E, Park S, Kim B, Chae Y, Cho S, Yi H, et al. Determination of the esculetin contents of medicinal plants by liquid chromatography-tandem mass spectrometry. Biomed Chromatogr, 2012;26:1247-1251. Doi: 10.1002/bmc.2686

33. Salih EYA, Fyhrquist P, Abdalla AMA, Abdelgadir AY, Kanninen M, Sipi M, et al. LC-MS / MS Tandem Mass Spectrometry for Analysis of Phenolic Compounds and Pentacyclic Triterpenes in Antifungal Extracts of *Terminalia brownii* (Fresen). Antibiotics, 2017;6(37):1-22. Doi: 10.3390/antibiotics6040037

34. Rezende FM, Ferreira MJP, Clausen MH, Rossi M, Furlan CM. Acylated flavonoid glycosides are the main pigments that determine the flower colour of the brazilian native tree *Tibouchina pulchra* (Cham.) Cogn. Molecules, 2019;24(4):718-734. Doi: 10.3390/molecules24040718

35. Chen G, Li X, Saleri F, Guo M. Analysis of flavonoids in *Rhamnus davurica* and its antiproliferative activities. Molecules, 2016;21:1275. Doi: 10.3390/molecules21101275

36. Simirgiotis MJ, Benites J, Areche C, Sepúlveda B. Antioxidant capacities and analysis of phenolic compounds in three endemic *Nolan*a species by HPLC-PDA-ESI-MS. Molecules, 2015;20:11490-11507. Doi: 10.3390/molecules200611490

37. Sobral F, Calhelha RC, Barros L, Dueñas M, Tomás A, Santos-Buelga C, et al. Flavonoid composition and antitumor activity of bee bread collected in Northeast Portugal. Molecules, 2017;22:248. Doi: 10.3390/molecules22020248

38. Ibrahim LF, Elkhateeb A, Marzouk MM, Hussein SR, Abdel-Hameed ES, Kassem MES. Flavonoid investigation, LC–ESI-MS profile and cytotoxic activity of *Raphanus raphanistrum* L. (Brassicaceae). J Chem Pharm Res, 2016;8(7):786-793.

39. Gu D, Yang Y, Abdulla R, Aisa HA. Characterization and identification of chemical compositions in the extract of *Artemisia rupestris* L. by liquid chromatography coupled to quadrupole time-of- flight tandem mass spectrometry. Rapid Commun Mass Spectrom, 2012;26:83-100. Doi: 10.1002/rcm.5289

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Banana peels a contemptible source of dietary fiber and natural antioxidants

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ABSTRACT

The Musaceae family of herbaceous plants, which includes the genus *Musa*, includes the edible fruit known as the banana. It is one of the most important fruit crops in the world and extensively grown in tropical nations for its great food uses. Banana peel, a waste that makes up 40% of the banana's weight and it is abundant in nutrients, bioactive compounds and antioxidants. Physical characteristics of banana peels were assessed, including yield, pH, bulk density, nutritional and antioxidant potential in this study. Results showed that the yield of banana peel powder 12.20%, the pH 5.60 and the bulk density 62.86 g/100 ml. The nutritional analysis results showed the percentage concentrations of moisture, ash, crude fat, crude fiber, crude protein, carbohydrate and energy were 7.50±0.45, 6.70±0.35, 1.81±0.03, 29.52±1.30, 3.22±0.07, 51.25±2.50 and 234±5.60, respectively. The findings of antioxidant research showed that, all the extracts of banana peel powder have a strong ability to scavenge the free radicals, however the methanolic extract had a greater free radicals scavenging activity ranged from (33.98±2.08-82.03±4.30%) than water extract (21.30±1.40-69.01±3.60%) and chloroform extracts (6.02±0.50-38.02±2.15%). According to these findings, banana peel is a low-cost source of dietary fiber, carbohydrates, and natural antioxidants that may be successfully employed in the food, pharmaceutical and other industries. As a result, banana

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wastes could pave the way for future study in uncharted territory.

Keywords: banana peel, nutrients, fiber, antioxidants, DPPH

INTRODUCTION

The banana (*Musa sp.*, Musaceae family) is an important fruit crop used for food in tropical and subtropical parts of Asia, America, Africa and Australia[1]. In 2019, the global banana production was 116.8 million tons (Figure 1a % production) and are predominantly produced in Asia, America, Africa, Oceania and Europe (Figure 1b; yearly production) and it is one of the most important fruits, ranking second to grapes, tomatoes and apples in terms of economic value[2]. With carbohydrates making up 22-32% of the weight of the fruit, it serves as a vital food supply for millions of people in developing countries and is an excellent source of energy. It is rich in minerals including potassium, magnesium, phosphorus, and folate as well as vitamins A, B6 and C. The fruit also contains a lot of sugar and other acids in addition to antioxidants and vitamin A. Banana is a high fibre food that lowers blood cholesterol levels[3].

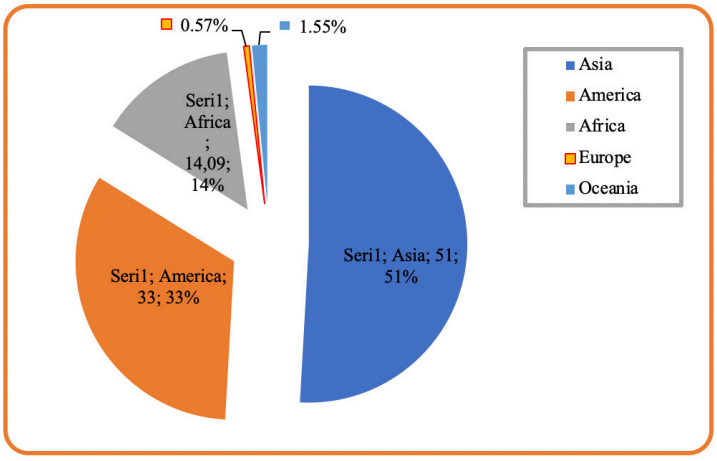

Figure 1a. % Production of banana predominantly

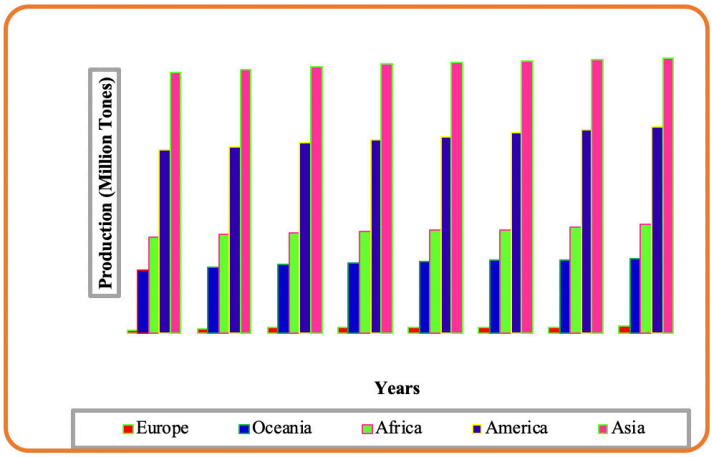

Figure 1b. Year wise production of banana predominantly

Fresh bananas make up 35–40% peels and if this enormous amount of waste is not properly disposed off, it might be hazardous to the environment[4]. However, as vegetables and fruits are rich in protein and important amino acids like leucine, valine, phenylalanine, and threonine (Figure 2), fibre and minerals, this by-product may be put to better use in order to reduce waste and provide a new source of food. The peel of a banana contains a lot of dietary fibre, which is proven to reduce the risk of conditions including diverticulosis, diabetes, colon cancer, irritable bowel syndrome and constipation[5]. Given the importance of bananas as a crop across the world create tones of leftover after each harvest season such as leaves, pseudo stems, stalks, and inflorescences[6]. Research on banana trash looked at the acceptability of each waste component, including the seeds and peels[7]. Banana peels have a strong antioxidant capacity and several ailments have been treated including burns, diarrhea, ulcers, and inflammation[8].

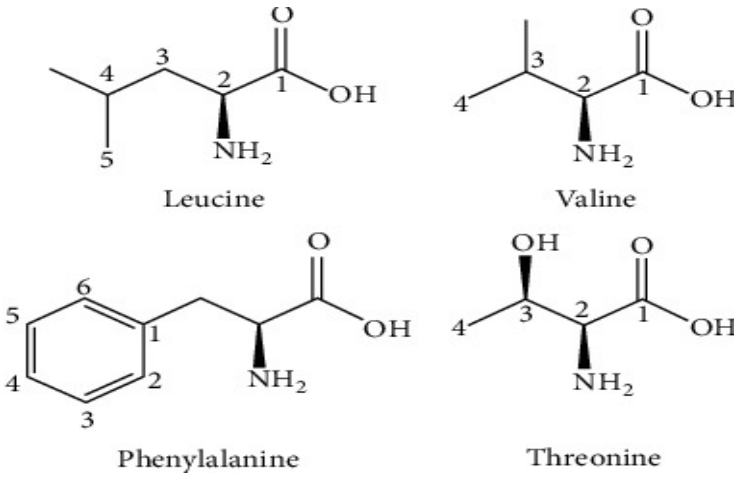

Figure 2. Essential amino acids in banana peel

Numerous epidemiological studies demonstrate the benefits of eating antioxidants that lower the incidence of illnesses related with oxidative stress, such as diabetes, cancer and heart disease[9]. By scavenging free radicals and reducing oxidative stress, dietary antioxidants may aid in the preventing and treating a variety of illnesses. The use of synthetic antioxidants is restricted due to potential health hazards so a latent substitute for synthetic antioxidants is dietary antioxidants[10]. Because of the cheap and enormous amounts of plant bio waste generated, their application may be expanded to the food sector, wherein they can be employed to produce new, useful meals like antioxidants.

When compared to other fruits, banana peels have higher quantities of phenols, an important secondary metabolite. The phenolic compounds gallic acid, catechin, epicatechin, tannins and anthocyanins are among those found in banana peel[11]. Figure 3 depicts the putative mechanism of these phenolic compounds' antioxidant activity through inhibiting reactive oxygen species production. ROS is essential for maintaining homeostasis and plays numerous functions including cellular signal transduction. But high degree of ROS concentration might produce aberrant cell signalling, which leads to cell damage. Numerous studies have connected ROS to a variety of chronic disorders, including neurodegeneration, cancer, diabetes and inflammation[12]. This study's objective was to assess the banana peel potential as a nutrients and natural antioxidants.

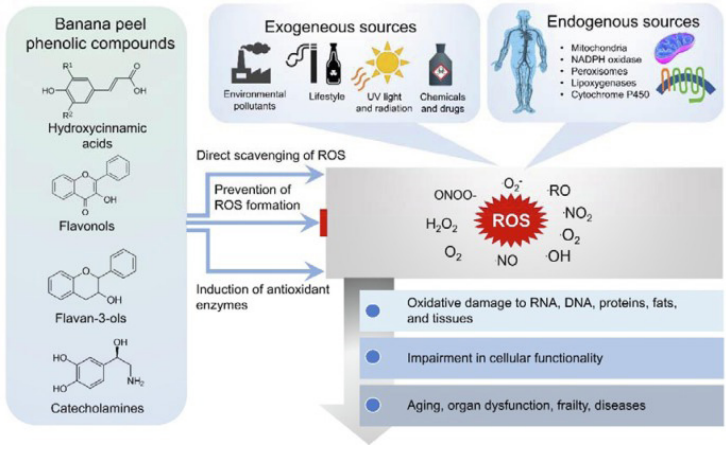

Figure 3. Putative mechanism of the antioxidant activity of phenolic compounds of banana peel[13]

METHODOLOGY

Chemicals and reagents

Analytical grade of chemicals Methanol, Chloroform, citric acid, DPPH and Folin's phenol reagent from Sigma, Aldrich & Fluka Chemical Co. (St. Ouis, Mo, USA). Distillation equipment was used to prepare the distilled water.

Materials

The bananas were acquired from a nearby market. The fruit was properly cleaned with tap water and then with distilled water before being separated into pulp and skins. The peels were chopped into small pieces and to decrease enzymatic browning soaked in a 0.5% (w/w) citric acid solution for 10 minutes. The solution was drained and dried to constant weight in a hot air oven at 45 °C. Using a milling blender, the dry peel was grinded into a homogeneous powder that passed through a 40 mesh screen sieve. The banana peel powder (Figure 4) was immediately packaged in polyethylene bags and refrigerated at 4±2 °C for analysis.

Figure 4. Banana, its peel and powder

Yield

The succumb of banana peel powder was calculated by dividing the amount of powder produced by the amount of fresh banana peel utilized and converting the results to gram of powder per 100g[14].

pH value

A suspension of banana peel powder (8% w/v) was made, agitated for 5 minutes and then let to stand for 30 minutes before filtering. Using a pH meter, the filtrate's pH value was determined by meter (InoLab pH Level-1, Germany)[15].

Bulk Density (BD)

A 100 mL graduated cylinder was filled with a 20 g sample. After tapping the cylinder ten times, the BD was determined by reading the final volume[16].

BD = Mass of Materials / Volume of Material after tapping

Nutritional content determination

The proximate composition (moisture, ash, fat, crude fiber, crude protein and carbohydrate) of the banana peel powder was assessed using established procedures AOAC, 2016[17]. The moisture content of the peel powder was evaluated by drying it in an oven at 105°C to a consistent weight. For six hours, the fat was extracted with hexane (40-60°C) using a soxhlet equipment. For protein determination, the Micro-Kjeldahl technique was used. Carbohydrate content was calculated by using following formula[18].

Carbohydrates % = 100- (moisture % + protein % + ash % + fat % + crude fiber %).

Gross energy determination

Using information from the proximate analysis, the samples' gross energy was estimated by dividing the percentages of crude protein, crude carbohydrate, and crude fat by 4.0 and 9.0, respectively which was expressed as Kcal/100g[19].

Extract preparation for antioxidant study

5gm of banana peel powder was added in 100ml of methanol & chloroform which was extracted in soxhlet apparatus. In the case of aqueous extraction, 5gm of sample with 100ml of distilled water was heated for 2 hours. The supernatant was collected after it had been filtered with filter paper for antioxidant study[20].

Antioxidant activity by DPPH assay

Using methanolic, aqueous and chloroform extracts of banana peel powder; the DPPH free radical scavenging activity was assessed by Brand-Williams

(1995) method[21] with slight modification[22]. Based on the stable 2, 2-diphenyl-1-picrylhydrazyl's capacity to scavenge free radicals, the antioxidant activity of banana peel extracts was evaluated. A solution of 0.004% DPPH in methanol was made, and 3 ml of this mixture was added to samples containing 1 to 5 mg/ml. After 30 minutes in the dark, these solution combinations' optical densities were measured at 517 nm using spectrophotometer (UV-Vis-1700, Shimadzu, Japan). As a blank, 3 ml of DPPH solution in 100 µl of methanol were used to measure antioxidant activity as fallows.

$$\text{Antioxidant activity \%} = 1 - [A_{sample}/A_{control}] \times 100$$

Statistical evaluation

The outcomes were shown as mean standard deviation (SD). The use of one-way analysis of variance to statistically evaluated the data. The Tukey test was carried out to see whether there were any changes between sample means that were significant at $p=0.05$[23].

RESULTS and DISCUSSION

Physical parameters

The results of yield revealed that the yield of banana peel powder was 12.50%. These findings were consistent with the prior report by Azza et al.[24], which stated that the yield of banana peel powder was 12.85%. The pH of banana peel powder was determined which 5.60. These findings are slightly higher than by Rodriguez-Ambriz et al.[25], who observed that the banana peel flour's average pH ranged from 4.80 to 5.47. The pH variations might be attributable to terminal residues within the starch molecules[26]. The bulk density was 62.86 g/100 ml which indicating that the particles were more compact. These values are comparable to those found by Ferreira et al.[27] who depicted the BD values of banana peel (62–66 g/100 ml). The BD represents the porosity of a food product that affects packaging design and wet ability, greater BD is a desired quality for amplified easiness with which flour can be dispensed[28]. Furthermore, the maximum BD of composite flour shows that this flour may be utilized as a thickening in the food processing sector, as well as in food preparation due to its potential to assist lower the thickness of paste which is a principal feature in restorative and child feeding[29].

Nutritional contents

Nutritional contents provide essential nutritional composition information and aids in determining sample quality. It provides information on moisture, ash, fat, fiber, protein, carbohydrate, energy[30] and the values of these contents

are shown in table 1. The moisture content of the banana peel powder was 7.50±0.45%[31] observed 11.56% moisture content in banana peel waste, while[32] reported 6-10% moisture content. It has been observed that low moisture content reduces the danger of mould growth and allows samples to be preserved for a longer period of time. The ash percentage was recorded 6.70±0.35%, that is comparable to other staples[33]. After heating eliminates water and organic compounds ash is the inorganic byproduct that is left. Adeyemi et al[34]. It is crucial to remember that the quantity of mineral elements in food is determined by the ash composition. The fat content of banana peel powder was 1.81 ± 0.03%, which was comparable to Morais et al.[35] but lower than Munguti et al.[36]. This might be due to variances in varietals or geographical factors.

According to our findings, the crud fiber content was 29.52±1.30 which is quite high and indicating that the banana peels powder is a rich source of fiber. The peels' high fiber content suggests that they may help alleviate constipation and promote overall health of human being[37]. Additionally, bile salts (which are made of cholesterol) were removed from the gut by dietary fiber, which helped lower blood levels of LDL cholesterol[38]. The content of carbohydrates was found to be 51.25±2.50%, which are one of the most significant elements in meals and raw materials. Carbohydrates are naturally added to food items to give nutrients which also improving the texture and overall quality of food products[39]. Banana peel has a significant amount of carbon and may be utilized as an absorbent to remove different contaminants from contaminated water[40,41]. The values of gross energy calculated in this study which indicating that banana peel powder has a excellent energy similar to those of other fruit leftovers, including citrus peels[42,43].

Table 1. Nutritional facts of banana peel powder

Sr. No.	Parameters	Values (g/100g)
1	Moisture	7.50 ± 0.45
2	Ash	6.70 ± 0.35
3	Crude fat	1.81 ± 0.03
4	Crude fiber	29.52 ± 1.30
5	Crude protein	3.22 ± 0.07
6	Carbohydrate	51.25 ± 2.50
7	Energy (Kcal/100g)	234 ± 5.60

Data are represented ± standard deviation

Antioxidant activity by DPPH assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) is widely employed to assess the capacity of dietary ingredients to scavenge free radicals[44,45]. The antioxidants activities of various extracts of banana peels powder showed that the free radical scavenging activity of methanol extract was superior to aqueous extract, followed by chloroform extracts. The % inhibition DPPH of banana peels in methanol extract was ranged from 34-82 (Figure 5) and the % inhibition DPPH of banana peels in aqueous extract was ranged from 21-69 (Figure 6) while the % inhibition DPPH of banana peels in methanol extract was ranged from 6-38 (Figure 7) at concentration 1-5 mg/ml. Our results are in accordance to given literature[46-48].

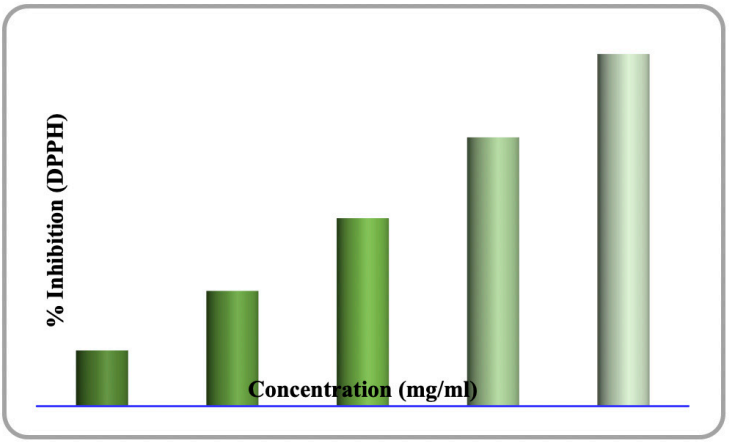

Figure 5. % Inhibition (DPPH) of chloroform extracts of banana peel powder

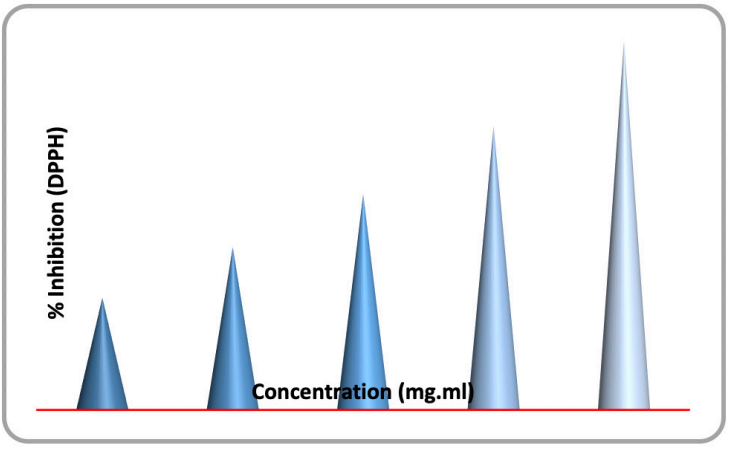

Figure 6. % Inhibition (DPPH) of water extract of banana peel powder

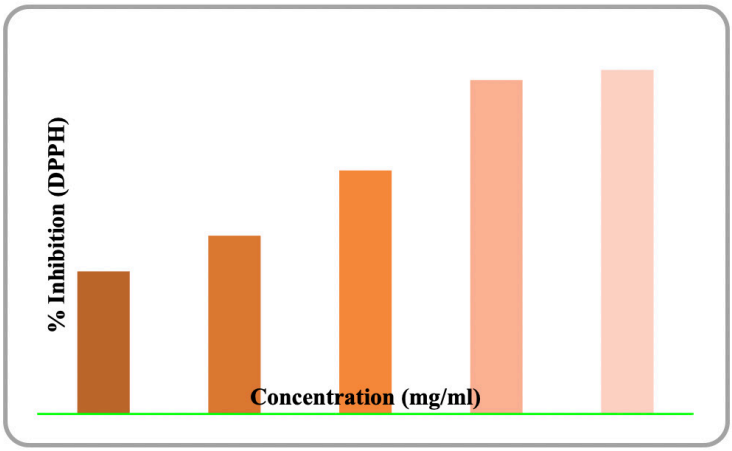

Figure 7. % Inhibition (DPPH) of methanol extract of banana peel powder

Although oxidative stress is a severe issue, utilizing plant material might reduce ROS damage through a variety of mechanisms[49]. We employed banana peel powder as a natural antioxidant for efficiently use of banana processing waste and this free radical scavenging capability of banana peel powder was due to presence of its polyphenolic contents. Banana peel is thought to have a total phenolic content that is three times greater than the fruit[50]. The phenolic constituents of banana peels may be separated into four classes, flavonols, hydroxycinnamic acids, flavan-3-ols, and catecholamines[51,52]. According to prior studies fruit peels are rich in dietary fiber and phytonutrients including triterpenes, sterols, active amines, polyamines, carotenoids, fatty acids and thus could be used in a variety of nutraceutical products[53,54].

CONCLUSION

From this study we conclude that the banana peel powder is a low-cost viable source of dietary fibre, which promote proper digestion of meals. Moreover our research has shown that banana peel has significant natural antioxidants which might aid the pharmaceutical industry and provide consumers a better knowledge of the manufacturing of value-added products.

STATEMENT OF ETHICS

All the necessary ethical rules were followed while performing research.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Not Applicable

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REFERENCES

1. Hikal WM, Kacaniova M, Husain Said-Al Ahl AH. Banana peels as possible antioxidant and antimicrobial agents. Asian J Res Rev Agri, 2021;3(3):35-45.

2. FAO. World Food and Agriculture - Statistical Yearbook. Rome: FAOSTAT; 2021. Available from: https://doi.org/10.4060/cb4477en

3. Oyeyinka BO, Afolayan AJ. Potentials of *Musa* species fruits against oxidative stress-induced and diet-linked chronic diseases: in vitro and in vivo implications of micronutritional factors and dietary secondary metabolite compounds. Molecule, 2020;25(1):10. Doi: 10.3390/molecules25215036

4. Azarudeen AM, Nithya R. Pharmaceutical Aspects of Banana peel: A Review. J Pharm Sci Res, 2021;13(2):112-117.

5. Zhang W, Li X, Jiang W. Development of antioxidant chitosan film with banana peels extract and its application as coating in maintaining the storage quality of apple. Int J Biol Macromolecul, 2020;154:1205-1214. Doi: 10.1016/j.ijbiomac.2019.10.275

6. Gomes S, Vieira B, Barbosa C, Pinheiro R. Evaluation of mature banana peel flour on physical, chemical and texture properties of gluten free. Rissol. J Food Proc Preserv, 2020;46(2). Doi: 10.1111/jfpp.14441

7. Zuorro A, Iannone A, Natali S, Lavecchia R. Green synthesis of silver nanoparticles using bilberry and red currant waste extracts. Processes, 2019;7:193. Doi: 10.3390/pr7040193

8. Khoozani AA, Birch J, Bekhit AE. Production, application and health effects of banana pulp and peel flour in the food industry. J Food Sci Technol, 2019;56:548-559. Doi: 10.1007/s13197-018-03562-z

9. Adin SN, Gupta I, Aqil M, Mohd Mujeeb M, Ahad A. BBD driven optimization of extraction of therapeutically active xanthanoid mangiferin from *Mangifera indica* L. leaves and its antioxidant activity. Pharmacog Res, 2023;15(1):84-93. Doi: 10.5530/097484900279

10. Saeed MK, Zahra N, Abidi SHI Q. Syed Phytochemical Screening and DPPH Free Radical Scavenging Activity of *Aloe vera* (*Aloe barbadensis* Miller) Powder. Int J Food Sci Agri, 2022;6(3):301-308. Doi: 10.26855/ijfsa.2022.09.010

11. Sidhu JS, Zafar TA. Bioactive compounds in banana fruits and their health benefits. *Food Qual Safety*, 2018;2(4):183-188. Doi: 10.1093/fqsafe/fyy019

12. Swiętek M, Lu YC, Konefał R, Ferreira LP, Cruz MM, Ma YH, Horak D. Scavenging of reactive oxygen species by phenolic compound-modified maghemite nanoparticles. Beil J of Nanotechnol, 2019;10(1):1073-1088. Doi: 10.3762/bjnano.10.108

13. Zaini HM, Roslan J, Saallah S, Munsu E, Sulaiman NS, Pindi W. Banana peels as a bioactive ingredient and its potential application in the food industry. J Functional Foods, 2022;92:1-12. Doi: 10.1016/j.jff.2022.105054

14. Romelle F, Ashwinim P, Manohar R. Chemical composition of some selected fruit peels. European J Food Sci Technol, 2016;4(4):12-21.

15. Pearson D. The Chemical Analysis of Foods. 7th edition. London: Church-Hill Livingstone. 1976.

16. FAO. "Natural additives from industrial wastes". Research Continues, Ain Shams University, 1982; 31(1):567-577.

17. AOAC. Official Methods of Analysis of the Association of Official Analytical Chemists. 20th ed. Published by the Association of Official Analytical Chemists, USA, 2016.

18. Speight J. Handbook of coal analysis, chemical analysis: A Series of Monographs on Analytical Chemistry and Its Applications. 2nd edition. John Wiley & Sons, New York USA, 2015.

19. Akinyeye RO, Oluwadunsin A, Omoyeni A. Proximate, mineral, anti-nutrients, phytochemical screening and amino acid compositions of the leaves of pterocarpus mildbraedi harms. Elec J Env Agric Food Chem, 2011;10:1848-1857.

20. Veluman S. Phytochemical screening and antioxidant activity of banana peel. IJARIIE, 2016;2(1):91-102.

21. Brand-Williams W, Cuvelier ME, and Berset CLWT. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol, 1995;28(1):25-30. Doi: 10.1016/S0023-6438(95)80008-5

22. Saeed MK, Ahmad I, Hina S, Zahra N, Kalim I. Physico-chemical analysis, total polyphenolic content and antioxidant capacity of yellow dye extracted from *Curcuma longa*. Biological Sciences-PJSIR, 2021;64(1):25-29. Doi: 10.52763/PJSIR.BIOL.SCI.64.1.2021.25.29

23. Sharoba AM, Farrag MA, Abd El-Salam AM. Utilization of some fruits and vegetables waste as a source of dietary fiber and its effect on the cake making and its quality attributes. J Agro Proces Technol, 2013;19(4):429-444. Doi: 10.21608/jfds.2013.72084

24. Azza A, Abou-Arab Ferial M, Abu-Salem B. Nutritional and anti-nutritional composition of banana peels as influenced by microwave drying methods. Int Scholar Sci Res Innov, 2017;11(12):845-852.

25. Rodriguez-Ambriz SL, Islas-Hernández JJ, Agama-Acevedo E, Tovar J, Bello- Pérez LA. Characterization of a fiber-rich powder prepared by liquefaction of unripe banana flour. Food Chem, 2008; 107:1515-1521. Doi: 10.1016/j.foodchem.2007.10.007

26. Eshak SN. "Sensory evaluation and nutritional value of balady flat bread supplemented with banana peels as a natural source of dietary fiber". Annals of Agric Sci, 2016;61:299-235. Doi: 10.1016/j.aoas.2016.07.002

27. Ferreira SL, Mariana S, Monica CP, Moro Thaısa MAJ, Basto Gabriela, Andrade MS, Goncalves R, Edira CBA. Formulation and characterization of functional foods based on fruit and vegetable residue flour. *J Food Sci Tech,* 2013;13197:1061-1064. Doi: 10.1007/s13197-013-1061-4

28. Abu-Bakar SSK, Ahmad N, Jailani F. Physicochemical properties and sensory evaluation of banana peel flour biscuits. Int J Engg Technol, 2018;7:253-256. Doi: 10.14419/ijet.v7i4.14.27576

29. Chandra S, Singh S, Kumari D. Evaluation of functional properties of composite Flours and sensorial attributes of composite flour biscuits, J Food Sci Technol, 2015;52(6):3681-3688. Doi: 10.1007/s13197-014-1427-2

30. Abubakar US, Yusuf KM, Safiyanu I, Abdullahi S, Saidum SR, Abdu GT and Indee AM, Proximate and mineral composition of corn cob, banana and plantain peels. Int J Food Sci Nut, 2016;1(6):25-27.

31. Pathak PD, Mandavgane SA, Kulkarni BD, Fruit peel waste: characterization and its potential uses. Curr Sci, 2017;113(3):444.

32. Kabenge I, Omulo G, Banadda N, Seay J, Zziwa A, Kiggundu N. Characterization of banana peels wastes as potential slow pyrolysis feedstock. J Sustainable Dev, 2018;2:14. Doi: 10.5539/jsd.v11n2p14

33. Anhwange BA, Ugye TJ, Nyiaatagher TD. Chemical composition of *Musa sapientum* (Banana) peels. EJEAFC, 2009;8(6):437-442.

34. Adeyemi OS, Oladiji AT. Compositional changes in banana (*Musa sp.)* fruits during ripening.African J Biotechnol, 2009;8(5):858-859.

35. Morais R, Rotta E, Sargi S, Bonafe E, Suzuki R, Souza N, Matsushita M, Visentainer J, Proximate composition, mineral contents and fatty acid composition of the different parts and dried peels of tropical fruits cultivated in Brazil. J Braz Chem Soc, 2017;28(2):308-318. Doi: 10.5935/0103-5053.20160178

36. Munguti JM, Liti DM, Waidbacher H, Straif M, Zollitsch W. Proximate composition of selected potential feedstuffs for *Nile tilapia* (*Oreochromis niloticus* L.) production in Kenya. Die Bodenkultur, 2006;57(3):131-141.

37. Anna C, Ewa Z, Dmytro Y, Zvenyslava Z, Izabela G, Sylwia K. Mineral content of the pulp and peel of various citrus fruit cultivars. Biol Trace Element Res, 2020;1193:555-563. Doi: 10.1007/s12011-019-01727-1

38. Olayinka BU, Etejere EO. Proximate and chemical compositions of watermelon (*Citrullus lanatus*) (thunb.) matsum and nakai cv red and Cucumber (*Cucumis sativus*). Int Food Res J, 2018;25(3):1060-1066.

39. Ashish B, Manish K, Panditand PR. Amelioration of in-vivo antioxidant activity by banana extracts. Int J Pharm Biol Arch, 2012;3(1):157-161.

40. Salim MR, Chowdhury KAJ, Rayathulhan R, Yunus K, Sarkar MZI. Biosorption of Pb and Cu from aqueous solution using banana peel powder. Desalin and Water Treat, 2016;57(1):303-314. Doi: 10.1080/19443994.2015.1091613

41. Kibria AA, Kamrunnessa MD, Rahman M, Kar A. extraction and evaluation of phytochemicals from banana peels (*Musa sapientum*) and banana plants (*Musa paradisiaca*). Malaysian J Halal Res (MJHR), 2019;2(1):22-26. Doi: 10.2478/mjhr-2019-0005

42. Sulekha G, Jaya G. Orange Peel: A potential source of phytochemical compounds. International J Chemtech Res, 2018;11(02):240-243.

43. Hassan Pyar, Peh K.K. Chemical Compositions of banana peels (*Musa sapientum*) Fruits cultivated in Malaysia using proximate analysis. Res J Chem Environ, 2018;22:108-113.

44. Saeed MK, Zahra N, Neelam S, Kiran S, Syed HIA, Quratulain S. Extraction of poppy seed (*Papaver somniferum* L.) oil and its antioxidant activity by DPPH Assay. LGU J Life Sci, 2022;6(2):95-106. Doi: 10.54692/lgujls.2022.0602211

45. Tawfeeq TA, Amani Tawfeeq AA, Eldalawy R, Ibraheem SK. Phytochemical analysis, GCMS identification, and estimation of antioxidant activity of Iraqi Vitex negundo L. J Med Chem Sci, 2023;6(4):876-883. Doi: 10.26655/JMCHEMSCI.2023.4.19

46. Okolie JA, Henry OE, Epelle EI. Determination of the antioxidant potentials of two different varieties of banana peels in two different solvents. Food Nut Sci, 2016;7:1253-1261. Doi: 10.4236/fns.2016.713115

47. Fidrianny I, Anggraeni NAS, Insanu M. Antioxidant properties of peels extracts from three varieties of banana (*Musa sp.)* grown in West Java-Indonesia. Int Food Res J, 2018;25(1):57-64.

48. Gbenga-Fabusiwa FJ, Yemisi A, Jeff-Agboola YA, Ololade ZS, Akinrinmade R, Agbaje DO. Waste-to-wealth; nutritional potential of five selected fruit peels and their health benefits: A review. Afr J Food Sci, 2022;16(7):172-183. Doi: 10.5897/AJFS2021.2138

49. Masood S, Rehman A, Ihsan MA, Shahzad K, Sabir M, Alam S, Ahmed W, Shah ZH, Alghabari F, Mehmood A, Chung G. Antioxidant potential and α-glucosidase inhibitory activity of onion (*Allium cepa* L.) peel and bulb extracts. Brazilian J Biol, 2023;83:1-9. Doi: 10.1590/1519-6984.247168

Pereira A, Maraschin M. Banana (*Musa spp.)* from peel to pulp: ethnopharmacology, source of bioactive compounds and its relevance for human health. J Ethnopharmacol, 2015;160:149-163. Doi: 10.1016/j.jep.2014.11.008

50. Vu HT, Scarlett CJ, Vuong, QV. Phenolic compounds within banana peel and their potential uses: A review. J Functl Foods, 2018;40:238-248. Doi: 10.1016/j.jff.2017.11.006

51. Shang C, Gu Y, Koyama T. Major triterpenes, cycloeucalenone and 31-norcyclolaudenone as inhibitors against both α-glucosidase and α-amylase in banana peel. Int J of Food Sci Technol, 2021;56(7):3519-3526. Doi: 10.1111/ijfs.14978

52. Swati G, Md. Akhlaqur R, Shanthy S. Citrus fruit as a potential source of phytochemical, antioxidant and pharmacological ingredients. J Sci Healthcare Explor, 2021;3(1). Doi: 10.2019/JSHE.2581.8473/202101001

53. Hashim M, Hamid Z, Gul Z, Akbar A. Functional, nutritional and medicinal potential of banana peel. Pure Appl Biol, 2023;12(1):470-490. Doi: 10.19045/bspab.2023.120049

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Assessment of patients' adherence to antihypertensive therapy in a teaching hospital in Ogun state, Nigeria

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ABSTRACT

Adherence is important in reducing morbidity and mortality associated with uncontrolled hypertension. The aim of the study was to assess adherence of hypertensive patients to therapy. A cross sectional study was carried out on 325 patients on antihypertensive therapy. Data collection tools were modified Hill Bone medication adherence subscale and Health Belief Model scale. Descriptive statistics, Pearson correlation and Multiple Regression Analysis were employed for data analysis at 5% level of significance. Most respondents were female, (56.3%), ≤ 60 years (63.7%), and married (62.2%). Patients differed significantly in their adherence based on age (p=0.032) and gender (p=0.025), severity (p=0.021) susceptibility (p=0.001), benefits (0.031), barriers (p=0.015) and cue to action (p=0.010). Adherence rate of 59.1% was reported among the respondents in the study. The study outcomes highlight the need for interventions by pharmacists that promote adherence to antihypertensive medications.

Keywords: hypertension, adherence, antihypertensive medication, modified Hill-Bone adherence subscale, health belief model

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INTRODUCTION

Hypertension is a chronic, non-communicable disease that affects individuals of various age groups, sex and socio-economic status[1]. Despite the advances made in prevention, detection, management and control of hypertension over the years, the disease remains a global public health concern[2]. It is a major risk factor for stroke, ischaemic heart disease, other cardiovascular diseases and chronic kidney disease. It is a major cause of mortality, causing more than 10 million deaths globally every year[3,4]. In 2021 an estimated 1.28 billion adults aged 30-79 years had hypertension globally with about two-thirds living in low- and middle-income countries (LMICs)[5]. The burden of hypertension has been on the rise over the past few decades in sub-Saharan Africa. In West Africa, hypertension prevalence ranges from 12%-68%, while prevalence of hypertension is between 12%-36.8% in Nigeria[6-9]. Medication adherence, which is 'the degree to which a person's behavior corresponds with the agreed recommendations from a healthcare provider is important, especially in management of chronic illnesses[10]. There have been documented cases of individuals who do not, however adhere to their medication regimen[11-14]. World Health Organization also reported that in developed countries, up to 50% of patients with chronic illnesses do not adhere to their medications, while the adherence rate is even lower in developing countries[10]. Hypertension is associated with one of the highest risks of premature mortality[15]. Poor adherence to antihypertensive therapy has been associated with various issues such as poor blood pressure control, re-hospitalization and increase healthcare costs[16,17]. Reports also show that blood pressure control is associated with marked reductions in cardiovascular events and reduced mortality[18]. Thus, it is important to improve patient adherence to antihypertensive therapy so as to achieve desirable blood pressure control[12]. The Health Belief Model (HBM) has been used to explain and predict the health behaviours of individuals for preventing and/or controlling diseases (such as hypertension) and their complications. It has shown efficacy in predicting health behaviors in individuals with or at risk of developing cardiovascular disease[19]. It was introduced in the 1950s by social psychologists Godfrey Hochbaum, Irwin Rosenstock and Stephen Kegels and is used to explain a wide range of health behaviour[20]. The Health Belief Model constructs can be used to study non-adherence to hypertension and other chronic diseases. It predicts health-related behaviour with six constructs, which are: perceived susceptibility to a health problem, perceived severity of the health problem, benefits of taking action, barriers to taking action, cues to action and self-efficacy[21]. The Health Belief Model postulates that for a person to avoid a disease, the individual should believe that he/she is susceptible to the disease and that

having the disease will cause a degree of severity of the disease on some aspect of the person's life. Also, undertaking a particular action would be favourable to health and the action would have to overcome barriers such as convenience, cost, discipline and time. Cues to action is the trigger for protective health behavior and can be internal (such as the appearance of the signs and symptoms of a disease) or external (such as the impact of mass media-radio, television, or advice from relatives, friends and health providers)[22]. The HBM has been used to assess people's behavior to diagnosed illnesses, especially with regards to adherence to medication regimens. The Hill Bone medication adherence subscale assesses hypertensive patient's behavior with regard to medication adherence[23]. Several countries have carried out studies on adherence to antihypertensive medication using the health belief model and Hill Bone medication adherence subscale[22-25]. Limited studies have, however been carried out on adherence in hypertensive patients in Nigeria using the Hill Bone medication adherence subscale and the health belief model[19]. The aim of this study was to assess adherence of hypertensive patients to therapy using the Hill Bone medication adherence subscale and evaluate the influence of the health belief model on adherence at the Medical Out-Patient Department of Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun state, Nigeria.

METHODOLOGY

Study setting

The study was conducted at the Medical Outpatient Department (MOPD) of Olabisi Onabanjo University Teaching Hospital, Sagamu**.** Olabisi Onabanjo University Teaching Hospital is a tertiary care facility located in Sagamu, a suburban town with a population of 253.412[26].

Study design

The study was a descriptive cross-sectional design which was conducted by pharmacists for 12 weeks from July to October, 2018.

Study population

All patients with hypertension, who were receiving antihypertensive therapy and met the following criteria:

Inclusion criteria

Hypertensive patients who were 18 years and above at the time of the study, had been on antihypertensive therapy for at least 6 months and who consented to participate in the study.

Exclusion criteria

Hypertensive patients less than 18 years of age, yet to be placed on therapy, those too sick to participate and those who did not consent to participate in the study.

Sample size determination

A sample size of 333 was calculated for this study using Kirkwood[27] sample size determination formula:

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where:

n= sample size

p=expected proportion in population based on previous studies or pilot studies (31.8%)

d= margin of sampling error acceptable (0.05)

Z= Standard normal deviate corresponding to 95% confidence level=1.96.

Sampling procedure

Hypertensive patients attending clinics between 8 am and 2 pm from Monday to Friday, who met the inclusion criteria, were randomly sampled for the study.

Ethical approval

Ethical approval was obtained from the Human Research Ethics Committee of Olabisi Onabanjo University Teaching Hospital with reference number OOUHREC/PHARM/B/000123.

Data collection tool

The data collection tool was a pre-tested, self-report questionnaire which comprised of seven sections- A, B, C, D, E, F and G. Section A contained 10 items on demographic variables of participants. Section B contained 8 items on adherence to anti-hypertensive therapy adapted from the Hill-Bone medication adherence subscale. Section C contained 6 items on perceived severity of hypertension in patients. Section D contained 6 items on patients' perception on susceptibility. Section E contained 6 items on perceived benefits of adherence to anti-hypertensive therapy. Section F contained 5 items on perceived barriers of non-adherence to anti-hypertensive therapy. Section G contained 7 items on patients' perception on cue to action.

The items in section B (the Hill-Bone medication adherence subscale) were on a four-point Likert scale of daily (1), frequently (2), rarely (3) and never (4) with total score ranging from 8 (minimum) to 32 (maximum). Sections C, D, E, F and G (the Health Belief Model) were on a four-point Likert scale which were: strongly agree (SA), agree (A), disagree (D) and strongly disagree (SD). The scores attached to these scales were: SA = 4; A = 3; D = 2; SD = 1.

Validity

The questionnaire was translated from English to Yoruba language which is the language spoken predominantly in the area. After translation it was back-translated and compared to the original questionnaire to check for differences and make necessary revisions in order to ensure that the meaning of the original questionnaire is retained.

For content validity, the questionnaire was examined by two cardiologists working at Cardiac unit at General Hospital, Ikorodu, Lagos State. The researcher held discussions with the cardiologists to look into issues of clarity, specificity of variables to be measured and relevance of the contents of the questionnaire in Nigerian context. The study instrument was pretested using 10 patients on antihypertensive therapy at another hospital not used in the main study (General Hospital in Ikorodu, Lagos State).

Reliability

The Cronbach Alpha values obtained were 0.840, 0.729, 0.710, 0.682, 0.675 and 0.680 for adherence, perceived severity, perceived susceptibility, perceived benefit, perceived barriers and perceived cue to action respectively of which adherence, perceived severity, perceived susceptibility are above the reference value of 0.7[28].

Data collection

Data were collected by the researcher and two trained assistants. The questionnaire consisted of closed ended questions and was written in English language that is understood and well-spoken by most Nigerians. The Yoruba version of the questionnaire was given to those who didn't understand English. The time used to complete one form was about 15 minutes. Data was collected within a period of 12 weeks.

Outcome measure

The main outcome measure was the percentage of patients with adherence levels to antihypertensive medication ≥80% using the modified Hill Bone medication adherence subscale[24]. Secondary outcome measures were predictors of adherence to antihypertensive therapy.

Data analysis

Data were entered into the computer using SPSS version 17.0. Data were summarized using frequency tables and cross tabulations. Descriptive statistics such as mean, standard deviation and simple percentage analysis were employed to compare rate of adherence. Pearson correlation analysis was done to determine the relationship between the independent variables and the dependent variable in the study. Multiple regression analysis was adopted to determine the combined effect of psycho-social variables suggested by Health Belief Model on adherence to antihypertensive therapy. The test was conducted at 5% level of significance.

RESULTS and DISCUSSION

Socio-demographic data of respondents

Out of 333 questionnaires distributed, a total of 325 (97.6% response rate) were retrieved. Most of the respondents (63.7%) were 60 years and below, female (56.3%) and married (62.2%). Sixty-nine respondents (21.2%) had no formal education, while only forty two (12.9%) had tertiary education as shown in Table 1.

Table 1. Socio-demographic variables of Respondents

Variable	Frequency	%
Age		
≤60 years	207	63.7
> 60 years	118	36.3
Gender		
Male	142	43.7
Female	183	56.3
Marital Status		
Married	202	62.2
Separated	51	15.7
Widowed	72	22.1
Highest Educational Qualification		

None	69	21.2
Primary Education	85	26.2
Secondary Education	129	39.7
Tertiary education	42	12.9
Occupation:		
Unemployed	166	51.1
Employed	159	48.9

Adherence to therapy based on socio-demographic variables

Most respondents (75.8%) who were 60 years and below were adherent to their medications. The adherence rates of male and female patients were 46.5% and 68.8% respectively. The adherence rate was higher among married patients (69.8%) than separated and widowed patients (45.1% and 38.9% respectively). Adherence rate was also higher among patients with tertiary education (76.2%) than those with primary, secondary or no education. Patients who were employed had higher adherence rate (78.0%) than those who were not employed (40.1%).

Age and gender were significantly associated with adherence (p=0.032 and p=0.025 respectively) as shown in Table 2.

Table 2. Adherence to therapy based on socio-demographic variables

Variables	Therapy Adherence				p-value
	Non-Adherent	%	Adherent		
	Frequency		Frequency	%	
Age					
≤60 years	50	24.2	157	75.8	0.032*
>60 years	83	70.3	35	29.7	
Gender					
Male	76	53.5	66	46.5	0.025*
Female	57	31.1	126	68.8	
Marital Status					
Married	61	30.2	141	69.8	0.071
Separated	28	54.9	23	45.1	
Widowed	44	61.1	28	38.9	
Educational Background					
None	24	41.7	35	58.3	0.068
Primary Education	41	48.2	44	51.8	
Secondary Education	57	44.2	72	55.8	
Tertiary Education	10	23.8	32	76.2	
Occupation					
Unemployed	98	59	68	40.1	0.081
Employed	35	22	124	78	

*p<0.05 is significant

Patients' adherence to antihypertensive medications

One hundred and ninety-two patients (59.1%) were adherent to their antihypertensive medications as shown in Figure 1.

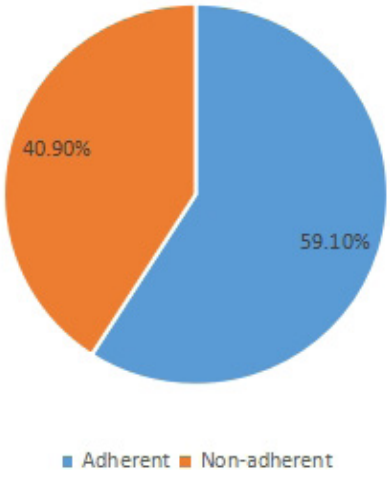

Figure 1. Patient adherence to antihypertensive medications

Adherence to therapy based on psycho-social variables

Patients with high perception of severity of their health condition had higher adherence rate of 79.7% compared to patients with low perceived severity. Adherence rates of hypertensive patients with low and high perceptions of susceptibility of having hypertension were 47.7% and 72.2% respectively. The adherence rates of hypertensive patients with low and high perceptions on the benefits of using antihypertensive medications were 39.2% and 78.1% respectively. Patients with low perception of barriers to treatment had higher adherence rate to antihypertensive therapy of 70.2% compared to those with high perception of barrier of 49.4%. Hypertensive patients with high perceived cues to action had higher adherence rate (71.0%) than patients with low perceived cues to action.

Adherence to therapy was significantly associated with patients' perception on severity, susceptibility, benefits, barriers and cue to action as shown in Table 3 ($p=0.021$, $p=0.001$, $p=0.031$, $p=0.0015$ and $p=0.010$ respectively).

Table 3. Adherence to therapy based on psycho-social variables

Patients Perceptions on	Therapy Adherence		
	Non-Adherence	Adherence	
	Frequency(%)	Frequency(%)	p-Value
Severity			
Low	109(52.7)	98(47.3)	0.021*
High	24(20.3)	94(79.7)	
Susceptibility			
Low	91(52.3)	83(47.7)	0.001*
High	42(27.8)	109(72.2)	
Benefits			
Low	96(60.8)	62(39.2)	0.031*
High	37(21.9)	132(78.1)	
Barriers			
Low	45(29.8)	106(70.2)	0.015*
High	88(50.6)	86(49.4)	
Cue to Action			
Low	88(51.8)	82(48.2)	0.010*
High	45(29.0)	110(71.0)	

*$p<0.05$ is significant

Patients' reasons for non-adherence to therapy

Reasons for non-adherence among the patients included forgetting to take medications, sense of feeling better, perceived deteriorating health despite the use of therapy, perceived ineffectiveness of the medication and cost of medications as shown in Figure 2.

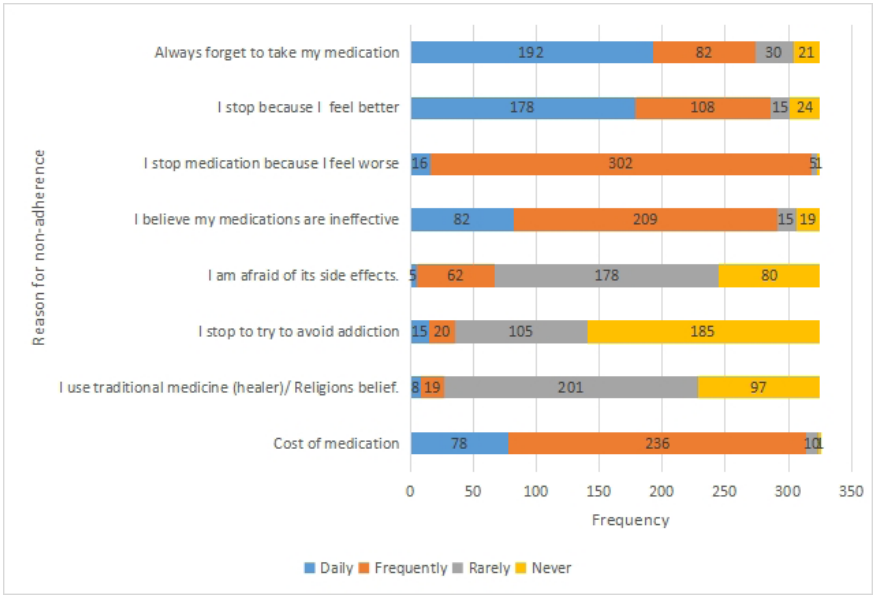

Figure 2. Patients' reasons for non-adherence to therapy

Relationship between psycho-social variables of health belief model and adherence to therapy by hypertensive patients

There was significant (positive but low) relationship between therapy adherence and perceived severity ($r= 0.104$; $p<0.05$). Furthermore, the result revealed that there was no significant relationship between adherence to therapy and perceived susceptibility ($r= 0.141$; $p>0.05$).

Likewise, there was significant (positive but low) relationship between perceived benefits and adherence to therapy ($r= 0.274$; $p<0.05$) However, perceived barriers had significant (negative but moderate) relationship with adherence to therapy ($r= -0.528$; $p<0.05$). Perceived cue to action had significant (positive but low) relationship with adherence to therapy ($r= 0.197$; $p<0.05$). More so, there was significant (positive but low) relationship between perceived severity of hypertension and perceived susceptibility ($r = 0.285$; $p<0.05$). Also, perceived severity had significant (positive but low) relationship with cues to action ($r = 0.202$; $p<0.05$). Perceived benefit of using medication showed significant negative, moderate relationship with perceived barriers ($r = -0.45$; $p<0.000$). Also perceived benefits of using medication showed moderate positive relationship with cues to action ($r = 0.323$; $p<0.05$).

Table 4. Relationship between psycho-social variables suggested by HBM and adherence to therapy (n=325)

Variables	1	2	3	4	5	6
Adherence to therapy (1)	1.000					
Perceived Severity (2)	.104*	1.000				
Perceived Susceptibility (3)	.141	.285**	1.000			
Perceived Benefits (4)	.274**	.090	-.062	1.000		
Perceived Barriers (5)	-.528**	-.090	.061	-.449**	1.000	
Perceived Cue to Action (6)	.197*	.202*	.180*	.323*	.323	1.000

* Correlation is significant at the 0.05 level (2-tailed).

** Correlations is significant at 0.01 level (2-tailed).

The composite effect of psycho-social variables on adherence to therapy

The predictor variables were perceived severity, perceived susceptibility, perceived benefit, perceived barriers and cues to action. The result indicated significant model fit for the data ($F = 12.911$; $p<0.05$). The amount of variance in therapy adherence which is accounted for by the predictors is 32.8% ($R^2 = 0.328$) while other variables accounted for 67.2%. Perceived barrier being the strongest predictor of adherence to therapy by patients suffering from hypertensive ($\beta = -0.477$; $p=0.000$). A negative beta coefficient indicates a negative association between perceived barriers and adherence to therapy. Other predictor variables such as perceived severity, perceived susceptibility, perceived benefit and cues to action were not statistically associated with adherence of patients to antihypertensive therapy.

Reported adherence rate to antihypertensive therapy among the patients in this study was 59.1%. There was a significant association between age, gender of patients and adherence to antihypertensive medications while marital status, educational level and occupation of patients had no significant association with adherence. Also, there was significant association in adherence to therapy by patients according to their perceptions on severity, susceptibility, benefits, barriers and cue to action. Reasons for non-adherence to therapy reported in the study included forgetting to take medication, sense of feeling better, deteriorating

health despite the use of therapy, ineffectiveness of the therapy and cost of medications. Furthermore, the result revealed that psycho-social variables had significant combined effect on adherence rate to therapy and that perceived barrier was the only psycho-social variables potently predicting adherence to therapy.

The rate of adherence in this study is similar to that reported by Takahashi et al.[29] who conducted a study in 3 district hospitals in South-Eastern Asia and reported that half of the hypertensive patients (50%) adhered to antihypertensive medication. Ambaw et al.[30] reported a higher adherence rate of 64.6% in a hospital in Ethiopia while Algabbani and Algabbani[3], and Hussein et al.[12] reported lower adherence to antihypertensive therapy by patients of 42.2% and 46.12 respectively.

The study found that more hypertensive patients 60 years and below adhered to their antihypertensive medication compared to older hypertensive patients. Similarly, Joho[31] who conducted a cross-sectional study of hypertensive patients in 3 district hospitals in Dar es Salaam Tanzania reported that hypertensive patients who were less than 64 years had higher adherence rate than those who were 65 years and above. However, Hussein et al.[12] reported that younger age (<40 years) was a significant predictor of adherence compared to older patients. In contrast, Lee et al.[32] conducted a study in South Korea and found that older people adhered more to antihypertensive therapy compared to younger people. Probable reason could be perceived susceptibility and severity. However, in a cross-sectional study in Saudi Arabia conducted among hypertensive patients at primary health clinics in Prince Sultan Medical City there was no relationship between age and medication adherence[3]. There was a higher rate of adherence reported by female patients compared to male patients in the study which was significant. This finding was similar to reports by Joho,[31] who reported that females had higher adherence to antihypertensive therapy compared to males. The low rate of adherence to antihypertensive therapy by male patients may be due to fear of the side effect of antihypertensive drugs, one of which is erectile dysfunction[33]. Algabbani and Algabbani[3] reported no significant relationship between gender and adherence.

Hypertensive patients with tertiary education in the study had higher adherence rate compared to those with little or no educational background, which is similar to the studies by Hussein et al.[12] and Joho[31], in which patients with higher education were more adherent to their medication. The probable reason could be because education plays an important part in comprehension, retention, recollection and application of health information and knowledge and patients with higher level of education may have a better understanding of the importance of controlling their blood pressure and the consequences of

poor drug adherence[25]. Married hypertensive patients in the study reported higher adherence rate than separated and widowed patients. The help, care and support patients received from spouses could be a reason why there was higher rate of adherence among married patients compared to those who were not. This result supported the findings of Joho[31] who reported that married participants were more adherent to treatment than non-married participants. A study by Najjuma et al.[34] of Southwest Ugandan patients also reported that patient's family support contributed to medication adherence. A meta-analysis by Abegaz et al.[35] showed that interventions adapted to family engagement can improve antihypertensive adherence.

Reasons reported for non-adherence to antihypertensive therapy reported in the study included forgetfulness, cost of the medications, feeling better, fear of the side effects, avoiding addiction to drugs and use of traditional medicine. Similar reasons were reported by Takahashi et al.[29] in an observational study of hypertensive patients for not adhering to their medication regimen.

The study reported significant relationship between drug therapy adherence and perceived severity. This implies that the higher the perceived severity of hypertension the greater the adherence to therapy. There was however no significant relationship between adherence to therapy and perceived susceptibility. There was a significant relationship between perceived benefits and adherence to therapy. This implies that the higher the perceived benefit of therapy, the higher the adherence to treatment. Perceived barriers had significant negative relationship with adherence to therapy, meaning the higher the perceived barrier, the less the adherence. Perceived cue to action was significantly related with adherence to therapy meaning that when individuals receive more reminders of the importance of treatment adherence, they are more likely to adhere to medication. More so, there was significant relationship between perceived severity of hypertension and perceived susceptibility. This implies that the higher the perceived severity of hypertension the higher the perception of being vulnerable to the complications of hypertension. Also, perceived severity had significant relationship with cues to action implying that the higher the perceived severity of hypertension the higher the inclination to follow the cues to action (reminders). Perceived benefit of using medication showed significant negative relationship with perceived barrier, this meant that the higher the perception of benefit the lower the perception of barriers. Also perceived benefit of using medication showed positive association with cues to action, meaning that the higher the perception of benefit, the higher the inclination to heed reminders**.** Similarly**,** Joho[31] reported significant relationship between perceived susceptibility, perceived benefits of therapy, perceived barriers to

treatment, cues to action and adherence to antihypertensive therapy by hypertensive patients. In contrast, in the study reported by Osamor and Ojelabi[19], only perceived susceptibility, perceived benefit of medication and perceived barriers to treatment had significant relationship with adherence.

The study reported that out of all the five HBM variables, only perceived barriers to adherence significantly predicted the adherence rate of hypertensive patients to antihypertensive medication. In contrast, Osamor and Ojelabi[19] reported that only perceived susceptibility was a significant predictor of adherence.

A limitation of the study was that the sample size used was less than the calculated sample size. This can be improved in subsequent studies by making allowance for attrition. Also, test-retest reliability was not carried out which should have further helped to determine the reliability of the test instrument used in the study. Another study limitation is that the study was conducted in only one healthcare facility. Multi-centre studies will ensure that the results can be generalized.

In the study, 59.1% of patients adhered to therapy. Age and gender were significantly associated with adherence. With the exception of perceived susceptibility, all the health belief constructs correlated with adherence to therapy. Perceived barriers to adherence significantly predicted adherence of patients to therapy. Pharmacists should educate patients on importance of drug adherence for better blood pressure control.

STATEMENT OF ETHICS

Ethical approval was obtained from the Human Research Ethics Committee of Olabisi Onabanjo University Teaching Hospital with reference number OOUHREC/PHARM/B/000123.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

OAA was involved in study design, data collection and analysis. TOA and HO were involved in data collection and preparation of manuscript draft. All authors contributed to revision of the draft, reading and approval of the final manuscript.

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REFERENCES

1. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, Clement D, Coca A, De Simone G, Dominiczak A, Kahan T. 2018 Practice Guidelines for the management of arterial hypertension of the European Society of Cardiology and the European Society of Hypertension. Blood Press, 2018;27(6):314-340. Doi: 10.1080/08037051.2018.1527177

2. Bappah BS, Shittu A, Usman JS, Nuhu JM, Sumaila FG, Ahmad AA, Mohammed J. Prevalence and correlates of undiagnosed hypertension among staff of a Nigerian university community. Pan Afri Med J, 2022;42(80). Doi: 10.11604/pamj.2022.42.80.26464

3. Algabbani FM and Algabbani, AM. Treatment adherence among patients with hypertension: findings from a cross-sectional study. Clin Hypertens, 2020;26(1):1-9. Doi: 10.1186/s40885-020-00151-1

4. Murray CJL, Aravkin AY, Zheng P, Abbafati C, Abbas KM, Abbasi- Kangevari M, Abd-Allah F, Abdelalim A, Abdollahi M Abdollahpour I and Abegaz KH. Global burden of 87 risk factors in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. The Lancet, 2020;396(10258):1223-1249. Doi: 10.1016/s0140-6736(20)30752-2

5. World Health Organization. Hypertension. https://www.who.int/news-room/fact-sheets/detail/hypertension. Accessed 11 March 2023.

6. Bosu WK. The prevalence, awareness and control of hypertension among workers in West Africa: a systematic review. Glob Health Action, 2015;8(1):26227. Doi: 10.3402/gha.v8.26227

7. Vincent Onabajo GG, Mohammad HS, Umeonwuka C. Prevalence of undiagnosed hypertension among a cohort of university workers in Nigeria. Int J Community Med Public Health, 2016;3:1963-1967. Doi: 10.18203/2394-6040.ijcmph20162073

8. Adedoyin RA, Awotidebe TO, Borode AO, Ativie RN, Akindele MA, Adeyeye VO, E Johnson, O. Comparison of blood pressure patterns of teaching and non-teaching staff of a Nigeria university. Int J Clin Med, 2016;7(7):454-460. Doi: 10.4236/ijcm.2016.77049

9. Egbi OG, Rotifa S, Jumbo J. Prevalence of hypertension and its correlates among employees of a tertiary hospital in Yenogoa, Nigeria. Ann Afr Med, 2015;14(1):8-17. Doi: 10.4236/ijcm.2016.77049

10. World Health Organization. Adherence to long-term therapies: evidence for action. Geneva; World Health Organization, 2003. Accessed 11 March 2023.

11. Mendes R, Martins S, Fernandes L. Adherence to medication, physical activity and diet in older adults with diabetes: its association with cognition, anxiety and depression. J Clin Med Res, 2019;11(8): 583-592. Doi: 10.14740/jocmr3894

12. Hussein A, Awad MS, Mahmoud HEM. Patient adherence to antihypertensive medications in Upper Egypt: a cross-sectional study. Egypt Heart J, 2020;72:1-8. Doi: 10.1186/s43044-020-00066-0

13. Foley L, Larkin J, Lombard-Vance R, Murphy AW, Hynes L, Galvin E, Molloy GJ. Prevalence and predictors of medication non-adherence among people living with multimorbidity: A systematic review and meta-analysis. BMJ Open, 2021;11(9):e044987. Doi: 10.1136/bmjopen-2020-044987

14. Suleiman IA, Aremu TO, Akinsanya AK. Adherence to medication among hypertensive diabetic patients in a secondary health institution in Lagos State. *JBSPR*, 2022;2(4):22-32. Doi: 10.52968/27453111.

15. Stanaway JD Afshin A, Gakidou E, Lim SS, Abate D, Abate KH et al. Global, regional and national comparative risk assessment of 84 behavioral, environmental, occupational and metabolic risks or clusters of risks for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet, 2018;392(10159):1923-1994. Doi: 10.1016/s0140-6736(18)32225-6

16. Borzecki AM, Oliveria SA, Berlowitz DR. Barriers to hypertension control. Am Heart J, 2005;149:785-794. Doi: 10.1016/j.ahj.2005.01.047

17. Cutler RL, Fernandez-Llimos F, Frommer M, Benrimoj C, Garcia-Cardenas V. Economic impact of medication non-adherence by disease groups: a systematic review. BMJ Open, 2018;8(1):e016982. Doi: 10.1136/bmjopen-2017-016982

18. Ettehad D, Emdin CA, Kiran A Anderson SG, Callender T, Emberson J, Chalmers J, Rodgers A, Rahimi K. Blood pressure lowering for prevention of cardiovascular disease and death: a systematic review and meta-analysis. The Lancet, 2016;387(10022):957-967. Doi: 10.1016/s0140-6736(15)01225-8

19. Osamor PE, Ojelabi OA. Health belief model and hypertension treatment compliance. Accessed 11 March 2023.

20. Onoruoiza SI, Musa A, Umar BD, Kunle YS. Using health beliefs model as an intervention to non compliance with hypertension information among hypertensive patients. Int Organ Sci Res J Human Sci, 2015;20(9):11-16. Doi: 10.9790/0837-20951116

21. Rosenstock IM. Historical origins of the health belief model. Health Educ Behav, 1974;2(4):328-335. Doi: 10.1177/109019817400200403

22. Rajkumar E, Romate J. Application of a Health Belief Model to hypertension within rural India. Ind J Pub Health Res Dev, 2020;11(1):782-790. Doi: 10.37506/v11/i1/2020/ijphrd/193923

23. Culig J, Leppee M. From Morisky to Hill-Bone; self-reports scales for measuring adherence to medication. *Coll. Antropo.,* 2014;38 (1):55-62.

24. Nashilongo MN, Singu B, Kalemeera F, Mubita M, Naikaku E, Baker A, et al. Assessing adherence to antihypertensive therapy in primary health care in Namibia: findings and implications. Cardiovasc Drugs Ther, 2017;31:565-578. Doi: 10.1007/s10557-017-6756-8

25. Alsolami F, Correa-Velez I. Hou XY. Factors affecting antihypertensive medications adherence among hypertensive patients in Saudi Arabia. Am J Med Med Sci, 2015;5(4):181-189. Doi: 10.5923/j.ajmms.20150504.07

26. Sagamu Local Government-Remo Voices Aloud-WordPress.com. Accessed 11 March 2023.

27. Kirkwood BR and Sterne JA. Calculation of required sample size in: Essential Medical Statistics, Blackwell Science, Oxford (2003), 420-421. ISBN: 978–0–86542–871–3

28. Taber KS. The use of Cronbach's Alpha when developing and reporting research instruments in science education. Res Sci Educ, 2018;48:1273-1296. Doi: 10.1007/s11165-016-9602-2

29. Takahashi E, Vilay P, Chanthakoummane K, Pongvongsa T, Kounnavong S, Kano S, Kobayashi J, Nonaka D. Adherence to antihypertensive medications in rural Lao PDR: a prospective observational study. Trop Med Health, 2021;49:88. Doi: 10.1186/s41182-021-00374-4

30. Ambaw AAD, Alemie GA, Yohannes SMW, Mengesha ZB. Adherence to antihypertensive treatment and associated factors among patients on follow up at University of Gondar Hospital, Northwest Ethiopia. BMC Public Health, 2012;12:282. Doi: 10.1186/1471-2458-12-282

31. Joho AA. Using the Health Belief Model to explain the patient's compliance to anti-hypertensive treatment in three district hospitals- Dar es Salaam, Tanzania: a cross section study. East Afr Health Res J, 2021;5(1):50-58. Doi: 10.24248/eahrj.v5i1.651

32. Lee H, Yano Y, Cho SMJ, Heo JE, Kim DW, Park S, et al. Adherence to antihypertensive medication and incident cardiovascular events in young adults with hypertension. Hypertension, 2021;77(4):1341-1349. Doi: 10.1161/HYPERTENSIONAHA.120.16784

33. Hernandez-Cerda J, Bertoneu-Gonzalez V, Zuazola P, Cordero A. Understanding erectile dysfunction in hypertensive patients: The need for good patient management. Vasc Health Risk Manag, 2020;231-239. Doi: 10.2147/VHRM.S22331

34. Najjuma JN, Brennaman L, Nabirye RC, Ssedyabane F, Maling S, Bajunirwe F, Muhindo R. Adherence to antihypertensive medication: an interview analysis of Southwest Ugandan patients' perspectives. Ann Glob Health, 2020;86(1). Doi: 10.5334/aogh.2904

35. Abegaz TM, Shehab A, Gebreyohannes EA, Bhagavathula AS, Elnour AA. Nonadherence to antihypertensive drugs: A systemic review and meta-analysis. Medicine, 2017;96(4):e5641. Doi: 10.1097/MD.0000000000005641

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Physical/Chemical modifications of *Oryza glaberrima* and *Digitaria exilis* starches: Effect on packing and compression properties of ibuprofen tablet formulations

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ABSTRACT

Imported grain starches are in high demand but are expensive, and their supply is unreliable. To address the need for innovative formulators, the development and use of native starches or the synthesis of modified starches with predetermined functions from locally sourced underused plants as excipients in pharmaceutical industries is critical. The primary goal of this research is to explore the influence of physical and chemical modification on the compressional and packing features of dual blends of Ibuprofen with *Oryza glaberrima* and *Digitaria exilis* starches in oral tablet formulation. Different ratios of starches and Ibuprofen were used in the direct compression method to prepare the tablets. From the native starch forms, pregelatinized and carboxymethylated starches were produced. The manufactured tablets' compressional features were investigated using the Heckel, Gurham, and Kawakita equations, as well as density measurements. Pregelatinization resulted in a faster onset but a lower amount of plastic deformations than native and carboxymethylated starch formulations. Increasing the particle size of these starches substantially impacts densification, rearrangement of particles, fragmentation propensity, and elastic/plastic deformation. The modified starches would make acceptable excipients because they increased tablet densification compared to the native forms.

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Keywords: carboxymethylation, compressional properties, direct compression, pregelatinization, starch

INTRODUCTION

For many decades, several plant starches have been investigated as pharmaceutical excipients[1]. Due to their affordability, inertness, and capacity to serve as a binding, gliding, disintegrating, and filling agent for solid dosage forms, starches are among the most readily available and widely utilized excipients in the drug industry to prepare tablets[2, 3]. Native or untreated starches are weak structurally and have limited functional options when making tablets; their function must be increased through modifications. Modifying or treating native starches through physical, chemical, or enzymatic techniques can be used to obtain desired functionalities or improve their physiochemical properties[3,4].

Understanding powders' packing, cohesive, and compressional characteristics are crucial in developing and manufacturing solid dosage forms like powders, tablets, and capsules of pharmaceutical standards; this is critical when combining powders, filling capsules with powders or granules, and dies in the course of tableting[5]. Several models characterizing powder blends have drawbacks, such as requiring a spherical shape for model validation[6], working only with a small particle size fraction[7], or losing accuracy as an additional powder component is added[8]. As a result, model failure will arise when the particle size distribution is wider or skewed[5].

Various techniques, such as compaction stimulators or instrumented production presses, can be used[9] to evaluate the compaction characteristics of pharmaceutical dosage forms. The compaction equation can demonstrate the link between powder parameters such as volume, porosity, density, void space, and compaction pressure. Constructing a linear plot by fitting the experimental data to an equation is necessary to make comparisons between several data sets easier[10].

The association between volume and compression pressure is used to generate a mathematical model of the compaction process[11]. Thus, increasing the compression force or pressure causes the volume of powders to decrease during powder compression; however, this compression process may be well described by monitoring changes in powder porosity as the compression pressure is increased[12]. The manufactured tablets' compressional features were studied using the Heckel, Gurham, and Kawakita equations and density measurements[13, 14, 15]. Various equations provide a comprehensive picture of the powder com-

paction process and excipient behavior. Tablets should also be strong enough to withstand post-compaction stress during handling and transportation[16].

The Heckel equation depicts the relationship between the powder's relative density (D) and the compaction pressure (P). The equation is expressed as:

$$\ln [1 \div (1 - D)] = KP + A \quad \text{..... [1]}$$

K is the plasticity slope of the compressed powder and the reciprocal of the mean yield pressure (Py). The constant A, commonly known as the equation's intercept, is related to the tableting method, which comprises die filling, powder particle rearrangement, and deformation. Using the equation below, the value of relative density (D_A or D), also known as the overall degree of densification and rearrangement of powder particles, can be derived from constant A[11,17].

$$D_A = 1 - e^{-A} \quad \text{..... [2]}$$

The initial rearrangement phase of densification due to die filling is described by the powder's relative density at the point where the applied pressure equals zero (D_o). The difference between D_A and D_o, known as the relative density D_B, represents the rearrangement when low pressures are applied to the powder bed:

$$D_B = D_A - D_o \quad \text{.....} \qquad [3]$$

Powder compression can be analyzed using the Kawakita equation and the amount of volume decrease (C), which is stated as:

$$C = (V_o - V_P) \div V_o = abP \div (1 + bP) \quad \text{..... [4]}$$

The preceding equation can be rewritten as follows:

$$P \div C = (P \div a) + (1 \div ab) \quad \text{..... [5]}$$

Where V_o is the initial bulk volume of the powder, and V_P is the bulk volume after compression. The constant a represents the material's lowest porosity before compression, while the constant b represents its plasticity. P_k is the pressure necessary to lower the powder bed by half, defined by the reciprocal of b[12,17,18].

The Gurnham equation states that a fractional increase in pressure increases apparent mass density relative to the prior pressure[19]. The association is as follows:

$$\frac{dP}{P} = A\, dD \quad \text{..... [6]}$$

P represents pressure, D represents apparent density based on solid weight and total volume, and A represents a constant.

Volume decrease can be expressed as porosity (ε) in pharmaceutical powder compaction, as follows:

$$Porosity = 1 - \frac{(Apparent\ density)}{(True\ density)}$$

$$\varepsilon = 1 - \frac{D}{\rho_t} \quad \text{.....[7]}$$

Where ρ_t denotes the material's particle or actual density.

Previous studies have shown that excipient compressional qualities can be utilized to validate the role of excipients in medication formulations[16, 20, 21]. The Heckel, Kawakita, and Gurnham compressional equations were employed to examine Ibuprofen tablets made by direct compression utilizing native, pregelatinized, and carboxymethylated starches from *Oryza glaberrima* (African/Ofada rice) and *Digitaria exilis* (Fonio/Acha). Ofada rice is the generic name of the indigenous rice species *Oryza glaberrima,* Steud Family Poaceae, which is mainly cultivated in Southwest Nigeria[22]. Rice has high starch content making it a potentially inexpensive source of starch for the pharmaceutical industry[23]. Also, *Digitaria exilis* (Acha), a food grain consumed in many parts of Africa and India, belongs to the same subfamily as maize, sorghum, and pearl millet. The starch from its grains is comparable in structure and physicochemical properties to starch from conventional cereal grains. However, Acha starch shows a higher water binding capacity than wheat, rice or maize starches[24], making it suitable for several pharmaceutical applications.

The principal goal of this study was to establish the packing, flow and cohesive characteristics of Ibuprofen tablet formulations containing untreated and treated versions of these locally sourced starches as filler binders. This study used Ibuprofen as the benchmark drug to determine how starch-based excipients affected tablets made from drugs with poor compaction characteristics[25]. Several studies reported that Ibuprofen bulk powder has poor flow properties, inadequate compaction behavior, and adheres to the surfaces of punch and die, making tablet formulation development difficult [26, 27, 28].

METHODOLOGY

Ibuprofen powder BP, sodium chloride and acetic anhydride were sourced from BDH Chemicals Limited. Magnesium stearate was acquired from Aldrich Chemical Company Inc., USA. Acetone was obtained from Merck Limited,

Germany. All the active and inactive pharmaceutical ingredients used in this study were of pharmaceutical standard and analytical grade.

Production of the native starches

The *Oryza glaberrima* and *Digitaria exilis* grains were acquired locally in Nigeria. The pure starch polymers were generated by aqueous extraction using Odeniyi[29] method with modification. In a nutshell, each sample's grains were soaked in distilled water for 2-3 days. The mixture was blended using an Osterizer Dual range Pulse Matic Milling blender (John Oster Manufacturing Co., Racine, Wisconsin, USA) into a slurry before being strained through a muslin cloth. The filtrate was allowed to settle after being suspended in distilled water. The obtained supernatant was decanted at 12-hour intervals, and the starch slurry was re-suspended in distilled water. After 72 hours, the cake was collected and milled on a local milling machine; then dried for 48 hours in a 50 °C oven (Laboratory oven TT-9083, Techmel and Techmel, TX, USA) before being milled to smaller particles with the Osterizer Dual range Pulse Matic Milling blender. A sieve with a mesh size of 0.315 mm was used to obtain the fine powder. The powder that resulted was then sealed in an airtight container. A sieve with a mesh size 120 was used to sift dry whitish end-products.

Synthesis of the pregelatinized starches

The two native excipients were pregelatinized in the laboratory according to the method by Okunlola and Adewusi[30]. 100 g of dry starch powder was dissolved in 100 mL of distilled water to create an aqueous slurry of each starch, which was then heated at 55 °C while being stirred every 10 minutes. The derived paste was crisp-dried for 48 hours at 60 °C in a hot air laboratory oven (TT-9083, Techmel and Techmel, TX, USA). The dried mass was ground into powder in a laboratory mill (Christy and Norris Ltd., Chelmsford, UK). Before use, all the starches were run through a sieve with a number 120 mesh (125 μm). These modified excipients were kept in airtight amber containers.

Synthesis of the carboxymethylated starches

A 100 g sample of native starch powder was combined with 400 mL of a 7.5 % w/v monochloroacetic acid solution in 1-propanol. The starch suspension was mixed with 10 mL of a 30 % w/v sodium hydroxide solution and heated on a hot plate for 20 minutes at 50 °C with constant stirring (200 revolution per minute). The reaction was then neutralized with glacial acetic acid before filtering through filter paper. The remaining sediment was washed with 80 % methanol, then 100 % methanol. The obtained starch was dried for six hours in an oven at 50 °C. The dehydrated starch fragments recovered were crushed into

a fine powder and sifted utilizing a British standard sieve with a mesh size of 120 mesh (125 μm). The powdered starch was weighed and kept in airtight vessels[31].

Analysis of particle size

The light microscope with batch number BH-2 BHS and manufactured by Olympus, Tokyo, Japan, was used for determining the particle size, with approximately 200 particles per sample being viewed. Each starch form's mean diameter, d, was ascertained by plotting the cumulative number of percent oversize versus particle size.

Determination of moisture content

Using an Ohaus infrared moisture content analyzer (Ohaus Scale Corporation, New Jersey), the percentage moisture content of 10 mg of each starch form was determined and recorded.

Densities measurements and compressibility characteristics

Using xylene as the displacement fluid, the particle density of each starch form was determined using the pycnometer method by Ayorinde et al[32]. Each starch powder's bulk density was ascertained using established procedures from the previous study[31]. Tapped density was determined by applying 100 taps at a standardized rate of 38 taps per minute to 30 g of each starch sample in a graduated cylinder. The calculations were carried out in triplicate. Each starch powder's relative density, D_o, was calculated by dividing its bulk density by its particle density. Previous research on these specific starches forms generated Hausner's ratio and Carr's index values[31].

The preparation of tablets

Binary blends of drug and excipients were made for direct compression, as illustrated in Table 1.

Each formulation containing the appropriate amounts of starch and Ibuprofen was well combined. The powder combinations (total of 400 mg per tablet) were compacted by utilizing a Carver Hydraulic Hand Press (Model C, Fred S. Carver Inc., Menomonee Falls, Wisconsin, USA) equipped with a calibrated pressure gauge. The flat-faced punch and 12.5 mm die were lubricated with a 2 % w/v magnesium stearate in acetone before each compression to prevent the tablet from sticking to the surface of the punch and die. The compressional pressures used were from 0.25 to 1.5 metric tonnes, with a dwell period of sixty seconds. After being carefully removed from the assembly, the pills were stored in sealed containers atop silica gel for 24 hours for elastic recovery before determining their properties.

Table 1. Drug and excipient composition in tablet forms

Formulation	Ibuprofen	Excipient
	%	%
F_1	90.0	10.0
F_2	75.0	25.0
F_3	50.0	50.0
F_4	25.0	75.0
F_5	00.0	100.0

Establishment of Heckel relationships for the native and modified starches

The ln 1/1-D was plotted versus applied pressure P for the different types of starches, also at different amounts of starch in the formulations. The extended linear plots' slope and intercept on the y-axis were K and A. Equation 2 was used to calculate total pre-compression density D_A at zero and low pressures, whereas mean pressure P_y was derived as a reciprocal of K. D_B (relative density at low pressures) was calculated by subtracting D_A from D_o, the powder bed's relative density at zero pressure (Equation 3)[16,20].

$D_A = 1 - e^{-A}$ ..... [2]

$D_B = D_A - D_o$ ..... [3]

Determination of Kawakita relationships for the different starches

The constant C, which signifies the level of volume reduction, was estimated utilizing Equation 4. P/C was plotted versus applied pressure P for the native and modified starches in the preparations at the varied starch concentrations. The constants a and ab were calculated using the slope and intercept of the straight line from Equation 5. Regression plots with Equation 6 were used to calculate P_k, the pressure needed to drop the powder bed volume half, and D_i, the packed beginning relative density[16,20].

$C = (V_o - V_p) \div V_o = abP \div (1 + bP)$ ..... [4]

$P \div C = (P \div a) + (1 \div ab)$ ..... [5]

$\frac{dP}{P} = A\, dD$ ..... [6]

Establishment of Gurnham relationships for the native and modified starches

Percent porosity (% ε) was plotted against lnP (natural logarithm of applied pressure) for different starches in the formulation at various concentrations. As previously stated, the slope of the regression line derived from each plot was used to calculate the value of c, a term for compressibility that signifies the influence of change in pressure on porosity, and d, which corresponded to the enhanced compressibility features[16, 20].

Statistical analysis

The data derived from the formulations were statistically analyzed using the Students' t-test and ANOVA, with $P < 0.05$ regarded as the importance level (GraphPad Software Inc., San Diego, USA)

RESULTS and DISCUSSION

Physical properties of the untreated and treated starches

Particle sizes of the starches had almost doubled following modifications (Table 2). Particle size study revealed that the native form had the smallest diameter, *d*, for the two different starches used in this study. Native African rice starch granules (7.24 μm) proved to be of a smaller size than pregelatinized (15.37 μm) and carboxymethylated (13.13 μm) granules. Native Fonio starch granules (3.16 μm) were also smaller than those that had been pregelatinized (4.98 μm) and carboxymethylated (7.69 μm). The mean diameter of native African rice and Fonio starch increased considerably after modification. The swelling of the starch granules brought on by gelatinization and the subsequent amylose leaching could be the source of the pregelatinized starches' larger particle size. The loss of amylose content after gelatinization results in enhanced amylopectin activity, improving starch swelling capacity. Several investigations have found that swelling power is closely related to amylose and its characteristics. Therefore, it was suggested that the degree of amylose lipid complexation, the amount of amylose that has been leached, and the phosphate content all substantially impact swelling power. Amylose lipid complexes limit swelling power, but the presence of phosphate groups in starch improves starch's water binding ability and, therefore, its swelling power[16,31,33]. These events are likely responsible for the high solubility, swelling power, and water absorption indices observed in pregelatinized starches[31]. Previous research has confirmed that pregelatinized starch has more excellent water absorption, swelling capacity, and solubility than native starch due to hydrogen bond breakdown and amylose leaching caused by gelatinization[34,35]. The highest value for anticipated

particle diameter was found in pregelatinized African rice. Larger particle sizes improve powder flow, which should improve compressibility[36,37].

The particle diameter of the two starches was also increased by carboxymethylation, which disrupts the starch granule structure and increases amylose leaching, resulting in starch granule enlargement. Adding the carboxymethyl group makes these starches more hydrophilic and aids in water holding, expanding the particle dimension of the chemically modified starches[16,38].

Table 2. Physical properties of the pure and modified starches (n =3, mean ± s.d)

Starch Source	Starch form	Mean Diameter, d (μm)	Particle density (gcm^{-3})	Hausner's Ratio	Carr's index	Moisture Content (%)
African Rice	Native	7.24±3.78	1.56±0.002	1.23±0.05	19.35±4.80	11.00
	Pregelatinized	15.37±13.17	1.47±0.01	1.18±0.02	14.62±4.66	10.44
	Carboxymethylated	13.13±7.15	1.53±0.02	1.21±0.05	17.50±4.97	9.48
Fonio	Native	3.16±1.85	1.48±0.002	1.25±0.06	19.90±6.04	10.12
	Pregelatinized	4.98±3.02	1.47±0.001	1.19±0.04	16.26±4.00	9.93
	Carboxymethylated	7.69±3.99	1.52±0.003	1.33±0.02	23.55±1.42	10.23

Table 2 also shows the different starches' physical properties, their respective particle densities, Hausner's ratios, and Carr's indices. The particle density of Ibuprofen was 1.062 gcm^{-3}, while its mean particle diameter was 44.15 μm.

The particle density of Ibuprofen powder was very low (1.063 gcm^{-1}), and the mean particle diameter was exceptionally high (44.15 μm). Ibuprofen's weak flow properties and elevated cohesion explain its poor compression qualities and, thus, the necessity for acceptable excipients with good flow and compression capabilities[16]. Ibuprofen demonstrates poor flow, compaction (tableting), and dissolution profile because of its hydrophobic structure and high cohesive, adhesive, and viscoelastic characteristics; therefore, it should be combined with excipients with superior physicochemical properties to enhance its compression and dissolution behavior[39]. Except for the carboxymethylated fonio, pure starch forms from the starches used in this study showed lesser particle density values than the treated forms. During powder mixing, the powder density had an impact, and segregation could occur due to size and shape. The behavior of the starch during packing affects unit operations like die, capsule filling, and compression[40].

Flowability test using the Hausner ratio and Carr's index (compressibility index) revealed lower values for the pregelatinized and carboxymethylated forms of African rice starch compared to their native form (Table 2), suggesting superior flow characteristics to their untreated form[35]. Pregelatinized Fonio flowed better than its native form, whereas carboxymethylated Fonio exhibited poor flow properties (Table 2). The Hausner and Carr's indices for starches were ranked in the following order: African rice; pregelatinized < carboxymethylated < native and Fonio; pregelatinized < native < carboxymethylated. The flowability of botanical starches was generally ranked in the order of African rice > Fonio. From the previous study on these native and modified forms of these starches by Omoteso et al.[31], the larger particle size of these modified starch granules may be attributed to the improved flow of pregelatinized and carboxymethylated starches. Larger particles flow better due to superior density and gravitational influences, but finer particles are more cohesive as a result of surface effects[35].

The native and the modified starches exhibited Hausner ratios more prominent than 1.11 and Carr's indices greater than 10. Values below 15 on Carr's index denote good flowability, while values above 25 denote poor flowability. Additionally, Hausner ratio values higher than 1.25 indicate poor flowability. The values of these indices will help the formulator in the judicious selection of excipients to prevent impeding the movement of powder into the die cavities through the hopper, which could affect the weight uniformity of the produced tablets[20,31,41,42]. Pregelatinized starches exhibiting lower Hausner's ratio values than native starch indicate improved flowability[20,35,40]. Therefore, starch modification, particularly pregelatinization, increases the flowability of native starch. Carboxymethylated starch also demonstrated outstanding flow properties. The most common pharmaceutical-modified starch is pregelatinized starch. Based on earlier research on pregelatinized starch, this treatment enhances starch flowability, disintegration, and hardness[43]. Generally, there was a direct relationship between the particle density, Carr's index, and Hausner ratio values between the native and modified starches (Table 2).

The moisture level of the samples that were examined ranged from 9.48 % to 11.00 % (Table 2). Except for carboxymethylated Fonio starch, which has a slightly greater % moisture content than native Fonio starch, native starches were shown to have higher moisture contents than their modified counterparts. The moisture content of native African rice was the highest (11.00 %), while carboxymethylated African rice had the lowest moisture content (9.48 %). Because starch is typically absorptive, the minor increase in carboxym-

ethylated Fonio's moisture content from 10.12 % to 10.23 % may be the result. However, all experimental starch samples' moisture content ranges were within the normal ranges anticipated at 50 % relative humidity[38,44].

The Heckel relationships of the pure and treated starches of African rice and Acha

Heckel relationships in Table 3 and Figure 1 yielded the following conclusions. The type A Heckel relationship was achieved due to the plot of ln (1/1-D) against applied pressure for pure starches (100 % starch) being linear and nearly parallel. Plastically deformed materials do this[45]. All formulations with experimental starch excipients produced linear plots with correlation coefficients over 0.970.

The slope and intercept of the extrapolated linear plots determine K and A, respectively. P_y, the pressure needed to distort particles, was computed as a reciprocal of K and measured plasticity. Low P_y values suggest higher and faster initiation of plastic deformation, whereas high P_y values indicate the opposite. P_y values were found to be usually lower in formulations comprising pregelatinized starch. Also, untreated starch formulations had lower P_y values than carboxymethylated ones but higher than the pregelatinized ones; this implies that pregelatinized starches stimulated faster commencement of plastic flow than other forms of starches[16], with pregelatinized < carboxymathylated< native for African rice starch and pregelatinized < native < carboxymethylated for Fonio. P_y values in African rice formulations primarily increased as the amount of starch excipients rose; however, there were differences in the values obtained for Fonio formulations. Table 3 demonstrates that the plasticity of the formulations appears to decrease as the amount of starch in the preparations increases. The order of P_y values by the source was mainly Fonio > African rice. Low P_y values suggest higher and faster initiation of plastic deformation, whereas high P_y values indicate the opposite.

The constant A is related to the particle rearrangement and filling of the die prior to the deformation and bonding of the particle. D_o is the powder bed's relative density when no pressure is exerted. It describes the early rearrangement stage of densification and is calculated from the relationship between loose bulk density and particle or true density. The entire degree of densification accomplished at zero and low pressures following rearrangement processes before any significant amount of inter particulate bonding is the relative density D_A of the material during densification at which a cohesive unbroken tablet has just been generated. The phase of densification is the powder's relative density

under low-pressure D_B, which occurs after using low pressures because of particle rearrangement or fragmentation before significant particle deformation occurs[16]. Tablet formulations containing pregelatinized starch had the highest D_A and D_o values[20] and the lowest D_B values.

In contrast, carboxymethylated starch formulations had intermediate D_o and D_B values and the lowest D_A values. Tablets containing natural starch exhibited moderate D_A values, the highest D_B values, and the lowest D_o values. The D_o values for the various tablet preparations declined as the amount of starch increased.

The values for D_o, D_A, and D_B for two botanical sources decrease with increasing the amount of starch in the formulations with minor variances. Greater values of these factors indicate a higher level of early packing in the die, a higher overall densification, and higher particle rearrangement during the initial stages of compression, respectively. The perceived drop in D_B values showed that powder particle rearrangement in the initial stages of compression declined at these starch amounts for formulations with increasing starch contents.

The decreasing D_o values as the quantity of starch adjuvants in the preparations grew suggested that as the starch content increased, the initial packing of the powder particles in the preparations because of die-filling decreased. D_o values rose in formulations, including modified starches, with the highest levels in formulations utilizing pregelatinized starch. Pregelatinized and carboxymethylated starches with larger powder particles were expected to have greater D_o values in their formulations. Previous researches have described this pattern[16,46]. As a result, pregelatinization of these two starches generated the optimum early packing of the formulation particles in the die, followed by carboxymethylated particles.

In native and carboxymethylated Fonio and native African rice starch and Ibuprofen tablet formulations, D_B values were higher than D_o values, representing the particle rearrangement stage at the preliminary step of compression; this might be ascribed to powder particle fragmentation caused by the use of low pressures, resulting in the stuffing of inter particulate void spaces that were primarily in existence at zero pressure; this promotes compaction[16, 47].

Table 3. Parameters calculated from Density measurements and Heckel plots for drug-native and modified starch blends

Starch Source	Conc. (%w/w)	Native				Pregelatinized				Carboxymethylated			
		P_y (MNm^{-2})	D_0	D_A	D_B	P_y	D_0	D_A	D_B	P_y (MNm^{-2})	D_0	D_A	D_B
Fonio starch (Digitaria exilis)	10	357.14	0.326	0.904	0.578	70.42	0.618	0.906	0.288	833.33	0.361	0.878	0.517
	25	588.24	0.309	0.850	0.541	555.56	0.587	0.913	0.326	126.58	0.340	0.706	0.366
	50	555.56	0.284	0.850	0.566	476.19	0.542	0.887	0.345	714.29	0.310	0.734	0.424
	75	416.67	0.262	0.733	0.471	263.16	0.503	0.773	0.270	588.24	0.285	0.763	0.478
	100	500.00	0.244	0.713	0.469	434.78	0.470	0.766	0.296	666.67	0.264	0.780	0.516
African rice (Oryza glaberrima)	10	294.12	0.387	0.814	0.427	204.08	0.648	0.951	0.303	166.67	0.562	0.870	0.308
	25	344.83	0.362	0.868	0.506	222.22	0.607	0.865	0.258	200.00	0.532	0.840	0.308
	50	454.55	0.328	0.707	0.379	322.58	0.549	0.830	0.281	666.67	0.489	0.803	0.314
	75	588.24	0.300	0.732	0.432	238.10	0.502	0.869	0.367	285.71	0.452	0.674	0.222
	100	625.00	0.276	0.642	0.366	250.00	0.462	0.720	0.258	312.50	0.421	0.760	0.339

P_y, Mean yield pressure/ mean pressure; D_o, Relative density at zero pressure; D_A, Overall degree of densification and rearrangement of powder particles or total pre-compression density at zero and low pressures; D_B, Relative density at low pressure.

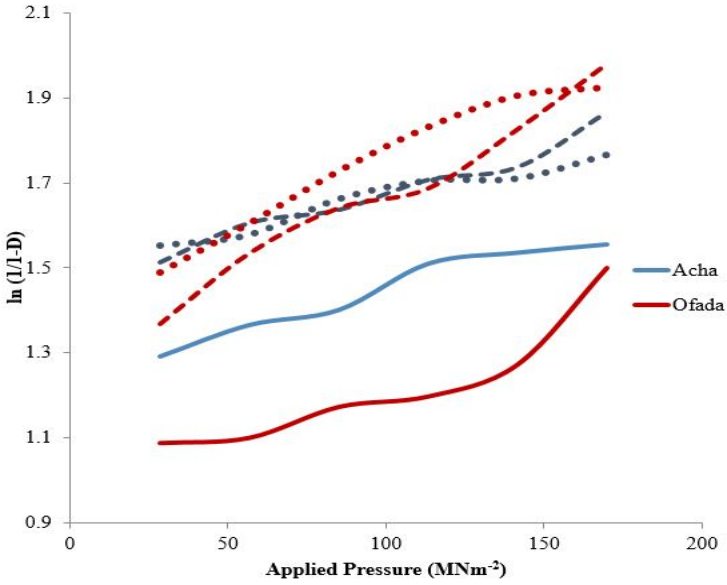

Figure 1. Overlays of Heckel plots for tablet preparations comprising Native (-), Pregelatinized (- - - - -), and Carbokxymethylated (. . . . .) starches of African rice (Ofada) and Fonio (Acha): 100%

Kawakita relationships for the untreated and treated starches of African rice and Fonio

Since no single expression has been proven to be perfect for describing powder compatibility, the Kawakita expression is frequently employed in examining the compressibility of pharmaceutical powders. Figure 2 showed linear relationships for all formulations and applied pressures, with a correlation value greater than 0.999. Thus, the densification mechanisms of the formulation of Ibuprofen tablets can be explained by equation[23].

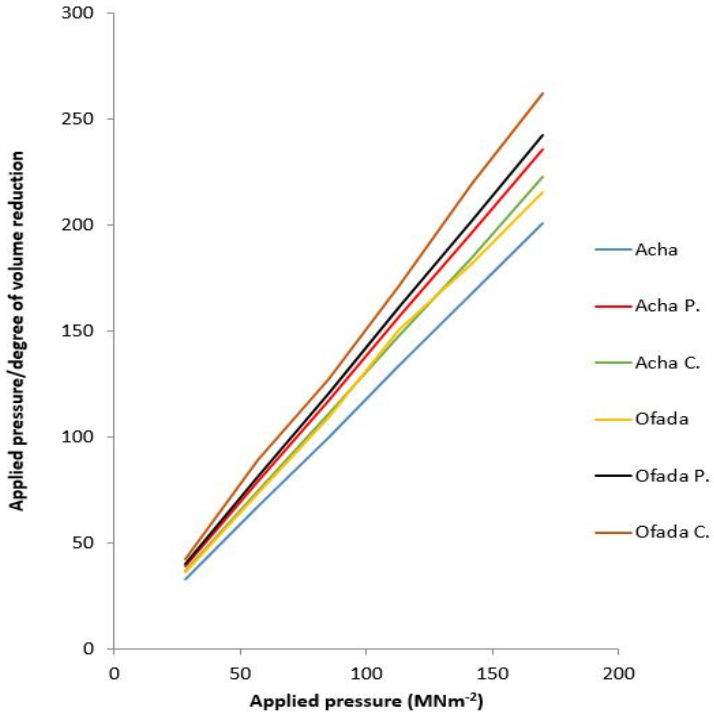

Figure 2. Overlay of Kawakita plots for Ibuprofen tablet preparations containing pure and treated starches (Pregelatinized and Carboxymethylated) of African rice (Ofada) and Fonio (Acha) at 10% w/w

The Heckel equation parameter P_y differs from the Kawakita parameter P_k in that the latter (Heckel) seems to correspond to the overall amount of plastic deformation happening in the course of compression, whilst the previous (Kawakita) is related to the commencement of plastic deformation in the course of compression[47]. Since P_k measures the inverse plastic deformation during compression, a reduced P_k value indicates enhanced or greater overall plastic deformation [47]. In the untreated and treated starch forms and different quantities of starch in the preparations, the level of P_k values by botanical starch origin varied.

The discrepancies in P_k values reported between starch formulations and Ibuprofen tablet preparations can be related to variations in characteristics throughout the preparation process, as Ibuprofen formulations, unlike starch, are several-component systems. In a one-component system, specifically native starch, deformation capacity is free from other components; however, plastic deformation starts whenever any component's yield point is surpassed in a several-component system, like Ibuprofen tablet formulations. The defor-

mation of any component after its yield value in the latter system may activate the deformation of other components in the system. Since the type of the speed and amount of plastic deformation are more complex for several components than for a single component, it may be hard to forecast the deformation parameter of multiple component systems and identify its characteristics from those of its single components[48]. However, the presence of Ibuprofen in the formulation is responsible for the changes observed in the binary formulation established in this work.

The pure form of the starch increased the P_k values. Pregelatinization reduced overall plastic deformation in the formulation of two botanical sources. Also, pregelatinized starches had the highest P_k values (Table 4). At 10 % starch concentration, pregelatinized Fonio and African rice starch showed extremely high P_k values. The carboxymethylated P_k value was also high at 10 % starch content of Fonio starch. P_k values for pure starches were in the order African rice > Fonio by botanical origin at 10 % starch concentration. The P_k values for pregelatinized and carboxymethylated starch tablets were Fonio > African rice in that order at 10 % starch concentration. A higher P_k number indicated lesser overall plasticity, whereas a lower value indicated increased total plasticity.

D_i (packed initial relative density) values varied with the rise in starch quantity in the Ibuprofen formulations, including the different starches, except native and pregelatinized Fonio starch, where D_i values increased with increasing starch concentration. D_i levels were often more significant in treated than in pure starch preparations. The formulations comprising pregelatinized starch had lower values than those including carboxymethylated starches. Thus, carboxymethylation and pregelatinization increased initial particle packing in Ibuprofen preparation. Furthermore, modification of starches at greater concentrations of starch resulted in higher packed initial relative density values of the Ibuprofen preparations compared to lower packed initial relative density values at smaller concentrations of starch content in the preparations.

D_i and D_o (loose initial relative density) values from the Kawakita and Heckel parameters (Tables 3 and 4) showed no clear trend or pattern in identifying the higher value. Although D_i had the most significant and lowest numbers, D_o's values were in the middle. D_i values represent the packed primary relative density of formulations when modest pressure or tapping is applied, whereas D_o values represent the loose initial relative density caused only by die filling. In the corresponding formulations, the Heckel parameter D_B, which pertains to densification at low pressures, had both greater and lower values than D_i. Particle size, morphology, and packing geometry of powder affect the two parameters.

Table 4. Features calculated from Kawakita plots for the drug-native and modified starch blends

Starch Source	Starch Conc. (%w/w)	Native		Pregelatinized		Carboxymethylated	
		P_k (MNm^{-2})	D_i	P_k (MNm^{-2})	D_i	P_k (MNm^{-2})	D_i
Fonio *(Digitaria exilis)*	10	3.578	0.186	29.281	0.388	15.594	0.308
	25	7.016	0.198	2.955	0.407	0.505	0.354
	50	4.923	0.199	1.455	0453	6.290	0.374
	75	2.846	0.227	0.793	0.454	0.729	0.339
	100	16.163	0.232	0.500	0.466	6.966	0.345
African rice *(Oryza glaberrima)*	10	0.871	0.274	26.632	0.428	1.385	0.546
	25	7.118	0.269	0.569	0.500	4.014	0.544
	50	0.799	0.306	1.299	0.508	2.508	0.632
	75	11.899	0.323	0.939	0.475	0.405	0.809
	100	1.002	0.347	0.668	0.525	0.441	0.679

P_k, Pressure necessary to lower the powder bed by 50%; D_i, Packed initial relative density.

Gurnham relationships of the untreated and treated starches of African rice and Acha

The Gurnham equation is another way to determine the compressibility of bulk powders. A rise in pressure causes a proportionate elevation in the apparent density of a substance[16,49]. The apparent density D and the natural logarithm of applied pressure, ln P, can thus have a linear relationship. Porosity ε is commonly used to express volume reduction. Then, porosity and ln P are linearly related. The slope and intercept are represented by the inferred linear plot's constants c and d, respectively. The slope constant c is a metric of excipient compressibility, describing the influence of pressure variation on compact porosity.

Table 5. Features calculated from Gurnham plots for drug-native and modified starch blends

Starch Source	Starch Conc. (%w/w)	Native		Pregelatinized		Carboxymethylated	
		c	d	c	d	c	d
Fonio *(Digitaria exilis)*	10	3.77	40.48	2.59	14.58	0.94	15.00
	25	3.81	38.10	1.02	11.88	7.74	48.85
	50	1.91	23.32	3.81	27.28	2.41	33.87
	75	1.54	19.53	4.49	35.72	2.71	32.04
	100	3.68	22.43	3.27	33.34	2.28	29.17
African rice *(Oryza glaberrima)*	10	3.13	27.33	1.34	9.06	3.43	22.75
	25	2.13	19.48	3.95	26.55	4.12	28.32
	50	4.40	43.26	3.07	26.22	1.73	24.74
	75	3.40	37.90	2.75	21.13	6.58	52.54
	100	3.84	47.57	6.09	46.17	4.74	38.69

c, Slope; d, Intercept.

Table 5 and Figure 3 demonstrate Gurnham correlations for formulations with 75 % starch excipients. There was a decrease in porosity with the increased applied pressure and starch concentration in the starch-Ibuprofen formulation. As pressure increases due to the powder's densification, pores close and porosity decreases. This result is corroborated by previous research[50]. Porosity ε plots vs ln P revealed a linear association with negative correlation coefficients $r > 0.920$, indicating a reverse link amid porosity and applied pressure. More significant slope (c) values were frequently reported for African rice starch formulations than for Fonio starch preparations, signifying that African rice starch formulations had more significant densification than acha starch. The slope values of the two starch sources' untreated, pregelatinized, and carboxymethylated preparations differed significantly ($p < 0.05$).

The intercept (d) was most significant in carboxymethylated starch preparations, smallest in pregelatinized, and intermediate in native. It has been proposed that increased compressibility properties correspond to the influence of d on material compressibility[20].

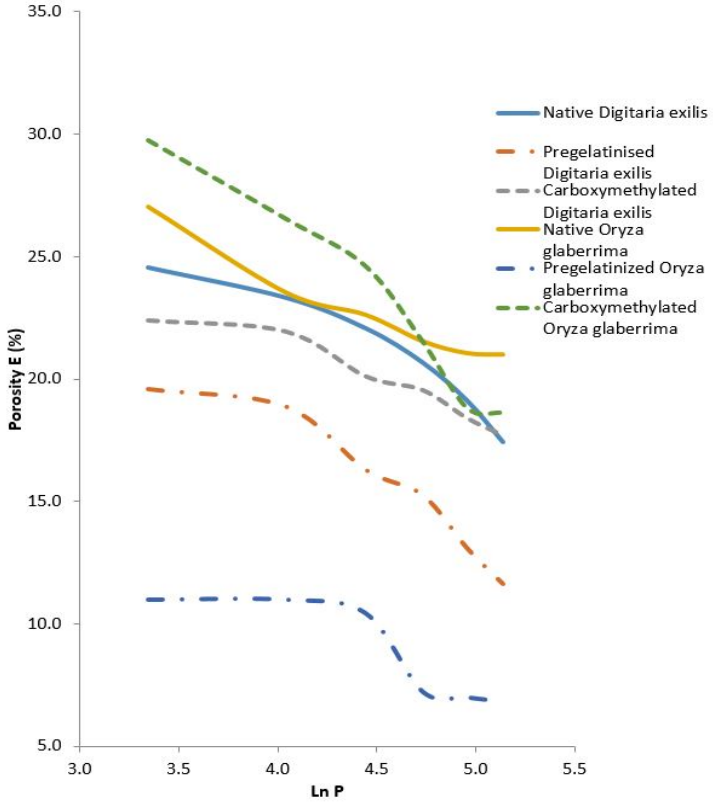

Figure 3. Overlay of Gurnham plots for Ibuprofen preparations comprising Native (-), Pregelatinized (- -) and Carboxymethylated (. . .) starches of Ofada rice and Acha: 75 % w/w

In conclusion, Pregelatinized and carboxymethylated African rice and Fonio starches were successfully synthesized from their native starch forms. Pregelatinization induced faster commencement of plastic deformations but lowered the overall quantity of plastic deformations in formulations. Modified starch forms, particularly pregelatinized ones, would make effective excipients because they increased tablet densification. The amount of pregelatinized starch used in tablet production is less than that of regular starch. Ibuprofen tablets with African rice starch had stronger densification than those utilizing Fonio starch.

STATEMENT OF ETHICS

This study did not include any human or animal subjects.

CONFLICT OF INTEREST STATEMENT

Not Applicable

AUTHOR CONTRIBUTIONS

OAO (Omobolanle Ayoyinka Omoteso) conducted experiments, interpreted the results and wrote the draft of the manuscript and formatted the manuscript to Journal specifications. MAO (Michael Ayodele Odeniyi) designed the research concept, provided some of the materials for the experiments, supervised the conduct of all experiments and reviewed the manuscript. All authors read and approved the final manuscript.

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REFERENCES

1. Guru PR, Kar RK, Nayak AK, Mohapatra S. A comprehensive review on pharmaceutical uses of plant-derived biopolysaccharides. Int J Biol Macromol, 2023;233:123454. Doi: 10.1016/j.ijbiomac.2023.123454

2. Charoenthai N, Sanga-ngam T, Kasemwong K, Sungthongjeen S, Puttipipatkhachorn S. Characterization of hydroxypropyl tapioca starch and its pregelatinized starch as tablet disintegrants. Starch, 2022;74(5-6):2100263. Doi: 10.1002/star.202100263

3. Odeniyi MA, Ayorinde JO. Effects of modification and incorporation techniques on disintegrant properties of wheat (triticum aestivum) starch in metronidazole tablet formulations. Polim Med, 2014;44(3):147-155.

4. Ali I, Ahmad M, Ridha S, Iferobia CC, Lashari N. Dual modification approach for tapioca starch using gamma irradiation and carboxymethylation. Hyb Adv, 2023;3:100071. Doi: 10.21203/rs.3.rs-2616192/v1

5. Podczeck F, Sharma M. The influence of particle size and shape of components of binary powder mixtures on the maximum volume reduction due to packing. Int J pharm, 1996;137(1):41-47. Doi: 10.1016/0378-5173(95)04420-5

6. Westman AR, Hugill HR. The packing of particles. J Am Ceram Soc, 1930;13(10):767-779. Doi: 10.1111/j.1151-2916.1930.tb16222.x

7. Newton JM, Bader F. The prediction of the bulk densities of powder mixtures and its relationship to the filling of hard gelatin capsules. J Pharm Pharmacol, 1981;33(1):621-626. Doi: 10.1111/j.2042-7158.1981.tb13887.x

8. Staple WJ. The influence of size distribution on the bulk density of uniformly packed glass particles. Soil Sci Soc Am J, 1975;39(3):404-408. Doi: 10.2136/sssaj1975.03615995003900030017x

9. Sinka IC, Cunningham JC, Zavaliangos A. Analysis of tablet compaction. II. finite element analysis of density distributions in convex tablets. J Pharm Sci, 2004;93(8):2040-2053. Doi: 10.1002/jps.20111

10. Denny PJ. Compaction Equations: A comparison of the Heckel and Kawakita equations. Powder Technol, 2002;127(2):162-172. Doi: 10.1016/S0032-5910(02)00111–0

11. Svačinová P, Macho O, Jarolímová Ž, Kuentz M, Gabrišová Ľ, Šklubalová Z. Evaluation of gravitational consolidation of binary powder mixtures by modified Heckel Equation. Powder Technol, 2022;408:117729. Doi: 10.1016/j.powtec.2022.117729

12. Rashid I, Haddadin RR, Alkafaween AA, Alkaraki RN, Alkasasbeh RM. Understanding the implication of Kawakita model parameters using in-die force-displacement curve analysis for compacted and non-compacted API powders. AAPS Open, 2022;8(1):1-20. Doi: 10.1186/s41120-022-00053-6

13. Heckel RW. Density-pressure relationship in powder compaction. Trans Met Soc AIME, 1961a;221:671-675.

14. Heckel RW. An analysis of powder compaction phenomena. Trans Met Soc AIME, 1961b;221:1001-1008.

15. Kawakita K, Lüdde KH. Some Considerations on Powder Compression Equations. Powder Technol,1971;4(2):61-68. Doi: 10.1016/0032-5910(71)80001-3

16. Omoteso OA, Adebisi AO, Odeniyi MA. Impact of thermal and chemical modifications on the compression and release properties of bambara nut starches in directly compressed tablet formulations. Starch, 2018;70(11-12):1700308. Doi: 10.1002/star.201700308

17. Tofiq M, Nordström J, Persson AS, Alderborn G. Effect of excipient properties and blend ratio on the compression properties of dry granulated particles prepared from microcrystalline cellulose and lactose. Powder Technol, 2022;399:117207. Doi: 10.1016/j.powtec.2022.117207

18. Patani BO, Akin-Ajani OD, Kumaran A, Odeku OA. Material and compressional properties of Irvingia Gabonensis (O'Rorke) Bail Polymers. J Excip Food Chem, 2022;13(2):64-76.

19. Gurnham CF, Masson HJ. Expression of Liquids from Fibrous Materials. Ind Eng Chem, 1946;38(12):1309-1315. Doi: 10.1021/ie50444a026

20. Lawal MV, Odeniyi MA, Itiola OA. The effect of thermal and chemical modifications of excipients on the compressional properties of paracetamol tablet formulations including maize, cassava and sweet potato starches as filler-binders. J Excip Food Chem, 2016;6(3):65-82.

21. Adedokun MO, Ayorinde JO, Odeniyi MA. Compressional, mechanical and release properties of a novel gum in paracetamol tablet formulations. Curr Issues Pharm Med Sci, 2014;27(3):187-194. Doi: 10.1515/cipms-2015-0013

22. Danbaba N, Anounye JC, Gana AS, Abo ME, Ukwungwu MN. Grain quality characteristics of Ofada Rice (*Oryza sativa* L.): cooking and eating quality. Int Food Res J, 2011;18:629-634.

23. Okunlola A. Flow, Compaction and Tableting Properties of Co-Processed Excipients Using Pregelatinized Ofada Rice Starch And HPMC. J Excip Food Chem, 2018;9(1):4-15.

24. Jideani IA, Takeda Y, Hizukuri S. Structures and physicochemical properties of starches from Acha (*Digitaria exilis*), Iburu (*D. iburua*) and Tamba (*Eleusine coracana*). Cereal Chem, 1996;73:677-685.

25. Nada AH, Al-Saidan SM, Mueller BW. Crystal modification for improving the physical and chemical properties of ibuprofen. Pharml Technol, 2005;29(11):90-101.

26. Gandhi P, Patil S, Aher S, Paradkar A. Ultrasound-assisted preparation of novel ibuprofen-loaded excipient with improved compression and dissolution properties. Drug Dev Ind Pharm, 2016;42(10):1553-1563. Doi: 10.3109/03639045.2016.1151035

27. Matji A, Donato N, Gagol A, Morales E, Carvajal L, Serrano DR, et al. Predicting the critical quality attributes of ibuprofen tablets via modelling of process parameters for roller compaction and tabletting. Int J Phar, 2019;565:209-218. Doi: 10.1016/j.ijpharm.2019.05.011

28. Al-Karawi C, Lukášová I, Sakmann A, Leopold CS. Novel aspects on the direct compaction of ibuprofen with special focus on sticking. Powder Technol, 2017;317:370-380. Doi: 10.1016/j.powtec.2017.05.014

29. Odeniyi MA, Adepoju AO, Jaiyeoba KT. Native and modified digitaria exilis starch nanoparticles as a carrier system for the controlled release of naproxen. Starch, 2019;71(9-10):1900067. Doi: 10.1002/star.201900067

30. Okunlola A, Adewusi SA. Development of theophylline microbeads using pregelatinized breadfruit starch (artocarpus altilis) as a novel co-polymer for controlled release. Adv Pharm Bull, 2019;9(1):93. Doi: 10.15171%2Fapb.2019.012

31.Omoteso OA, Adebisi AO, Kaialy W, Asare-Addo K, Odeniyi MA. Effect of pregelatinization and carboxymethylation on starches from african rice and fonio: influence on release of low melting-point drug. Br J Pharm, 2019;4(2):1-15. Doi: 10.5920/bjpharm.645

32. Ayorinde JO, Itiola OA, Odeniyi MA. Effects of material properties and speed of compression on microbial survival and tensile strength in diclofenac tablet formulations. Arch Pharm Res, 2013;36:273-281. Doi: 10.1007/s12272-013-0027-4

33. Han H, Hou J, Yang N, Zhang Y, Chen H, Zhang Z, et al. Insight on the changes of cassava and potato starch granules during gelatinization. Int J Biol Macromol, 2019;126:37-43. Doi: 10.1016/j.ijbiomac.2018.12.201

34. Nawaz H, Waheed R, Nawaz M, Shahwar D. Physical and chemical modifications in starch structure and reactivity. Chem Properties of Starch, 2020;9:13-35.

35. Lawal MV, Odeniyi MA, Itiola OA. Material and rheological properties of native, acetylated, and pregelatinized forms of corn, cassava, and sweet potato starches. Starch, 2015;67(11-12):964-975. Doi: 10.1002/star.201500044

36. Espin MJ, Ebri JMP, Valverde JM. Tensile strength and compressibility of fine CaCO3 powders. Effect of nanosilica addition. *Chem Eng J,* 2019;378:122166. Doi: 10.1016/j.cej.2019.122166

37. Kudo Y, Yasuda M, Matsusaka S. Effect of particle size distribution on flowability of granulated lactose. Adv Powder Technol, 2020;31(1):121-127. Doi: 10.1016/j.apt.2019.10.004

38. Odeniyi M, Omoteso OA, Adebisi AO. Solid state characterization and rheological properties of native and modified bambara groundnut (*vigna subterranean*) starches. J Excip Food Chem, 2017;8(3):2578.

39. Abioye AO, Kola-Mustapha A. Formulation studies on ibuprofen sodium–cationic dextran conjugate: effect on tableting and dissolution characteristics of ibuprofen. Drug Dev Ind Pharm, 2016;42(1):39-59. Doi: 10.3109/03639045.2015.1024684

40. Kankate D, Panpalia SG, Kumar KJ, Kennedy JF. Studies to predict the effect of pregelatinization on excipient property of maize and potato starch blends. Int J Biol Macromol, 2020;164:1206-1214. Doi: 10.1016/j.ijbiomac.2020.07.170

41. Achor M, Oyeniyi JY, Musa M, Gwarzo MS. Physicochemical properties of cassava starch retrograded in alcohol. *J Appl Pharm Sci*. 2015;*5*(10):126-131. Doi: 10.7324/JAPS.2015.501021

42. Ayorinde JO, Odeniyi MA, Balogun-Agbaje O. Formulation and evaluation of oral dissolving films of amlodipine besylate using blends of starches with hydroxypropyl methyl cellulose. Polim Med, 2016;46(1):45-51. Doi: 10.17219/pim/65098

43. Garcia MA, Garcia CF, Faraco AA. Pharmaceutical and biomedical applications of native and modified starch: a review. *Starch,* 2020;72(7-8):1900270. Doi: 10.1002/star.201900270

44. Juarez-Enriquez E, Olivas GI, Zamudio-Flores PB, Ortega-Rivas E, Perez-Vega S, Sepulveda DR. Effect of water content on the flowability of hygroscopic powders. J Food Eng, 2017;205:12-17. Doi: 10.1016/j.jfoodeng.2017.02.024

45. Balla TB, Joseph NM, Belete A. Optimization of pregelatinized taro boloso-i starch as a direct compression tablet excipient. Biomed Res Int, 2023;2023. Doi: 10.1155/2023/9981311

46. Alebiowu G, Itiola OA. Effects of natural and pregelatinized sorghum, plantain, and corn starch binders on the compressional characteristics of a paracetamol tablet formulation. Pharm Technol, 2001;25(9):26-30.

47. Itiola OA, Pilpel N. Tableting Characteristics of Metronidazole Formulations. Int J Pharm, 1986;31(1-2):99-105. Doi: 10.1016/0378-5173(86)90218–8

48. Lawal MV. Evaluation of Natural, Acetylated and Pregelatinized Starches as Excipients in Directly Compressed Paracetamol Tablets. [Doctoral thesis. University of Ibadan, Nigeria; 2014.]

49. Zhao J, Burt HM, Miller RA. The Gurnham equation in characterizing the compressibility of pharmaceutical materials. Int J Pharm, 2006;317(2):109-113. Doi: 10.1016/j.ijpharm.2006.02.054

50. Akin-Ajani OD, Itiola OA, Odeku OA. Application of the Gurnham equation in characterizing the compressibility of fonio and sweet potato starches and their paracetamol tablet formulations. Niger J Pharm Res, 2018;14(1):25-33.

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The impact of an education program on the appropriate prescription of proton pump inhibitors in hospitalized internal medicine services patients

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ABSTRACT

The objective of this study was to determine the effect of an education program on physicians' knowledge, appropriate prescribing, and administration of proton pump inhibitors (PPIs) in hospitalized internal medicine patients. This quasi-experimental, prospective study was conducted in a university hospital over a period of three months, and included patients who received PPIs before (1 month) and after (1 month) the education program. A questionnaire was used to assess physicians' knowledge before and after the education program. In this study, a total of 215 patients and 32 physicians participated. The rate of appropriate PPI prescribing for indication and administration route increased from 46.4% to 49.5% and from 48.2% to 51.5%, respectively, after the education program ($p>0.05$). The mean number of correct answers on the knowl-

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edge questionnaire increased by 5 out of an average of 10 correct answers before the education to 15 correct answers after the education (p=0.001).

Keywords: proton pump inhibitors, education, internal medicine, pharmacists, medication review

INTRODUCTION

Proton pump inhibitors (PPIs) are medications that are used to treat a variety of gastrointestinal (GI) conditions, including peptic ulcer disease, gastroesophageal reflux disease, GI hemorrhages, dyspepsia, *Helicobacter pylori* eradication, Zollinger-Ellison syndrome (ZES), erosive gastritis, and esophagitis[1,2]. PPIs are sometimes prescribed without a clear indication or for unnecessarily long periods of time, which is referred to as overuse[3].

It is common for patients hospitalized in internal medicine departments to receive acid suppression therapy (AST). It has been observed that proton pump inhibitor (PPI) treatment often begins during a hospital stay and continues after the patient is discharged. However, the inappropriate use of PPIs has been linked to increased medication costs and an increase in undesirable side effects[1,4].

There is a high rate of inappropriate use of PPIs in hospitalized patients, according to several studies. De Rijdt et al. found that 43% of non-critical patients in their study were prescribed long-term AST without a proper indication[1]. Other studies have also found high rates of inappropriate AST use, including 69.2% in a study by Nachnani et al., 44% during hospitalization and 47% after discharge in a study by Sheik-Taha et al., and 69% of patients receiving PPI treatment in a study by Nasser et al. who did not have a valid indication for PPI use[5-7].

Another important issue regarding the inappropriate use of PPIs concerns the route of administration. In 2004, two hospital reports on PPI use indicated that 56% of patients receiving intravenous (IV) PPIs had inappropriate indications, most of which were indications for stress ulcer prophylaxis (SUP). On the other hand, oral PPIs have several advantages over IV formulation, including lower cost, less use of hospital resources, and fewer complications associated with IV administration[7,8]. Currently, IV PPIs are approved by the US Food and Drug Administration (FDA) to treat ZES patients with pathological hypersecretory conditions who cannot tolerate oral medications due to complicated erosive esophagitis. In practice, the use of IV PPIs is much more common. The decision to administer IV PPI depends on several factors such as the patient's

ability to swallow, gastric motility, intestinal transit, and permeability[9]. Given the potential complications and cost, IV PPIs should be considered among inappropriate uses in cases where oral PPIs could be used instead.

The aim of this study is to improve the knowledge of physicians regarding the appropriate use of PPIs in terms of indications and administration route, following an educational program on PPI prescribing in the internal medicine services.

METHODOLOGY

This study, which was a prospective, quasi-experimental design, was conducted at a university hospital between October 1, 2019, and January 15, 2020. The study was divided into two parts: a pre-education phase (October 1-30, 2019) and a post-education phase (December 15, 2019-January 15, 2020). During both periods, the researchers evaluated the treatment of patients hospitalized in the internal medicine services and assessed the appropriateness of PPI use. The study included adult patients who were admitted to the internal medicine services and used PPIs for any reason during their hospitalization. The goal was to include at least 200 patients in the study without any sampling, based on the number of hospitalized patients in the relevant services for the periods in which the study was conducted.

The patient's demographic information such as age, gender, comorbidities, reason for hospitalization, total hospital stay, recent hospitalization history in the last six months, smoking, and alcohol use were collected using a "Patient Profile Registration Form" Information regarding the patient's medication use and results of biochemical laboratory tests were obtained from the patient's medical records.

The appropriateness of the indication and method of administering PPIs was assessed by a clinical pharmacist and a gastroenterology specialist, using evidence-based criteria from previous studies[1,9–13]. To evaluate the appropriateness of the indication and administration route of PPIs, the medical records and drugs of inpatients were examined. The appropriateness of the indication was evaluated by considering accompanying risk factors (such as the use of non-steroidal anti-inflammatory drugs [NSAIDs], systemic corticosteroids, antiplatelet, and anticoagulant drugs) and information from the package insert of PPIs. When evaluating the appropriateness of the administration route, factors like tolerance of oral medications, pathological hypersecretion states, swallowing ability and gastric motility were taken into consideration.

In the second part of the study, an educational program was organized by gas-

troenterology specialists for internal medicine residents, lasting one hour. The program covered general information about PPIs, including mechanism of action, appropriate indications, recommended treatment duration, potential side effects, contraindications, drug interactions, common inappropriate uses, safety concerns, and options for administration route (IV or oral). The education was concluded with case studies and discussion. On the same day, a questionnaire which included a knowledge test was administered to the physicians, both before and after the education.

The questionnaire given to the participating physicians consisted of three sections and a total of 34 questions. The questionnaire, which was created by the authors, was self-structured and had 11 questions in total, with the first section asking about sociodemographic information and the second section containing general questions about PPI use (such as frequency of prescribing PPIs for hospitalized and discharged patients and recognizing adverse effects caused by PPIs). The third section, which evaluated the education, had 23 questions about PPIs, covering topics such as indications, side effects, safety, and drug interactions.

The primary outcome of this study is to enhance the knowledge level of internal medicine residents on the appropriate use of PPIs through education, and as a result, to decrease the inappropriateness of PPIs prescribed to patients in terms of indication and administration.

Statistical Analysis

As the data collected did not follow a normal distribution, non-parametric statistical tests were used for the analysis. The chi-square test was used to analyze categorical variables, while the Mann-Whitney U test was used for nonparametric numerical values. The McNemar-Bowker Test was used to compare the responses to the questionnaire before and after the education. The statistical analysis was performed using IBM SPSS Statistics for Windows, Version 25.0. (Armonk, New York: IBM Corp.). The results were considered statistically significant at a 95% confidence interval, with a $p<0.05$. There was no missing data in the study.

RESULTS and DISCUSSION

During the study period, 302 patients were admitted to the internal medicine services. A total of 215 patients who used PPIs during their hospitalization were included in the study, 112 in the pre-education period and 103 in the post-education period. Eighty-seven patients (28.8%) were not included as they did not use PPIs during the study period. Thirty-two physicians participated in the

education on appropriate PPI prescribing. The patients included in the study were 54% male, and 80,6% of the physicians participating in the education were female. There was no significant difference in sociodemographic characteristics between the patients included in the study before and after the education. Table 1 shows the sociodemographic information of the patients who were evaluated for PPI appropriateness in the first period of the study.

Table 1. Sociodemographic characteristics of patients*

	Before Education (n=112), n (%)	**After Education (n=103), n (%)**
Age, Median (IQR)	64.50 (52.25-74.75)	64.00 (47.00-75.00)
Gender **Male** **Female**	 60 (53.6) 52 (46.4)	 56 (54.4) 47 (45.6)
Smoking	17 (17.5)	23 (24.0)
Alcohol	3 (3.1)	1 (1.0)
History of hospitalization in the last 6 months	29 (61.7)	39 (61.9)

IQR: Interquartile range *There was no statistically significant difference in terms of sociodemographic characteristics.

The appropriateness of PPI uses for patients participating in the study was evaluated in terms of indication and administration route. When compared to before and after the education, the results for PPI appropriateness were not found to be statistically significant ($p>0.05$). When all periods were evaluated, it was determined that PPI was used with an inappropriate indication at a rate of 47.9% and by an inappropriate administration route at a rate of 49.7%. Other comparisons of PPI use before and after education are shown in Table 2.

Table 2. Comparison of PPI use appropriateness in the pre-and post-education periods

	Total	Before Education	After Education	p value
PPI indication compliance at admission, n (%)	103 (47.9)	52 (46.4)	51 (49.5)	0.651
PPI administration route compliance at admission	107 (49.7)	54 (48.2)	53 (51.5)	0.635
Prognosis, n (%) Intensive Care Unit Death Discharge	15 (6.9) 3 (1.4) 197 (91.6)	10 (8.9) 1 (0.9) 101 (90.2)	5 (4.9) 2 (1.9) 96 (93.2)	0.422
Total number of days using PPI, Median (IQR)	10 (7.00-14.00)	10 (7.00-14.00)	10 (6.00-14.00)	0.810
Total length of stay in hospital, Median (IQR)	11(8.00-17.00)	10 (8.00-17.00)	12 (8.00-18.00)	0.297

IQR: Interquartile range, PPI: Proton pump inhibitor

Before the education, the physicians participating in the survey were asked 5 general questions about the use of PPIs. According to their responses, the physicians reported that they frequently initiate PPI treatment (46.9%) in hospitalized patients. When it comes to patients who were already taking the drug at home, physicians stated that they mostly did not evaluate the appropriateness of indication (71%) or the duration of treatment. They also frequently prescribed PPIs at discharge. The distribution of other responses to the general questions about the use of PPIs is shown in Table 3.

The accuracy of the answers on the questionnaire given after the education were compared to the answers given before the education. The median (interquartile range) of correct answers before and after the education were 10 (8-11.25) and 15 (13-17) respectively. The mean number of correct answers increased by 5 (from 10 to 15) after the education, which was found to be statistically significant (p=0.001).

The questionnaires conducted before and after the education on PPIs were grouped into 4 categories. According to the group of questions, there was an average increase of 22.6% in correct answers for indication questions, 19,1% for interaction questions, 17.8% for side-effect questions, and 23.6% for safety questions. The comparison of the questionnaire questions before and after the education according to their categories is shown in Table 4.

In this study, the appropriateness of PPI treatment in terms of indication and administration route for patients admitted to internal medicine services was evaluated and the impact of education on these usage rates was assessed. It was determined that approximately half of the patients had inappropriate PPI use and administration route rates when all periods were evaluated.

Table 3. Distribution of physicians' opinions about PPI use before education

Questions	Answers	The number of participants, n (%)
How often do you start PPI treatment for your hospitalized patients?	Rarely	3 (9.4)
	Sometimes	14 (43.8)
	Often	15 (46.9)
In what percentage of patients do you start PPI treatment?	≤30%	6 (18.8)
	31-59%	9 (28.1)
	60-89%	12 (37.5)
	≥90%	5 (15.6)
How often would you evaluate the appropriateness of indication and treatment duration in a patient with PPI among the medications used at home?	Never	4 (12.5)
	Sometimes	23 (71.9)
	Always	5 (15.6)
How often do you write PPI treatment in the discharge prescription?	≤30%	5 (15.6)
	31-59%	17 (53.1)
	60-89%	7 (21.9)
	≥90%	3 (9.4)
Have you observed any PPI-related adverse reactions in your patients?	Yes	8 (25.0)
	No	24 (75.0)

PPI: Proton pump inhibitor

Pham et al. reported that while the rate of PPI use was 29% at hospital admission, this rate increased to 71% by the time of discharge, and that PPI use was prescribed for appropriate indications in only 9.9% of patients[2]. A 7-month retrospective review of a large teaching hospital in Australia found that only 37% of the inpatient population used PPIs for indications deemed acceptable by the

Australian Pharmaceutical Benefits Program[12]. Other studies also support the findings of this study regarding the inappropriate use of acid suppressants. Walker et al. reported that 67% of PPIs were prescribed for unapproved indications in their study involving hospitalized patients[13]. Other studies have reported rates of inappropriate PPI use as high as 57% and 60%[14,15]. Our findings, along with previous studies, suggest that the prescribing of acid-suppressing drugs is relatively common and often inappropriate. The rates of inappropriate PPI use reported in these studies were found to be higher than in this study. The differences in these rates may be due to variations in the guidelines, consensus, and sources used to evaluate indication appropriateness.

In this study, physicians reported that their PPI prescribing habits at hospital admission were moderate to high. The habit of prescribing PPIs without considering appropriate indication criteria at admission leads to high rates of inappropriate use[16]. A review reported that prophylaxis for gastro-duodenal ulcers in patients without risk factors, prophylaxis for stress ulcers in non-intensive care units, patients receiving steroid therapy alone without risk of gastric ulcers, and excessive treatment of functional dyspepsia are the main causes of inappropriate use of PPIs[15,17]. However, the development of stress ulcers is rare in general hospitalized patients, and guidelines recommend this practice only for intensive care patients[18].

Table 4. Distribution of the number of participants who answered the survey questions correctly before and after the education

	Participation (n)		Correct answer (%)		p value
Category of Questions	**Before**	**After**	**Before**	**After**	
Indication Questions					
Injectable PPI is always preferred for hospitalized patients	23	26	76.7	96.3	NS
There is no need for PPI use in a patient over 65 years of age who is taking low-dose aspirin for cardiovascular prevention	10	13	33.3	48.1	NS
A PPI should be added to the discharge prescription of a patient using steroids long term	6	18	20	66.7	NS
A PPI should be added to the discharge prescription of a patient using NSAIDs	11	19	36.7	70.4	NS
A PPI should be added to the discharge prescription of a patient with a history of ulcer	27	22	90	81.5	NS

Barret's esophagitis, idiopathic Helicobacter pylori/NSAID (-) ulcers, Zollinger-Ellison syndrome, NSAID use with a high risk of GI bleeding are examples of short-term PPI use indications	10	13	33.3	48.1	NS
Helicobacter pylori eradication, stress ulcer prophylaxis, functional dyspepsia, peptic ulcer treatment and maintenance are examples of long-term (PPI indications)	7	13	23.3	48.1	NS
The most effective medication group for the initial treatment of GERD is PPIs	26	27	86.7	100	NS
Patients using steroids (if they are not using NSAIDs), patients with portal hypertensive gastropathy and acute pancreatitis can be given as examples of patients who need to use PPIs for stress ulcer prophylaxis	4	13	13.3	48.1	0.011
PPI can be used for stress prophylaxis in patients outside the intensive care unit	7	15	23.3	55.6	0.0001
Average	13.1	17.9	43.6	66.2	
Interaction Questions					
It is recommended that a patient using omeprazole and levothyroxine take both drugs together on an empty stomach	25	22	83.3	81.5	NS
PPIs may increase the absorption and serum concentration of digoxin	17	22	56.7	81.5	NS
PPIs can increase the toxicity of warfarin and phenytoin	15	23	50	85.2	NS
PPIs can decrease serum concentrations of diazepam, theophylline, methotrexate	0	5	0	18.5	NS
Average	14.2	18	47.5	66.6	
Advers Effect Questions					
Clostridium difficile infection is not associated with PPI use	18	24	60	88.09	0,042
It was observed that the risk of osteoporosis did not increase in patients receiving long-term PPI therapy	22	24	73.3	88.9	NS
One of the conditions associated with the use of PPIs is the increased risk of community-acquired pneumonia	1	6	3.3	22.2	0,021
It is accepted that there is a relationship between PPI use and dementia	5	6	16.7	22.2	NS
In chronic PPI use, magnesium, calcium and B12 levels should be monitored once a year	24	27	80	100	NS
Average	14	17.4	46.6	64.4	
Safety Questions					
PPI use in pregnant women is safe	9	16	31	59.3	NS

Long-term use of PPIs may delay the diagnosis of gastrinoma in the patient	23	21	76.7	77.8	NS
Long-term use of PPI can be stopped suddenly	5	13	16.7	48.1	NS
The use of PPIs is considered safe in patients with cirrhosis	13	21	43.3	77.8	NS
Average	12.5	17.7	41.9	65.7	

GERD: Gastroesophageal reflux disease, GI: Gastrointestinal, NS: Not significant, NSAID: Non-steroidal anti-inflammatory drug, PPI: Proton pump inhibitor

Another issue of concern regarding the inappropriate use of PPIs in this study is the unnecessary use of IV PPIs in patients who are able to take them orally. Approximately half of the patients had such an inappropriate route of administration. IV PPIs are more expensive compared to oral PPIs and have only a few absolute indications. More than half of the hospitalized patients prescribed IV PPIs could have taken oral PPIs instead[19]. Recent studies have shown that IV PPI preparations are associated with gastric hypersecretion and ZES associated with neoplastic conditions, severe non-variceal upper GI bleeding cases that cannot take oral medication, GI bleeding with the risk of recurrent continuous bleeding, and high rates of GI bleeding in intensive care units without access to enteral nutrition or without oral intake. its use in risky patients is considered appropriate[20]. Inappropriate use of IV PPI has been observed in various studies, especially in cases with no high suspicion of upper GI bleeding[20-22]. Lai et al. reported inappropriate IV PPI use at 74.5%, and Alsultan et al. reported as high as 71.7%[20,23]. Alsultan et al. also noted differences between consultants, specialists, and practitioners in the inappropriate prescribing of IV PPI[20]. These rates are considerably higher than the rates found in our study.

In this study, the impact of education on PPI use was evaluated. The education was evaluated by a knowledge test containing 23 questions about PPIs, including indications, side effects, drug safety, and drug interactions. The physicians' knowledge level was found to have increased by an average of 5 correct answers after the education, with the largest increases observed in questions about PPI safety and indications. However, this statistically significant increase in the level of knowledge did not provide a positive change in the PPI prescribing habits of physicians in the post-training period. Previous studies have also attempted to reduce inappropriate PPI use through different methods. Odenthal et al. implemented a program led by a clinical pharmacist, which included patient education and follow-up, in a family medicine clinic to reduce inappropriate PPI use. The clinical pharmacist evaluated PPI-using patients through visits to determine whether the PPIs used were candidates for discontinuation. Of the

patients followed up on, 86% successfully discontinued the use of PPIs. This suggests that a program led by a clinical pharmacist, which includes detailed discontinuation instructions, patient education, and follow-up, can be effective in avoiding the prescribing of long-term PPI therapy[24]. In a study conducted at a university hospital in France, the prescribing of PPIs was analyzed over a period of three years, during which 132.890 prescriptions were evaluated. Out of these, 701 (4.6%) were identified as problems with PPIs. The most commonly reported issues were the lack of proper indications (24.4%) and inappropriate routes of administration (19.8%). To address these issues, recommendations were made to discontinue the drug or adjust the dosage in 40.5% of cases. The primary intervention was to discontinue the use of PPIs due to their inappropriate use. Through these pharmaceutical interventions during prescription analysis, the use of PPIs was optimized. The study highlights the importance of communication strategies to improve the education and practice of healthcare professionals, especially through the actions of pharmacists[25].

In this study, it was found that a one-day training program alone did not significantly decrease the rate of PPI use for inappropriate indications or administration. This may be due to the passive nature of the intervention and the need for more comprehensive and long-term approaches, such as implementing a national guideline, incorporating appropriate instructions for use into electronic systems, and providing continuing education for physicians and medical personnel, as previous studies have shown to be effective in reducing inappropriate PPI use[26-29]. It is important to note that continuing education is crucial in ensuring that physicians and medical personnel adopt generally accepted principles and use PPIs in a balanced manner[10].

One of the strengths of this study is that it demonstrated the effectiveness of a one-day education program in significantly increasing the knowledge level of physicians about PPIs. However, the study also has limitations, such as being dependent on the information present in medical records, which may be incomplete, and not reflecting the long-term impact of education. Additionally, the study was conducted in a single center academic tertiary hospital with a relatively small sample size, making it difficult to generalize the results to other hospitals and internal medicine services.

In conclusion, our study highlights the need for improvement in the appropriate prescribing and administration of PPIs among internal medicine residents. The results showed that a one-day education program can increase the knowledge level of physicians about PPIs, but this increase was not reflected in their prescribing and administration habits. To address this issue, hospitals should

implement guidelines on the use of PPIs, provide regular education to physicians by experts, and monitor the long-term effects of these interventions. Additionally, implementing controlled policies such as formulary restriction, restricting IV PPI administration to specific indications, and including drug discontinuation orders for certain indications may also help improve the appropriate use of PPIs.

STATEMENTS OF ETHICS

Approval from the ethics committee was obtained for this study on July 26, 2019, with the approval number 09.2019.686 from Marmara University Faculty of Medicine Clinical Research Ethics Committee.

CONFLICT OF INTEREST STATEMENT

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, shareholding, and similar situations in any firm.

AUTHOR CONTRIBUTIONS

Design: YEA, CE; Acquisition of data: YEA, CE, ÇK, AA, TKY; Analysis of data: YEA, CE, MS, BO, OCÖ; Drafting of the manuscript: YEA, MS; Critical revision of the manuscript: YEA, MS, OCÖ; Statistical analysis: YEA, MS; Technical or financial support; YEA, CE, ÇK, AA, TKY; Supervision: YEA, CE, ÇK, AA, TKY; Other (specify): YEA, CE, ÇK, AA, TKY.

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REFERENCES

1. De Rijdt T, Spriet I, Willems L, Blanckaert M, Hiele M, Wilmer A, et al. Appropriateness of acid suppression therapy. Ann Pharmacother, 2017;51(2):125-134. Doi: 10.1177/1060028016670414

2. Pham CQD., Regal RE, Bostwick TR, Knauf KS. Acid suppressive therapy use on an inpatient internal medicine service. Ann Pharmacother, 2006;40(7-8):1261-1266. Doi: 10.1345/aph.1G703

3. Agee C., Coulter L, Hudson J. Effects of pharmacy resident led education on resident physician prescribing habits associated with stress ulcer prophylaxis in non-intensive care unit patients. Am J Health Syst Pharm, 2015;72(11): 48-52. Doi: 10.2146/sp150013

4. Arthur Grube RR, May DB. Stress ulcer prophylaxis in hospitalized patients not in intensive care units. Am J Health Syst Pharm, 2007;64(13):1396-1400. Doi: 10.2146/ajhp060393

5. Nachnani JS., Bulchandani D, Moormeier J, Foxworth J. Patient and physician predictors of inappropriate acid-suppressive therapy (AST) use in hospitalized patients. J Hosp Med, 2009;4(8):10-14. Doi: 10.1002/jhm.492

6. Sheikh-Taha M, Alaeddine S, Nassif J. Use of acid suppressive therapy in hospitalized noncritically ill patients. World J Gastrointest Pharmacol Ther, 2012;3(6):93-96. Doi: 10.4292/wjgpt.v3.i6.93

7. Nasser SC, Nassif JG, Dimassi HI. Clinical and cost impact of intravenous proton pump inhibitor use in non-ICU patients. World J Gastroentero, 2010;16(8):982-986. Doi: 10.3748/wjg.v16.i8.982

8. Guda NM, Noonan M, Kreiner MJ, Partington S, Vakil N. Use of intravenous proton pump inhibitors in community practice: an explanation for the shortage? Am J Gastroenterol, 2004;99(7):1233-1237. Doi: 10.1111/j.1572-0241.2004.30894.x

9. Pang SH, Graham DY. A clinical guide to using intravenous proton-pump inhibitors in reflux and peptic ulcers. Therap Adv Gastroenterol, 2010;3(1):11-22. Doi: 10.1177/1756283X09352095

10. Agreus L, Borgquist L, Tsoposidis A, Wallenius V, Kostic S, Lundell L, et al. Stor överförskrivning och ökat bruk av protonpumpshämmare [Significant over- and misuse of PPIs]. Lakartidningen, 2021;118:20220. PMID: 34216475

11. Kaplan GG, Bates D, Mcdonald D, Panaccione R, Romagnuolo J. Inappropriate use of intravenous pantoprazole: extent of the problem and successful solutions. Clin Gastroenterol Hepatol, 2005;3(12):1207-1214. Doi: 10.1016/S1542-3565(05)00757-3

12. Naunton M, Peterson GM, Bleasel MD. Overuse of proton pump inhibitors. J Clin Pharm Ther, 2000;25(5):333-340. Doi: 10.1046/j.1365-2710.2000.00312.x

13. Walker NM, Mcdonald J. An evaluation of the use of proton pump inhibitors. Pharm World Sci, 2001;23(3):116-117. Doi: 10.1023/A:1011278030001

14. Ali O, Poole R, Okon M, Maunick S, Troy E. Irrational use of proton pump inhibitors in general practise. Ir J Med Sci, 2019;188(2):541-544. Doi: 10.1007/s11845-018-1891-1

15. Zink DA, Pohlman M, Barnes M, Cannon ME. Long-term use of acid suppression started inappropriately during hospitalization. Aliment Pharmacol Ther, 2005;21(10):1203-1209. Doi: 10.1111/j.1365-2036.2005.02454.x

16. Mat Saad AZ, Collins N, Lobo MM, O'Connor HJ. Proton pump inhibitors: a survey of prescribing in an Irish general hospital. Int. J. Clin. Pract, 2005;59(1):31-34. Doi: 10.1111/j.1742-1241.2004.00298.x

17. Savarino V, Marabotto E, Zentilin P. Furnari M. Bodini G, De Maria C, et al. Proton pump inhibitors: use and misuse in the clinical setting. Expert Rev. Clin. Pharmacol, 2018;11(11):1123-1234. Doi: 10.1080/17512433.2018.1531703

18. Armstrong TA, Coursin DB, Devlin J, Duke JS, Fish D, Gonzalez ER, et al. ASHP therapeutic guidelines on stress ulcer prophylaxis. Am J Health Syst Pharm, 1999;56(4):347-379. Doi: 10.1093/ajhp/56.4.347

19. Chin HL, Wallace P. Embedding guidelines into PMID direct physician order entry: simple methods, powerful results. Proc AMIA Symp, 1999:221-225. PMID: 10566353

20. Alsultan MS, Mayet AY, Malhani AA, Alshaikh MK. Pattern of intravenous proton pump inhibitors use in ICU and non-ICU setting: a prospective observational study. Saudi J Gastroenterol, 2010;16(4):275-279. Doi: 10.4103/1319-3767.70614

21. Perwaiz MK, Posner G, Hammoudeh F, Schmidt F, Neupane N, Enriquez D, et al. Inappropriate use of intravenous PPI for stress ulcer prophylaxis in an inner city community hospital. *J Clin Med Res*, 2010;2(5):215-219. Doi: 10.4021/jocmr447w

22. Craig DG, Thimappa R, Anand V, Sebastian S. Inappropriate utilization of intravenous proton pump inhibitors in hospital practice--a prospective study of the extent of the problem and predictive factors. QJM, 2010;103(5):327-335. Doi: 10.1093/qjmed/hcq019

23. Lai PS, Wong YY, Low YC, Lau HL, Chin KF, Mahadeva S. Unexplained abdominal pain as a driver for inappropriate therapeutics: an audit on the use of intravenous proton pump inhibitors. PeerJ, 2014;2:e451. Doi: 10.7717/peerj.451

24. Odenthal DR, Philbrick AM, Harris IM. Successful deprescribing of unnecessary proton pump inhibitors in a primary care clinic. J Am Pharm Assoc, 2020;60:100-104. Doi: 10.1016/j.japh.2019.08.012

25. Skalli S, Bertin C, Charhon N, Mouchoux C, Huet E, Charpiat B, et al. Interventions des pharmaciens sur les prescriptions d'inhibiteurs de la pompe à protons dans un centre hospitalier universitaire [Pharmacist's interventions on proton pump inhibitor prescriptions in a University Hospital]. J Pharm Belg, 2015;(2):20-29.

26. Wu J, Dickinson S, Elgebaly Z, Blogg S, Heaney A, Soo Y, et al. Impact of NPS MedicineWise general practitioner education programs and Choosing Wisely Australia recommendations on prescribing of proton pump inhibitors in Australia. BMC Fam Pract, 2020;21(1):85. Doi: 10.1186/s12875-020-01158-1

27. Del Giorno R, Ceschi A, Pironi M, Zasa A, Greco A, Gabutti L. Multifaceted intervention to curb in-hospital over-prescription of proton pump inhibitors: a longitudinal multicenter quasi-experimental before-and-after study. Eur J Intern Med, 2018;50:52-59. Doi: 10.1016/j.ejim.2017.11.002

28. Freedberg DE, Salmasian H, Abrams JA, Green RA. Orders for intravenous proton pump inhibitors after implementation of an electronic alert. JAMA Intern Med, 2015;175:452-454. Doi: 10.1001/jamainternmed.2014.6680

29. Bruno C, Pearson SA, Daniels B, Buckley NA, Schaffer A, Zoega H. Passing the acid test? Evaluating the impact of national education initiatives to reduce proton pump inhibitor use in Australia. BMJ Qual Saf, 2020;29(5):365-373. Doi: 10.1136/bmjqs-2019-009897

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Examination of the relationship between skin autofluorescence and lifestyle habits in young adults

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ABSTRACT

This study investigates the relationship between skin autofluorescence (SAF) levels and lifestyle habits in healthy young adults. The study was conducted between January 2022 and December 2022 with 300 healthy young adults. The questionnaire form in which the participants' sociodemographic characteristics, general habits and dietary habits were questioned, Mediterranean Diet Adherence Scale (MEDAS), International Physical Activity Questionnaire-Short Form (IPAQ-SF), Pittsburgh Sleep Quality Index (PSQI) were applied, anthropometric measurements were taken by the researcher, and SAF levels were measured. At the end of the study, the mean SAF levels of the participants were observed to be 1.48±0.21 AU. SAF levels were found to be 1.49±0.21 AU in women and 1.47±0.21 AU in men. It was found that SAF levels did not differ significantly by gender ($p>0.05$). Smokers' SAF levels ($x=1.60$ AU) were statistically significantly higher than non-smokers' SAF levels ($\bar{x}=1.43$ AU) ($p<0.05$). A significant correlation was observed between SAF levels and BMI (body mass index), waist circumference, hip circumference, waist height ratio, adherence to a Mediterranean diet and physical activity levels ($p<0.05$). No significant correlation was observed between participants' sleep quality and their SAF levels. In conclusion, adopting healthy eating and lifestyle habits reduces SAF levels.

Keywords: advanced glycation end products, healthy adults, lifestyle habits, skin autofluorescence

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INTRODUCTION

Advanced glycation end products (AGEs) are a heterogeneous group of compounds produced endogenously from the non-enzymatic glycation of proteins, lipids, and nucleic acids. In addition to the endogenous formation of these products, smoking and consumption of AGE-rich foods also contribute to the AGE pool exogenously[1,2].

Advanced glycation end-products are part of normal metabolism but can become pathogenic if their accumulation in tissues increases. The pathogenic effects of AGEs affect the body through two mechanisms: AGE receptor-mediated and AGE receptor-free. AGEs can directly cross-link with body proteins and change their functions and structures. In addition, they have pro-inflammatory effects through AGE receptors[3,4].

Various methods are currently used to detect AGEs in tissues, biological fluids, and foods. Skin biopsies are the gold standard for determining AGE levels in tissues. Skin autofluorescence (SAF) is recommended as a simple, noninvasive way of assessing AGE tissue accumulation[5,6].

It is predicted that lifestyle habits such as eating habits, physical activity level, smoking, and sleep quality may affect the formation and accumulation of AGEs in the human body. There are very few studies investigating SAF levels in the general population. These studies evaluated limited lifestyle habits.

There has been no study conducted on this subject among the Turkish population. With this study, it was planned to determine the mean SAF levels of young adults in the Turkish population. It was also planned to compare them with other countries' populations. The main purpose of the research is to evaluate the potential relationship between SAF levels and lifestyle habits in healthy young adults.

METHODOLOGY

This study is based on a cross-sectional research model. The study was conducted between January 2022 and December 2022 with 300 healthy young adults aged 18–30 years. Participants signed a consent form, stating that they voluntarily participated in the study. The inclusion criteria of the study were to be between 18-30 years old, volunteer to participate in the study. Exclusion criteria were having scars and/or tattoos on the inner arm and dark skin colour that has potential to interfere with SAF-AGE measurement, pregnancy and lactation, having chronic diseases and language barrier that makes it difficult to communicate.

The questionnaire form applied to the study participants consists of demographic characteristics, general health information, nutritional habits, anthropometric measurements, the Mediterranean Diet Adherance Scale (MEDAS), the International Physical Activity Questionnaire (IPAQ)-Short Form, and the Pittsburgh Sleep Quality Index (PSQI).

The skin autofluorescence levels were measured by the investigators using the "AGE Reader" device (DiagnOptics Technologies, Groningen, The Netherlands) from the dominant forearm, approximately 10-15 cm below the elbow bend, at room temperature and with the participants in a sitting position. The AGE Reader is a non-invasive device that uses the characteristic fluorescent properties of certain AGEs to calculate levels of AGEs deposited in the skin. Since the accumulation of AGEs in the body increases with age, each age has a normal AGE value. The software of the manufacturer considers a value lower than 1.53 AU in this age group as low risk.

The research data were analyzed using SPSS (Statistical Package for Social Sciences) for Windows 22.0 program. Numbers, percentages, means, and standard deviations were used as descriptive statistical methods. In the comparison of quantitative continuous data between two independent groups, the t-test was used. For comparing quantitative continuous data among more than two independent groups, the one-way ANOVA test was employed. Following the ANOVA test, Scheffe's post hoc analysis was conducted to determine the differences. Pearson correlation analysis was applied to the study's continuous variables. The significance level (p) for comparison tests was taken as 0.05. The correlation coefficients (r) are evaluated as follows: 0.00-0.25 very weak; 0.26-0.49 weak; 0.50-0.69 moderate; 0.70-0.89 high; 0.90-1.00 very high.

RESULTS and DISCUSSION

A total of 300 people, 150 women and 150 men, participated in the study. Table 1 shows the demographic characteristics and general habits of the participants.

The average body weight for women was 59.19±12.07 kg, and for men, it was 77.14±10.33 kg. The average height was 163.24±5.59 cm for women and 177.18±5.65 cm for men. The average BMI values were 22.22±4.45 kg/m^2 for females and 24.55±2.92 kg/m^2 for males. Furthermore, the average waist circumference for females was 71.09±8.39 cm, while for males, it was 86.11±8.22 cm. The average hip circumference was 94.76±9.76 cm for females and 102.32±7.38 cm for males. The waist/hip ratios were 0.75±0.05 for females and 0.84±0.05 for males. The waist/height ratios were 0.43±0.05 for females and 0.48±0.04 for males. The anthropometric measurements of men were found to be statistically higher ($p<0.05$).

According to BMI data, 64.7% of women and 54.7% of men were classified as normal. 41.3% of men were overweight. Waist circumference measurements showed that 89.3% of women and 82.7% of men were normal. In regards to waist-hip ratios, 98.7% of women and 82.0% of men were found to be normal. According to waist-to-height ratios, 68.0% of women were healthy, 59.3% of men were healthy, and 39.3% were overweight.

Table 1. The demographic characteristics and general habits of the participants

Demographic characteristics and general habits	Female (n=150)		Male (n=150)		Total (n=300)	
	(X±SD)		(X±SD)		(X±SD)	
Age (years)	22,07±1,91		23,76±3,41		22,92±2,89	
Marital status	n	%	n	%	n	%
Married	5	3,3	11	7,3	16	5,3
Single	145	96,7	139	92,7	284	94,7
Smoking						
Yes	23	15,3	62	41,3	85	28,3
No	127	84,7	88	58,7	215	71,7
Alcohol intake						
Yes	4	2,7	36	24,0	40	13,3
No	146	97,3	114	76,0	260	86,7

n: Number, X±SD: mean±standard deviation

The study examined the frequency of main meals, snack consumption, and skipped main meals among the participants. Results showed that 56.0% of the participants had two main meals, while 34.3% had three main meals. Among females, 59.4% had two main meals, and 25.3% had three main meals. Among males, 52.7% had two main meals, and 43.3% had three main meals. Regarding snacks, 44.0% of the participants had one snack, and 33.0% had two snacks. Furthermore, 64.9% of the participants skipped breakfast, while 30.7% skipped lunch.

According to the scoring of the MEDAS, the average score for females was 6.06 ± 2.38, while for males it was 6.25 ± 2.08 ($p>0.05$). In terms of adherence to the MD, 71.0% of the participants had inadequate adherence, 10.7% had acceptable adherence, and 18.3% had a high level of adherence. It was found that 72.0% of females and 70.0% of males had inadequate adherence to the Mediterranean diet.

The average MET score for females was 1426.46 ± 1371.55 MET-min/week, while for males it was 1922.01 ± 1767.34 MET-min/week ($p<0.05$). In terms of physical activity levels, 28.0% of females were classified as inactive, 64.0% as moderately active, and 8.0% as active. Among males, 29.3% were classified as inactive, 48.7% as moderately active, and 22.0% as active.

The average PSQI score for females was 7.14 ± 3.74, while for males it was 5.52 ± 4.19 ($p<0.05$). It was found that 34.0% of females had good sleep quality, while 66.0% had poor sleep quality. Among males, 55.3% had good sleep quality, while 44.7% had poor sleep quality.

The study found that the average total SAF level of the participants was similar between genders, with no significant difference observed ($p>0.05$). The SAF levels are provided in Table 2.

Table 2. Skin autofluorescence levels according to gender

SAF levels	Female (n=150)	Male (n=150)	Total (n=300)	p value
	(X±SD)	(X±SD)	(X±SD)	
SAF (AU)	1,49±0,21	1,47±0,21	1,48±0,21	0,470

Independent Samples T-Test, n: Number, X±SD: mean±standard deviation

Significant differences were found in SAF levels based on smoking status ($p<0.05$). The SAF levels of smokers ($\bar{x}$=1.60 AU) were higher than those of non-smokers ($\bar{x}$=1.43 AU). However, no significant difference was observed in SAF levels based on alcohol intake ($p>0.05$). The SAF levels based on smoking and alcohol intake status are provided in Table 3.

Table 3. Skin Autofluorescence levels based on smoking and alcohol intake

Variables	Age (AU) (X±SD)	p value
Smoking		
Yes	1,60±0,21	**<0.001**
No	1,43±0,19	
Alcohol intake		
Yes	1,50±0,18	0,550
No	1,47±0,22	

Independent Samples T-Test, X±SD: mean±standard deviation

The skin autofluorescence levels of the participants showed a significant difference according to their BMI values ($p<0.001$). The levels were highest in obese individuals ($\bar{x}=1.76$), above average in overweight individuals ($\bar{x}=1.54$), and lowest in both normal weight ($\bar{x}=1.43$) and underweight individuals ($\bar{x}=1.43$). There was no significant difference found between SAF levels and waist-hip ratio ($p>0.05$). However, there was a significant difference in SAF levels according to waist-to-height ratio ($p<0.05$). The SAF levels of individuals classified as overweight based on waist-to-height ratio ($\bar{x}=1.55$ AU) were higher than those classified as normal ($\bar{x}=1.44$ AU). The SAF levels based on anthropometric measurements are provided in Table 4.

Table 4. The skin autofluorescence levels according to the anthropometric measurements

Variables	AGE (AU)	p value
BMI[a] (kg/m²)	**(X±SD)**	
Underweight	1,43±0,17	**<0.001**
Normal	1,43±0,21	
Overweight	1,54±0,18	
Obese	1,76±0,19	
Waist-to-hip ratio[a]		
Normal	1,50±0,21	**0,063**
Risk	1,47±0,19	
Waist-to-height ratio[a]		
Underweight	1,50±0,18	
Healthy	1,44±0,22	**<0.001**
Overweight	1,55±0,19	

a: One-way analysis of variance (ANOVA) b: Independent samples t-test, X±SD: mean±standard deviation

The participants' SAF levels showed a significant difference according to their Mediterranean diet adherence ($p<0.05$). Participants with insufficient adherence to the Mediterranean diet had higher SAF levels ($x=1.50$) than those with acceptable adherence ($\bar{x}=1.41$). Additionally, participants with insufficient adherence had higher SAF levels ($x=1.50$) than those with strict adherence ($\bar{x}=1.41$). There was no significant difference found between SAF levels and physical activity levels based on IPAQ, as well as between SAF levels and sleep quality based on PSQI. Table 5 shows SAF levels according to different scales.

Table 5. Skin autofluorescence levels of the participants according to different scales

Variables	AGE (AU)	p value
	(X±SD)	
MEDAS[a]		
Insufficient adherence	1,50±0,21	**0,005**
Moderate adherence	1,41±0,18	
Strict adherence	1,41±0,20	
IPAQ[a]		
Inactive	1,52±0,23	**0,077**
Minimally Active	1,47±0,20	
Active	1,44±0,21	
PSQI[a]		
Good sleep	1,47±0,21	0,466
Poor sleep	1,48±0,21	

a: One-way analysis of variance (ANOVA) b: Independent samples t-test, X±SD: mean±standard deviation

When examining the correlation analyses, significant positive correlations were found between BMI, waist circumference, hip circumference, waist-to-height ratio, and SAF levels. Additionally, significant negative correlations were observed between adherence to the MD, physical activity levels, and SAF levels. The correlation relationships between other variables were not statistically significant ($p>0.05$). The correlation analysis between various variables and SAF levels is presented in Table 6.

Table 6. Pearson correlation coefficient (r) between skin autofluorescence and measured variables

Variables	Correlation coeffcient (r)	p value
BMI	**0.32****	**<0.001**
Waist circumference	**0.17****	**<0.001**
Hip circumference	**0.24****	**<0.001**
Waist-to-height ratio	**0.21****	**<0.001**
Waist-to-hip ratio	0,02	0,76
Meal frequency	-0,10	0,07
Snack frequency	0,05	0,41
MEDAS score	**-0.28****	**<0.001**
IPAQ score	**-0.14***	0,02
PSQI score	0,11	0,07

*<0.05; **<0.01; Pearson Correlation Analysis

This study represents a pioneering effort in establishing reference values for SAF in the young and healthy Turkish population. These reference values hold significant potential for application in various translational and clinical studies.

The average SAF values of individuals who participated in our study are lower compared to studies conducted in China, the Netherlands, and Japan[7-9]. It is uncertain whether these differences stem from demographic and/or ethnic variations among the studied groups.

The study found no significant differences in SAF levels between genders. This aligns with previous research that has also reported similar results, indicating that gender may not be a significant factor influencing SAF levels [8-10]. However, some studies have reported slight variations in SAF levels between genders, suggesting that there might be other factors associated with gender, such as skincare practices or hormonal differences, that could indirectly contribute to differences in SAF levels[11-12].

Consistent with previous research, our study found that smokers exhibited higher SAF levels compared to non-smokers, indicating a potential association between smoking status and increased accumulation of AGEs in the skin. The relationship between smoking and increased SAF levels has been consistently demonstrated in numerous studies[10-11]. Smoking contributes to the formation and accumulation of AGEs through several mechanisms. Firstly, smoking introduces exogenous AGEs into the body. Secondly, tobacco extracts and smoke

contain glycotoxins that enhance the formation of AGEs by interacting with proteins. Thirdly, smoking leads to increased oxidative stress, systemic inflammation, and endothelial dysfunction, all of which promote the formation of AGEs[12]. These mechanisms collectively contribute to the harmful effects of cigarette smoking on health.

In terms of anthropometric measures, our study revealed significant positive correlations between BMI, waist circumference, hip circumference, waist-to-height ratio, and SAF levels. These findings are in line with previous research, which consistently supports the link between adiposity measures and increased AGE accumulation reflected by higher SAF levels[11-13]. Adipose tissue, particularly in the abdominal region, is known to promote systemic inflammation, oxidative stress, and metabolic abnormalities, all of which contribute to the formation of AGEs[14].

In our study, we observed significant correlations between adherence to the MD and SAF levels. These findings are supported by other research indicating that the MD is inversely related to circulating AGE levels, particularly in populations with type 2 diabetes and older adults[15-17]. The diet's emphasis on fruits, vegetables, legumes, whole grains, nuts, and olive oil provides protective effects against AGE formation and oxidative stress [18]. Overall, adhering to a MD appears to be beneficial in reducing AGE levels and promoting better health outcomes.

Within the scope of our study, we discovered significant correlations between physical activity levels and SAF levels. Consistent with previous research, higher physical activity levels were associated with lower SAF levels, suggesting that regular physical activity may contribute to reducing AGE accumulation[17,19]. The benefits of physical activity in improving metabolism, insulin sensitivity, antioxidant defenses, and circulation likely play a role in reducing AGE formation and accumulation[20]. Incorporating regular physical activity into a healthy lifestyle can potentially contribute to maintaining health and minimizing AGE burden.

The absence of a significant correlation between PSQI and SAF levels in our study suggests that sleep quality may not directly impact the accumulation of AGEs, as reflected by SAF levels. The literature on the relationship between sleep quality and AGE accumulation reveals inconsistent findings. Some studies reported a positive association between poor sleep quality and high AGE accumulation[21], while others found no significant association[22]. These inconsistencies can be attributed to differences in study populations, methodologies

and/or other factors. Insufficient sleep time or poor sleep quality can increase oxidative stress and inflammation, potentially leading to higher AGE levels[23]. However, more research is needed to examine the relationship between sleep quality and SAF levels.

In our study, no statistically significant relationship was found between dietary habits and SAF levels. To date, limited research has been conducted to specifically investigate the potential correlation between eating frequency, meal skipping, and SAF levels. More studies are needed to explore the potential relationship between main meal frequency, snack consumption, and meal skipping, and SAF levels.

In conclusion, this study provides valuable information regarding SAF levels factors. The findings show that gender may not play a significant role in affecting SAF levels. In contrast, smoking, anthropometric measures, adherence to MD, and physical activity levels are associated with SAF levels. However, further research in larger and more diverse population samples is needed to confirm these findings and explore the underlying mechanisms.

STATEMENT OF ETHICS

The present study underwent a thorough and comprehensive ethical review by the Non-Interventional Clinical Research Ethics Committee of Istanbul Medipol University. The research protocol was granted approval, with the assigned approval number E 10840098 772.02 809, issued on 11/03/2021. This rigorous ethical review process ensured that the study design, procedures, and data collection methods met the required ethical standards and regulations. By obtaining ethical approval, the study prioritizes the protection of participants' rights, confidentiality, and overall well-being throughout the research process.

CONFLICT OF INTEREST STATEMENT

The authors of this paper declare no conflict of interest regarding its publication.

AUTHOR CONTRIBUTIONS

The authors of this paper made equal contributions to the study.

REFERENCES

1. Byun K, Yoo YC, Son M, Lee J, Jeong GB, Park YM, et al. Advanced glycation end-products produced systemically and by macrophages: a common contributor to inflammation and degenerative diseases. Pharmacol Ther, 2017;177(5):44-55. Doi: 10.1016/j.pharmthera.2017.02.030

2. van Dongen KCW, Kappetein L, Miro Estruch I, Belzer C, Beekmann K, Rietjens IMCM. Differences in kinetics and dynamics of endogenous versus exogenous advanced glycation end products (AGEs) and their precursors. Food Chem Toxicol, 2022;164:112987. Doi: 10.1016/j.fct.2022.112987

3. Jud P, Sourij H. Therapeutic options to reduce advanced glycation end products in patients with diabetes mellitus: a review. Diabetes Res Clin Pract, 2019;148(2):54-63. Doi: 10.1016/j.diabres.2018.11.016

4. Cooke J. Dietary Reduction of Advanced Glycation End Products: An Opportunity for Improved Nutrition Care. J Ren Nutr, 2017;27(4):23-26. Doi: 10.1053/j.jrn.2017.04.003

5. Atzeni IM, van de Zande SC, Westra J, Zwerver J, Smit AJ, Mulder DJ. The AGE Reader: a non-invasive method to assess long-term tissue damage. Methods, 2022;203(19):533-541. Doi: 10.1016/j.ymeth.2021.02.016

6. Meerwaldt R, Lutgers HL, Links TP, Graaff R, Baynes JW, Gans ROB, et al. Skin autofluorescence is a strong predictor of cardiac mortality in diabetes. Diabetes Care, 2007;30(1):107-112. Doi: 10.2337/dc06-1391

7. Isami F, West BJ, Nakajima S, Yamagishi SI. Association of advanced glycation end products, evaluated by skin autofluorescence, with lifestyle habits in a general Japanese population. J Int Med Res, 2018;46(3):1043-1051. Doi: 10.1177/0300060517736914.

8. Yue X, Hu H, Koetsier M, Graaff R, Han C. Reference values for the Chinese population of skin autofluorescence as a marker of advanced glycation end products accumulated in tissue. Diabet Med, 2011;28(7):818-823. Doi: 10.1111/j.1464-5491.2010.03217.x

9. Koetsier M, Lutgers HL, De Jonge C, Links TP, Smit AJ, Graaff R. Reference values of skin autofluorescence. Diabetes Technol Ther, 2010;12(5):399-403. Doi: 10.1089/dia.2009.0113

10. Kellow NJ, Coughlan MT, Reid CM. Association between habitual dietary and lifestyle behaviours and skin autofluorescence (SAF), a marker of tissue accumulation of advanced glycation endproducts (AGEs), in healthy adults. Eur J Nutr, 2018;57(6):2209-2216. Doi: 10.1007/s00394-017-1495-y

11. van Waateringe RP, Slagter SN, van der Klauw MM, van Vliet-Ostaptchouk J V., Graaff R, Paterson AD, et al. Lifestyle and clinical determinants of skin autofluorescence in a population-based cohort study. Eur J Clin Invest, 2016;46(5):481-490. Doi: 10.1111/eci.12627

12. Van Waateringe RP, Mook-Kanamori MJ, Slagter SN, Van Der Klauw MM, Van Vliet-Ostaptchouk J V., Graaff R, et al. The association between various smoking behaviors, cotinine biomarkers and skin autofluorescence, a marker for advanced glycation end product accumulation. PLoS One, 2017;12(6):0179330. Doi: 10.1371/journal.pone.0179330

13. Den Engelsen C, Van Den Donk M, Gorter KJ, Salomé PL, Rutten GE. Advanced glycation end products measured by skin autofluorescence in a population with central obesity. Dermatoendocrinol, 2012;4(1):33-38. Doi: 10.4161/derm.17999

14. Yuen MMA. Health Complications of Obesity: 224 Obesity-Associated Comorbidities from a Mechanistic Perspective. Gastroenterol Clin North Am, 2023;52(2):363-380. Doi: 10.1016/j.gtc.2023.03.006

15. Lopez-Moreno J, Quintana-Navarro GM, Camargo A, Jimenez-Lucena R, Delgado-Lista J, Marin C, et al. Dietary fat quantity and quality modifies advanced glycation end products metabolism in patients with metabolic syndrome. Mol Nutr Food Res. 2017;61(8). Doi: 10.1002/mnfr.201601029

16. Grahovac M, Kumric M, Vilovic M, Martinovic D, Kreso A, Ticinovic Kurir T, et al. Adherence to Mediterranean diet and advanced glycation end products in patients with diabetes. World J Diabetes, 2021;12(11):1942-1956. Doi: 10.4239/wjd.v12.i11.1942

17. Sánchez E, Betriu À, Salas-Salvadó J, Pamplona R, Barbé F, Purroy F, et al. Mediterranean diet, physical activity and subcutaneous advanced glycation end-products' accumulation: a cross-sectional analysis in the ILERVAS project. Eur J Nutr, 2020;59(3):1233-1242. Doi: 10.1007/s00394-019-01983-w

18. Finicelli M, Di Salle A, Galderisi U, Peluso G. The mediterranean diet: an update of the clinical trials. Nutrients, 2022;14(14):2956. Doi: 10.3390/nu14142956

19. Drenth H, Zuidema SU, Krijnen WP, Bautmans I, Smit AJ, Van Der Schans C, et al. Advanced glycation end products are associated with physical activity and physical functioning in the older population. J Gerontol A Biol Sci Med Sci, 2018;73(11):1545-1551. Doi: 10.1093/gerona/gly108

20. Pinckard K, Baskin KK, Stanford KI. Effects of Exercise to Improve Cardiovascular Health. Front Cardiovasc Med, 2019;6:69. Doi: 10.3389/fcvm.2019.00069

21. Tsuruta A, Zheng T, Ogura M, Yagi M, Takabe W, Yonei Y. Sleep quality, physical activity, and glycative stress in the elderly: The Yurin Study. Glycative Stress Res, 2019;6(1):39-48. Doi: 10.24659/gsr.6.1_039

22. Konishi S, Hatakeyama S, Imai A, Okita K, Kido K, Ozaki Y, et al. Effect of advanced glycation end products on nocturia or sleep disorders: A longitudinal study. BJUI Compass, 2022;3(2):162-168. Doi: 10.1002/bco2.114

23. Atrooz F, Salim S. Sleep deprivation, oxidative stress and inflammation. Adv Protein Chem Struct Biol, 2020;119(8):309-336. Doi: 10.1016/bs.apcsb.2019.03.001

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Phytochemical profile and antioxidant activity potential of *Lotus sanguineus* (Vural) D.D.Sokoloff

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ABSTRACT

The *in vitro* antioxidant activity and phenol, phenolic acid, flavonoid, and proanthocyanidin content of *Lotus sanguineus* (Vural) D.D.Sokolof extracts were investigated. The aerial parts of *Lotus sanguineus* have high antioxidant activity. While the ethyl acetate extract has the highest antioxidant activity, in contrast the hexane extract shows lowest activity. In addition, phytochemical antioxidant compounds, phenolic substance, phenolic acid, flavonoid and proanthocyanidin contents of the extracts were determined. The highest total phenolic, phenolic acid, flavonoid and proanthocyanidin contents were found in the ethyl acetate extract. The obtained data supported the result that the plant has antioxidant activity.

Keywords: antioxidant activity, *Lotus sanguineus*, Fabaceae

INTRODUCTION

The genus *Lotus* L. (Fabaceae) is represented by 125 species worldwide[1]. *Lotus* species semishrubs or rarely shrubs naturally distributed in Europe, Asia, Af-

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rica, Australia, some islands of the Atlantic and Pacific Oceans and the Socotra archipelago in the Indian Ocean[2,3]. *Lotus sanguineus* (Vural) D. D. Sokoloff (*Syn. Dorycnium sanguineum* Vural) is an endemic species and has a very limited distribution in Karaman, Turkey[4] and is locally called kızıl gernevük[5]. According to IUCN (2019), the status of *L. sanguineus* was evaluated as CR (Critically Endangered)[4].

In traditional medicine, aerial parts of *Dorycnium rectum* have been used to treat burns and wounds[6] and muscular-skeletal, cardiovascular, digestive and skin disorders[7]. *D. pentaphyllum* have been used as antidiarrhoeal[8]. *D. pentaphyllum* and *D. graecum* as antihemoroidal[9,10].

Previous studies indicate that the extracts and compounds of *Dorycnium rectum*[11-13], *D.* pentaphyllum[9,14-17], *D. herbaceum*[18,19] and *D. hirsutum*[20] have various biological activities such as antibacterial, antifungal, antiparasitic, anti-inflammatory, anthelmintic, cytotoxic and antioxidant activities.

Phytochemical components present in the genus *Dorycnium* have been reported such as sulfur-containing compounds, isoflavonoids, flavonoids, hydroquinone glucoside, phenylbutanone glucoside and polyphenolic compounds[10,18-28].

Oxidative stress caused by free radicals contribute to the development and progression of various chronic diseases such as diabetes, atherosclerosis, cancer, liver disease, neurodegenerative disorders and autoimmune diseases. An imbalance between antioxidant systems and the production of oxidants, including ROS, is known as oxidative stress[29]. Exogenous antioxidants such as flavonoids and polyphenols found in fruits, vegetables, and medicinal plants act as scavenging ROS, chelating metals, and regulating enzymatic and non-enzymatic systems[30,31]. Therefore, there has been increasing efforts to identify new antioxidants from natural resources[32].

There are no reports in the literature dealing with the phytochemical profile and bioactivities of *L. sanguineus*. Aim of this study to determine the *in vitro* antioxidant activity of *L. sanguineus* extracts and their phenol, phenolic acid, flavonoid, and proanthocyanidin content.

METHODOLOGY

Plant material

The aerial parts of *Lotus sanguineus* were collected in the vicinity of Karaman-Bucakkışla in July 2019 and identified by Ömer Çeçen. A voucher specimen (KNYA 28307) was deposited in the Herbarium of Selçuk University (KONYA).

Chemicals

All chemicals, enzymes and references used in the experimental protocols were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The quality of all chemicals was of analytical grade.

Extraction

Coarsely powdered aerial parts (50 g) of the plant were sequentially extracted at room temperature with hexane and 80% methanol. The extracts were individually concentrated in a rotary evaporator under reduced pressure to dryness. Hexane and methanol extracts of the aerial parts were 0.1975 g, 0.40% and 6.4958 g, 12.99%, respectively. The methanol extract was redissolved in a mixture of methanol/water (10:90) and then sequentially partitioned with dichloromethane and ethyl acetate; the resulting extracts were separately concentrated in vacuo to dryness and aqueous-methanol extract was lyophilized. Dichloromethane, ethyl acetate and aqueous-methanol extracts of the aerial parts were 0.63 g (1.26%, w/w), 0.1860 g (0.37%, w/w) and 6.4958 g (12.99%, w/w) respectively.

Quantitative assessment of phytochemical profile

Total phenolic content

The total phenolic content of the extract was determined according to the method of Singleton and Rossi[33]. 75 µL of Na_2CO_3 and 100 µL of Folin-Ciocalteu Reagent were added to 20 µL of freshly diluted extract. Then the composite was left to incubate in the dark at room temperature for 30 minutes. The absorbance was measured spectrophotometrically at 690 nm using a 96-well microplate reader. The total phenolic content of the extracts was expressed as mg gallic acid equivalents (GAE) in 1 g dry extract.

Total phenolic acid content

The total phenolic acid content of the extracts was detected spectrophotometrically with respect to the method declared by Mihailović et al. in 2016[34]. 1 mL of each of extract, Arnow reagent, 0.1 M HCl, and 1 M NaOH solutions were mixed. Later, the eventual volume was adjusted to 10 mL with distilled water. The absorbance of samples was measured immediately at 490 nm. The total phenolic acid content of the extracts was stated as caffeic acid equivalents (CAE) in 1 g dry extract.

Total flavonoid content

The total flavonoid content of the extracts was calculated according to the aluminum chloride colorimetric method developed by Woisky and Salatino[35]. 50 µL extracts were mixed with 150 µL of 75% ethanol, 10 µL of 10% aluminum chloride solution, 10 µL of 1M potassium acetate. The mixture was incubated in the dark at room temperature for 30 minutes. The absorbance was read spectrophotometrically at 405 nm. The results were expressed as quercetin equivalents (QE) in 1 g dry extract.

Total proanthocyanidin content

The total proanthocyanidin content of the extracts was explored with regard to the vanillin-HCl method emphasized by Ariffin et al.[36] 2.5 mL of 1% vanillin and 2.5 mL of 9 M HCl were added to 1 mL of the sample in a capped glass tube. The mixture was allowed to incubate in the dark for 20 minutes at 30 °C. The absorbance was read spectrophotometrically at 492 nm. The total proanthocyanidin content of the extracts was expressed as (-)-epicatechin equivalents (ECE) in 1 g dry extract.

Estimation of antioxidant activity based on free radical-scavenging activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity

The scavenging activity of the extracts against DPPH radical was assessed using the method described by Akter et al.[37,38] 25 µL of extracts were separately added to 200 µL 0.1 mM DPPH solution prepared in methanol before use. The mixture was incubated at room temperature in the dark for 50 minutes. The absorbance was read at 540 nm. MeOH was used in the control group and butylated hydroxy toluene (BHT) was used as the reference material.

DMPD (N, N'-dimethyl-p-phenylenediamine) radical-scavenging activity

The scavenging activity of the extracts against DMPD radical was expressed by using the method described by Fogliano et al. in 1999[39]. 15 µL of extracts were separately added to 210 µL of the DMPD solution prepared before use. The mixture was incubated at room temperature in the dark for 50 minutes. The absorbance was measured at 492 nm. The results were given as mg Trolox equivalent (TE) per g material.

Estimation of antioxidant activity based on metal-related activity

Ferric reducing antioxidant power (FRAP)

FRAP assay was performed according to a method described by Benzie and Strain[40]. 10 µL of extracts and 30 µL of distilled water were mixed with a working FRAP reagent in a microplate. The mixture was incubated at 37°C for 30 minutes. Later, the absorbance was recorded at 593 nm using a 96-well microplate reader. BHT was used as the reference substance. The results were expressed as µM $FeSO_4.7H_2O$ per g material.

Cupric reducing antioxidant capacity (CUPRAC)

CUPRAC activity was determined according to the method found by Apak et al. with some modifications[41]. 85 µL of each of 10 mM $CuSO_4$, 7.5 mM neocuproine, and 1 M ammonium acetate buffer (pH 7.0) solutions were mixed in a microplate. Later, 51 µL of distilled water and 43 µL of extracts were added respectively. The mixture was incubated at room temperature for 1 hour. After the incubation period, the absorbance was read at 450 nm. The results were given as mg ascorbic acid equivalent (AAE) per g material.

Determination of total antioxidant capacity by phosphomolybdenum method (TOAC)

The total antioxidant capacities of the samples were measured with regard to the phosphomolybdenum method found by Prieto et al. with small modifications[42]. 300 µL of the reagent solution were mixed with 30 µL of warrantably diluted extracts. The microplate containing the mixture was incubated at 95°C for 90 minutes in a water bath. After the incubation period, the samples were cooled to room temperature, and the absorbance was read at 690 nm using a 96-well microplate reader. The results were given as mg ascorbic acid equivalent (AAE) per g material.

Statistics

The experiments were performed in triplicate. The results were assessed as mean ± standard deviation. Statistical comparisons were made using one-way analysis of variance (ANOVA) followed by Students–Newman– Keuls post hoc test for multiple comparisons. In addition, Pearson correlation coefficients were calculated. The statistically significant difference was detected as $p < 0.05$.

RESULTS and DISCUSSION

This work is the first report concerning the *in vitro* antioxidant activity and phenol, phenolic acid, flavonoid, and proanthocyanidin contents of the aerial

parts of *Lotus sanguineus* (i.e., *Dorycnium sanguineum*). *D. herbaceum* acetone, ethyl acetate, and ethanol extracts were investigated for their *in vitro* antioxidant activity with quantification of phenolic compound contents and the ethanol extract showed highest antioxidant activity and total phenolic content in a previous study[18]. The total polyphenolic and flavonoid content and the antioxidant effect of the hydroalcoholic extract of *D. herbaceum* were also investigated and the total polyphenolic content was found higher than the flavonoid content and the extract showed significant antioxidant activity in earlier studies[19]. The total flavonoid content and the antioxidant effects of the methanol, ethyl acetate and n-butanol extracts of *D. hirsutum* were determined in previous studies. The amounts of flavonoids in the ethyl acetate extract was found considerably higher than in the other extracts and, showed highest antioxidant activity[20].

The phytochemical profiles observed with the extracts of *L. sanguineus* are shown in Table 1. The highest total phenolic, phenolic acid, flavonoid and proanthocyanidin contents were found in the ethyl acetate extract.

Table 1. Total phytochemical profile of the extracts

Extracts	1	2	3	4	5
Total Phenolic Content[A]	320.5 ± 15.08[a]	339.4 ± 4.29[b]	340.5 ± 9.45[c]	422.5 ± 3.79[c]	381.8 ± 8.33[c]
Total Phenolic Acid Content[B]	117.4 ± 2.80[a]	254.4 ± 9.43[b]	238.3 ± 0.79[c]	283.3 ± 9.49[c]	60.6 ± 0.79[c]
Total Flavonoid Content[C]	28.1 ± 5.35[a]	34.5 ± 5.43[a]	58.5 ± 2.71[b]	90.1 ± 5.60[b]	3.2 ± 1.86[b]
Total Proanthocyanidin Content[D]	117.03 ± 2.29[a]	160.1 ± 6.05[b]	131.3 ± 3.15[c]	324.6 ± 2.10[c]	153.7 ± 0.52[d]

1: MeOH extract; 2: Hexane extract; 3: CH_2Cl_2 extract; 4: EtOAc extract; 5: aqueous-methanol extract

[A] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg gallic acid equivalents (GAE) in 1 g dry extract. [B] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg caffeic acid equivalents (CAE) in 1 g dry extract. [C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg quercetin equivalents (QE) in 1 g dry extract. [D] Results were expressed as the mean of triplicates ± stand-

ard deviation (S.D.) and as mg (-)-Epicatechin equivalents (ECE) in 1 g dry extract. [a-d] Values with different letters within a row were significantly different ($p<0.05$)

In vitro antioxidant activity potential observed with the extracts of *L. sanguineus* are shown in Table 2.

Table 2. *In vitro* antioxidant activity potential of the extracts expressed as EC50 values (µg/mL)

Name of the analysis	1	2	3	4	5
DPPH radical-scavenging activity[A]	570.4 ± 3.73[a]	1846.3 ± 36.57[b]	673.6 ± 20.06[c]	550.4 ± 1.41[c]	584.8 ± 13.68[d]
DMPD radical-scavenging activity[B]	113.0 ± 1.56[a]	-	29.5 ± 3.40[c]	119.8 ± 1.63[c]	143.8 ± 2.96[c]
Ferric reducing antioxidant power[C]	1189.3 ± 27.11[a]	298.5 ± 8.33[b]	922.9 ± 61.58[c]	3880.2 ± 84.85[c]	1344.1 ± 23.35[c]
Cupric reducing antioxidant capacity[D]	289.9 ± 1.71[a]	54.3 ± 4.51[b]	235.4 ± 15.40[c]	758.3 ± 51.94[c]	222.6 ± 6.26[c]
Total antioxidant capacity[D]	122.8 ± 3.89[a]	155.6 ± 14.93[b]	117.9 ± 7.50[c]	228.6 ± 5.89[c]	92.1 ± 5.56[c]

1: MeOH extract; 2: Hexane extract; 3: CH_2Cl_2 extract; 4: EtOAc extract; 5: Aqueous-methanol extract

P.S. 1) EC_{50} value of the reference compound "butylated hydroxytoluene (BHT)" in DPPH scavenging activity is found to be 827.41 ± 1.66. 2) FRAP activity of the reference compound "butylated hydroxytoluene (BHT)" is found to be 2556.85 ± 50.24 µM FeSO4 eq. in 1 g dry extract. [A]Results were expressed as the mean of triplicates ± standard deviation (S.D.) and DPPH activity was expressed as EC_{50} in µg/mL equivalents. [B]Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg Trolox equivalents (TE) in 1 g dry extract. [C]Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as µM $FeSO_4$ equivalents in 1 g dry extract. [D]Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg ascorbic acid equivalents (AAE) in 1 g dry extract. [a-d] Values with different letters within a row were significantly different ($p < 0.05$).

The results obtained from this study showed that the aerial parts of *L. sanguineus* have high antioxidant activity. Extracts have been shown to have free radical scavenging activity and the capacity to reduce metal ions involved in

free radical production by *in vitro* antioxidant tests. While the ethyl acetate extract has the highest antioxidant activity, in contrast the hexane extract shows lowest activity. The data obtained for the phenol, phenolic acid, flavonoid, and proanthocyanidin contents of the extracts supported the result that the plant has antioxidant activity.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, Fatma Tosun, Esra Acar Şah, Sümeyye Albayrak and Ömer Çeçen; methodology, Esra Acar Şah and Sümeyye Albayrak; formal analysis and investigation, Esra Acar Şah and Sümeyye Albayrak; resources, Fatma Tosun, Sümeyye Albayrak, Esra Acar Şah and Ömer Çeçen; writing—original draft preparation, Fatma Tosun, Esra Acar Şah and Sümeyye Albayrak; writing—review and editing, Fatma Tosun, Sümeyye Albayrak, Esra Acar Şah and Ömer Çeçen. All authors have read and agreed to the published version of the manuscript.

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REFERENCES

1. POWO (2023). Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet.
http://www.plantsoftheworldonline.org/. Accessed 16 March 2023.

2. Kramina TE, Degtjareva GV, Samigullin TH, Valiejo-Roman CM, Kirkbride Jr JH, Volis S, et al. Phylogeny of *Lotus* (Leguminosae: Loteae): Partial incongruence between nrITS, nrETS and plastid markers and biogeographic implications. Taxon, 2016;65(5):997-1018. Doi: 10.12705/655.4

3. Kramina TE, Lysova MV, Samigullin TH, Schanzer IA, Özbek MU, Sokoloff DD. Phylogenetic placement and phylogeography of large-flowered *Lotus* species (Leguminosae) formerly classified in *Dorycnium*: Evidence of pre-pleistocene differentiation of western and eastern intraspecific groups. Plants, 2021;10(2):260. Doi: 10.3390/plants10020260

4. Subaşı Ü, Şekerciler F, Vural M. Conservation assessment and action on *Lotus sanguineus* (Fabaceae), a critically endangered species endemic to Turkey. Hacquetia, 2020;19(2):307-315. Doi: 10.2478/hacq-2020-0003

5. Asal D, Yaşarkan O. New Turkish scientific names for 171 taxa where duplications have occurred in the "Checklist of the Flora of Turkey (Vascular Plants)." Bağbahçe Bilim Dergisi, 2017;4(2):1-7.

6. Pieroni A, Giusti ME, De Pasquale C, Lenzarini C, Censorii E, Gonzáles-Tejero MR. et al. Circum-Mediterranean cultural heritage and medicinal plant uses in traditional animal healthcare: a field survey in eight selected areas within the RUBIA project. J Ethnobiol Ethnomed, 2006;2(1):16. Doi: 10.1186/1746-4269-2-16

7. González-Tejero MR, Casares-Porcel M, Sánchez-Rojas CP, Ramiro-Gutiérrez JM, Molero-Mesa J, Pieroni A, et al. Medicinal plants in the Mediterranean area: synthesis of the results of the project Rubia. J Ethnopharmacol, 2008;116(2):341-357. Doi: 10.1016/j.jep.2007.11.045

8. Rigat M, Bonet MÀ, Garcia S, Garnatje T, Valles J. Studies on pharmaceutical ethnobotany in the high river Ter valley (Pyrenees, Catalonia, Iberian Peninsula). J Ethnopharmacol, 2007;113(2):267-277. Doi: 10.1016/j.jep.2007.06.004

9. Bremner P, Rivera D, Calzado MA, Obón C, Inocencio C, Beckwith C, et al. Assessing medicinal plants from South-Eastern Spain for potential anti-inflammatory effects targeting nuclear factor-Kappa B and other pro-inflammatory mediators. J Ethnopharmacol, 2009;124(2):295-305. Doi: 10.1016/j.jep.2009.04.035

10. Movsumov IS, Yusifova DY, Suleymanov TA, Garaev EA. Biologically active compounds from Dorycnium graecum growing in Azerbaijan. Chem Nat Compd, 2020;56:953-954. Doi: 10.1007/s10600-020-03198-6

11. Sivakumaran S, Molan AL, Meagher LP, Kolb B, Foo LY, Lane GA, et al. Variation in antimicrobial action of proanthocyanidins from *Dorycnium rectum* against rumen bacteria. Phytochemistry, 2004;65(17):2485-2497. Doi: 10.1016/j.phytochem.2004.08.046

12. Molan AL, Waghorn GC, Min BR, McNabb WC. The effect of condensed tannins from seven herbages on *Trichostrongylus colubriformis* larval migration *in vitro*. Folia Parasitol, 2000;47(1):39-44. Doi: 10.14411/fp.2000.007

13. Waghorn TS, Molan AL, Deighton M, Alexander RA, Leathwick DM, McNabb WC, et al. In vivo anthelmintic activity of *Dorycnium rectum* and grape seed extract against Ostertagia (Teladorsagia) circumcincta and Trichostrongylus colubriformis in sheep. N Z Vet J, 2006;54(1):21-27. Doi: 10.1080/00480169.2006.36599

14. Stefanović O, Radojević I, Vasić S, Čomić L. *Antibacterial activity of naturally occurring compounds from selected plants*. Antimicrobial Agents, Chapter 1, Books on Demand, Hırvatistan, 2012; pp. 1-25.

15. Usta C, Yıldırım AB, Türker AU. Antibacterial and antitumour activities of some plants grown in Turkey. Biotechnol Biotechnol Equip, 2014; 28(2): 306-315. Doi: 10.1080/13102818.2014.909708

16. Türker H, Yıldırım AB. Screening for antibacterial activity of some Turkish plants against fish pathogens: a possible alternative in the treatment of bacterial infections. Biotechnol Biotechnol Equip, 2015;29(2):281-288. Doi: 10.1080/13102818.2015.1006445

17. Koygun G, Arslan E, Zengin G, Orlando G, Ferrante C. Comparison of anticancer activity of *Dorycnium pentaphyllum* extract on MCF-7 and MCF-12A cell line: Correlation with invasion and adhesion. Biomolecules, 2021;11(5):671. Doi: 10.3390/biom11050671

18. Stefanović OD, Tešić JD, Čomić LR. *Melilotus albus* and *Dorycnium herbaceum* extracts as source of phenolic compounds and their antimicrobial, antibiofilm, and antioxidant potentials. J Food Drug Anal, 2015;23(3):417-424. Doi: 10.1016/j.jfda.2015.01.003

19. Nicolaescu O, Varut RM, Patruna S. Determination of the polyphenolic content of *Dorycnium herbaceum* plant extract and testing *in vivo* antioxidant effect, comparative with Bilberry. Rev Chim, 2019;70(2):667-670.

20. Pistelli L, Noccioli C, Martera M, Giamperi L, Bucchini A, Fraternale D, et al. Antioxidant flavonol glycosides from *Dorycnium hirsutum*. Chem Nat Compd, 2006;42(3):281-284. Doi: 10.1007/s10600-006-0099-1

21. Zurabishvili TS, Kemertelidze EP. Flavonoids of *Dorycnium intermedium*. Chem Nat Compd, 1974;10:265. Doi: 10.1007/BF00563640

22. Kazantzoglou G, Magiatis P, Panoutsopoulos G, Skaltsounis AL. Dorycnioside, a new phenylbutanone glucoside from *Dorycnium pentaphyllum* subsp. *herbaceum*. Z Naturforsch C, 2004;59(1-2):23-26. Doi: 10.1515/znc-2004-1-205

23. Hanganu D, Vlase L, Olah N. Phytochemical analysis of isoflavons from some Fabaceae species extracts. Not Bot Horti Agrobot Cluj-Napoca, 2010;38(1):57-60.

24. Varut RM, Rotaru LT. GC-MS, HPLC, TLC of *Dorycnium herbaceum* tincture species and synergistic/antagonist effect testing in combination with antibiotics. Rev Chim, 2017;68(2):228-231.

25. Uysal Ş, Aktümsek A, Picot CM, Sahan A, Mollica A, Zengin G, et al. A comparative in vitro and in silico study of the biological potential and chemical fingerprints of *Dorcycinum pentapyllum* subsp. *haussknechtii* using three extraction procedures. New J Chem, 2017;41(22):13952-13960. Doi: 10.1039/C7NJ03497K

26. Aliyazicioğlu Y, Demir S, Yaman SÖ, Şener SÖ, Demir EA, Aliyazicioğlu R, et al. Phytochemical analysis of *Dorycnium pentaphyllum* and its antiproliferative effect on cervix cancer cells. J Agric Nat, 2019;22(2):365-373. Doi: 10.18016/ksutarimdoga.vi.579938

27. Demir S, Turan İ, Mısır S, Aliyazicioğlu Y. Selective cytotoxic effect of *Dorycnium pentaphyllum* extract on human breast, liver, and lung cancer cells. KSU J Agric Nat, 2019;22(3):473-479. Doi: 10.18016/ksutarimdoga.vi.497868

28. Demir S, Özer Yaman S, Şener SÖ, Ayazoğlu Demir E, Aliyazıcıoğlu R, Özgen U, at al. *Dorycnium pentaphyllum* extract has antiproliferative effect on human cervix and colon cancer cells. Nutr Cancer, 2020;72(3):504-512. Doi: 10.1080/01635581.2019.1636100

29. Davalli P, Mitic T, Caporali A, Lauriola A, D'Arca D. ROS, cell senescence, and novel molecular mechanisms in aging and age-related diseases. Oxid Med Cell Longev, 2016;2016. Doi: 10.1155/2016/3565127

30. Nazir N, Nisar M, Zahoor M, Uddin F, Ullah S, Ullah R, et al. Phytochemical analysis, in vitro anticholinesterase, antioxidant activity and in vivo nootropic effect of *Ferula ammoniacum* (*Dorema ammoniacum*) D. Don. in scopolamine-induced memory impairment in mice. Brain Sci, 2021;11:259. Doi: 10.3390/brainsci11020259

31. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chem, 2006;99(1):191-203. Doi: 10.1016/j.foodchem.2005.07.042

32. Nediani C, Giovannelli L. Oxidative stress and inflammation as targets for novel preventive and therapeutic approches in non communicable diseases. Antioxidants, 2020;9(4):290. Doi: 10.3390/antiox9040290

33. Singleton VL, Rossi JAJ. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acids reagents. Amer J Enol Viticult, 1965;16:144-158. Doi: 10.5344/ajev.1965.16.3.144

34. Mihailović V, Kreft S, Benković ET, Ivanović N, Stanković MS. Chemical profile, antioxidant activity and stability in stimulated gastrointestinal tract model system of three *Verbascum* species. Ind Crops Prod, 2016;89:141-151. Doi: 10.1016/j.indcrop.2016.04.075

35. Woisky RG, Salatino A. Analysis of propolis: some parameters and procedures for chemical quality control. J Apic Res, 1998;37(2):99-105. Doi: 10.1080/00218839.1998.11100961

36. Ariffin F, Heong Chew S, Bhupinder K, Karim AA, Huda N. Antioxidant capacity and phenolic composition of fermented *Centella asiatica* herbal teas. J Sci Food Agric, 2011;91(15):2731-2739. Doi: 10.1002/jsfa.4454

37. Akter MS, Ahmed M, Eun JB. Solvent effects on antioxidant properties of persimmon (*Diospyros kaki* L. cv. Daebong) seeds. Int J Food Sci Techno, 2010;45(11):2258-2264. Doi: 10.1111/j.1365-2621.2010.02400.x

38. Celep E, Aydin A, Yesilada E. A comparative study on the *in vitro* antioxidant potentials of three edible fruits: Cornelian cherry, Japanese persimmon and cherry laurel. Food Chem Toxicol, 2012;50(9):3329-3335. Doi: 10.1016/j.fct.2012.06.010

39. Fogliano V, Verde V, Randazzo G, Ritieni A. Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. J Agric Food Chem, 1999;47(3):1035-1040. Doi: 10.1021/jf980496s

40. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": the FRAP assay. Anal Biochem, 1996;239(1):70-76. Doi: 10.1006/abio.1996.0292

41. Apak R, Güçlü K, Özyürek M, Karademir SE. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. J Agric Food Chem, 2004;52(26):7970-7981. Doi: 10.1021/jf048741x

42. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdinum complex: specific application to the determination of vitamin E. Anal Biochem, 1999;269(2):337-341. Doi: 10.1006/abio.1999.4019

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In vitro anti-inflammatory activities of *Tanacetum parthenium* L. extract and its major metabolite parthenolide

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ABSTRACT

Tanacetum parthenium L. (Feverfew) is daisy-like Asteraceae plant carrying sesquiterpene lactones; used for the treatment of migraine and anti-inflammatory effect. This study aims to evaluate *T. parthenium* extract and major metabolite parthenolide for *in vitro* COX-1/COX-2, LOX inhibitory activity. The extract analyzed by HPTLC. To evaluate COX-1/COX-2 inhibition assays, studied with commercial kits (20µg/mL concentration for extract, 5 µg/mL for parthenolide). The major component of extract characterized as parthenolide. IC_{50} values for COX-1/COX-2 inhibition of extract were 10.45 and 9.81µg/mL, for parthenolide; 4.86 and 1.90µg/mL. SI values of *T. parthenium* extract and parthenolide were 0.93 and 0.39. Extract showed selective COX-2 inhibitory activity. The inhibition value of extract on LOX was 80% and inhibition value of parthenolide was 41.13%. The results suggested that *T. parthenium* extract showed selective potential for COX-2 enzyme inhibition. To the best of our knowledge, the extract was tested with COX-1, COX-2, and LOX enzymes for the first time.

Keywords: COX, LOX, anti-inflammatory, *Tanacetum parthenium*, feverfew

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INTRODUCTION

Tanacetum parthenium L., (Syn: *Matricaria parthenium* L.) also known as 'Feverfew' is an Asteraceae family plant and it has daisy-like flowers. *Tanacetum* sp. grows in a wide range of area, native to the Balkan Peninsula. It easily grows along roadsides, woods and fields in North Africa, Europe, Australia, Turkiye, China and Japan. Feverfew was mentioned in Materia Medica by Dioscorides and used as a traditional remedy for inflammation and fever[1,2].

T. parthenium extracts and preparations were used as a prophylaxis against migraine, also against fever, psoriasis and arthritis ethnobotanically. Feverfew was called as 'medieval aspirin' in 1770's[1]. The plant was used since ancient times for headache, menstrual difficulties and any kind of inflammation. Another usage of feverfew is using the fresh flowering heads as insect repellent[2]. Feverfew tincture was also useful for swellings caused by insect bites. Feverfew also was a popular herb for menstrual cycle and hormones. It was known as "general strenghtener of the wombs". Traditionally, feverfew was used for asthma, pain, stomachache, threathened misscariage, spasm, tinnitus and vertigo[3].

According to previous *in vivo* and *in vitro* studies, aerial parts of feverfew has anti-inflammatory[4], antinociceptive[5], antileishmanial[6-8], antioxidant[9], antiprotozoal[10,11], α-glucosidase inhibitory[12], anti-HSV-1[13], anti-migraine[14] and insecticide[2] activities. These activities are mainly based on the sesquiterpene lactone content of the plant. Some of these compounds are germacrane type sesquiterpene lactone; parthenolide[9], guaianolide (11,13-dehydrocompressanolide)[7] and α-methylenebutyrolactone[15].

Inflammation is a kind of defence mechanism caused by the action of inflammatory agents in the body[16]. Nowadays, anti-inflammatory drugs are widely used in order to relief the symptoms of inflammation. Regular usage of anti-inflammatory drugs can cause gastrointestinal side effects, caused by the cyclooxygenase (COX) enzyme mechanism in the body. Lypoxygenase (LOX) enzyme is another important factor for inflammation cascade. Excessive accumulation of reactive oxygene species can cause release of cytokines and activation of LOX[17].

The aim of this present study was to evaluate *in vitro* anti-inflammatory activity of *T. parthenium* extract obtained by the aerial parts of the plant and parthenolide. Standardization of *T. parthenium* extract was searched according to European Pharmacopoeia by High Performance Thin Layer Chromatography (HPTLC). Anti-inflammatory activity was evaluated by the COX-1, COX-2 and LOX assays, respectively.

METHODOLOGY

Materials

Lypooxygenase (1.13.11.12, type I-B, Soybean), linoleic acid and test materials were purchased from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). COX-1 and COX-2 enzyme assay kits were purchased from Cayman Chemical Company (Ann Arbor, Michigan). All used chemicals were of analytical grade or higher if not otherwise stated.

Plant material and extraction

Dried aerial parts of *T. parthenium* as certified Pharmacopoeial quality were obtained from Caelo. Dried plant material was grounded to fine powder. 100 g of the powdered material were weighed and extraction was carried out with methanol for 1 hour with shaking. At the end of 1 hour, the extract was filtered through Whatman no:1 filter paper and concentrated with the help of a rotary evaporator. The process was repeated 3 times in total.

HPTLC analysis

HPTLC fingerprint analysis was held with an accepted methodology determined by HPTLC Association[18]. HPTLC analyzes were performed with CAMAG HPTLC system. Glass HPTLC plates coated by silica gel was used as the stationary phase, cyclohexane:ethylacetate (50:50, v:v) was used as the mobile phase. The substances were applied to the HPTLC plate using the Linomat 5 applicator system. Samples were applied to the plates as 7-mm bands, 8 mm apart. *T. parthenium* extract was dissolved in methanol as 50mg/mL density, and applied 5µL to the plate. 0,2 mg/mL parthenolide solution was used as an analytical standart. Anisaldehyde derivatization reagent was used and spots belonged to the extract were observed under the sunlight.

Enzyme inhibition assays

LOX enzyme inhibition

The *in vitro* LOX enzyme inhibition capacity was determined by using modified spectrophotometric method described by Baylac and Racine[19].

Lypoxygenase (1.13.11.12, type I-B, Soybean), linoleic acid and test materials were purchased from Sigma (St. Louis, MO, USA). Potassium phospohate buffer solution (1,94 mL; 100 mM; pH9.0), 40 µl test solution and 20 µl lypoxygenase enzyme solution were prepared and incubated for 10 minutes in 25°C environment. 50 µl linoleic acid solution was added to the reaction and measured the absorbance change at 230 nm for 20 minutes. Test materials and

Nordihydroguairatic Acid (NDGA), which was used as a positive control were dissolved in methanol. All kinetic studies were performed using quartz cuvettes. The 50% inhibition (IC_{50}) values of the test substances were calculated.

COX enzyme inhibition

COX enzyme inhibition results were screened by using colorimetric method. For this experiment, commercial COX-1 and COX-2 enzyme kits were used and the experimental protocol was carried out under the conditions determined by the company that supplied the purchased kit[20]. The concentration used for the *T. parthenium* extract was 20μg/mL, for parthenolide was 5 μg/mL.

Statistical analysis

Statistical analysis was assessed by using GraphPad Prism 8 (GraphPad Software, Inc., San Diego, California; Version 8.4.3). One-way analysis of variance (ANOVA) and Dunnett's or Tukey post hoc tests were used for the statistical assesment to multiple comparisons. Significance limit was stated as $P < 0.05$ and all repeated tests were in triplicate.

RESULTS and DISCUSSION

Standardization

HPTLC analysis was used for standardization of the *T. parthenium* extract and its major metabolite, parthenolide. HPTLC chromatograms of the parthenolide and *T. parthenium* extract are given in Fig. 1. As a result, parthenolide was detected between 12 spots in the *T. parthenium* extract at $R_F \approx 0.45$.

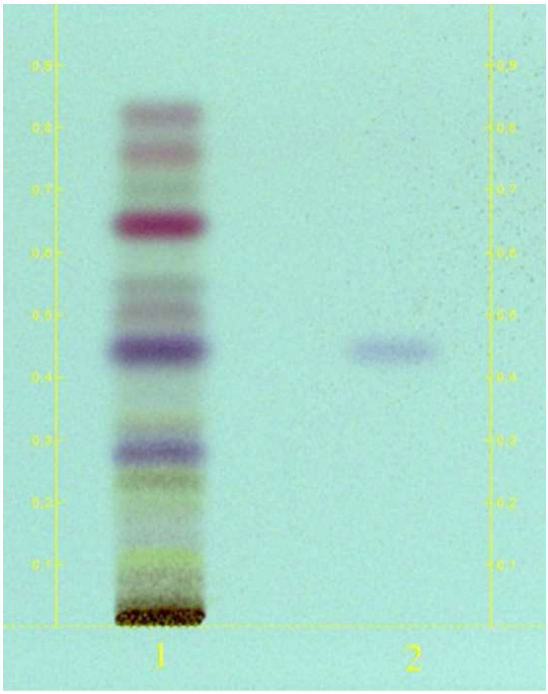

Figure 1. HPTLC chromatogram of *T. parthenium* methanolic extract (1. *T. parthenium* extract, 2. Parthenolide)

Since European Pharmacopoeia states that standardized *T. parthenium* extract should contain parthenolide, the extract was analysed and approved according to parthenolide by HPTLC system. Until now, only the essential oil of *T. parthenium* was examined with HPTLC method[21], most studies preferred to work with TLC and HPLC method[22]. This is the first study using HPTLC method for *T. parthenium* extract. In a previous study, *T. parthenium* collected from Florina was extracted by acetonitrile and examined by HPLC. Retention time was 35 min after the final purification of parthenolide [22]. Also there were studies obtaining parthenolide but using another plant instead of *T. parthenium*. *Tarchonanthus camphoratus* leaves was used and HPTLC analysis was the choice of method for parthenolide. By using BDD-run (behavior-driven development), $R_F \approx 0.15$ was detected for parthenolide[23].

COX-1, COX-2 and LOX enzyme inhibition

Anti-inflammatory activity of *T. parthenium* extract and parthenolide were examined by using the *in vitro* COX and LOX enzyme activities. COX-1 and COX-2 enzyme kits were used for measuring inhibition capacity of *T. parthenium* extract and parthenolide. IC_{50} (μg/mL) inhibition values and selective indexes are given in Table 1.

Indomethacine was used as positive control as COX-2 selective anti-inflammatory agent. The IC_{50} values of *T. parthenium* extract on COX-1 and COX-2

was 10.45 and 9.81, the IC_{50} values of parthenolide on COX-1 and COX-2 was 4.86 and 1.90, respectively. As given in Table 1, despite the inhibition power of parthenolide on COX-1 more than COX-2, *T. parthenium* extract was more powerful effect of inhibiting COX-2. The SI value of parthenolide was 0.39, hence the SI value of *T. parthenium* was 0.93. *T. parthenium* extract showed selective inhibitor effect on COX-2 compared to parthenolide.

In a previous study, parthenolide-depleted extract of feverfew inhibited the activity of pro-inflammatory enzymes 5-lipoxygenase, phosphodiesterase-3 and 4, on the other hand, the same extract was not effective on COX-1 and COX-2. In this case, the parthenolide is the responsible secondary metabolite from anti-inflammatory effect. The extract also inhibited the release of nitric oxide, PGE_2 (Prostoglandin E_2) and TNF-a from macrophages[24]. In another study, anti-inflammation activity of *T. parthenium* was studied in different pathways such as PGE_2 level, brain-derived neurotrophic factor (BDNF), interleukin-10 (IL-10), and IL-1β gene expression[4]. To the best of our knowledge, this is the first comparative study includes COX and LOX inhibiting potential of *T. parthenium* extract and parthenolide.

Regarding anti-inflammatory activity, COX-2 enzyme selectivity is important in order to avoid the side effects. COX-1 inhibition leads to classical NSAIDs side effects[25]. Since patients and health professionals avoiding from gastrointestinal side effects, selective COX-2 inhibition is very popular. The results of this study showed that ethnobotanical use of *T. parthenium* as an anti-inflammatory herb has an explanation.

In this present study, as a marker of anti-inflammatory activity, LOX inhibition capacities of *T. parthenium* extract and parthenolide was evaluated. Modified spectrophotometric method was used for determination of LOX enzyme capacity and the results support that parthenolide have a significant potential of inhibiting LOX enzyme. LOX inhibition results stated as % inhibition value which were belonged to parthenolide and *T. parthenium* extract were shown in Table 1. Parthenolide showed 41.13% and *T. parthenium* extract showed 80.12% inhibition on LOX enzyme. NDGA was used as positive control. LOX enzyme inhibition values prove that *T. parthenium* extract was a lot more powerful inhibitor compared to major component, parthenolide. This effect may be cause of synergistic mechanism of compounds beyond the standard compound, parthenolide. Isolated compounds were known as the source of biologic activity but recent studies showed that it is not true and synergistic effect usually stronger than only one molecule. A previous study stated the inhibiting capacity of *T. parthenium* extract fractions on leukocytes as strong[25].

In another research, the IC_{50} value of LOX inhibition of parthenolide-depleted extract was 11.8 ± 4.8 μg/ml[24].

Table 1. IC_{50} and SI values for COX-1 and COX-2 inhibition and % inhibition values for LOX of *T. parthenium* extract and parthenolide

Material	IC50 (μg/mL)		LOX inhibition value (%)	SI*
	COX-1	COX-2		
T. parthenium extract	10.45 ± 1.55	9.81 ± 1.07	80.12 ± 2.02	0.93
Parthenolide	4.86 ± 0.55	1.90 ± 0.14	41.13 ± 1.25	0.39
Indomethacine	1.03 ± 0.02 (μM)	7.44 ± 0.12 (μM)	NT	7.22
NDGA	NT	NT	99.8 ± 0.19	NT

*Selective Index= COX-2 IC_{50} / COX-1 IC_{50}.
*Not tested

As a conclusion, it was observed that *T. parthenium* methanol extract was effective on inflammation and it was selective on COX-2 enzyme. LOX inhibition of *T. parthenium* extract was promising. To the best of our knowledge, the extract was tested with COX-1, COX-2 and LOX enzymes for the first time. The *in vitro* biological activities may be due to sesquiterpene lactones, mainly parthenolide. Thanks to the findings of this present research, *T. parthenium* can be considered as a source of COX-2 selective anti-inflammatory compounds. These data indicated that *T. parthenium* standardized extract is a valuable plant material. Further pharmacological and clinical studies are needed for lightening the chemical reactions.

STATEMENT OF ETHICS

This study does not require any ethical approval.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Supervising: FD; Data collection and/or processing: AEK, SBK, EG; Analysis and/or interpretation: AEK, SBK, EG; Literature search and writing: RB.

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REFERENCES

1. Heptinstall S. Feverfew-an Ancient Remedy for Modern Times? J R Soc Med, 1988;81:373-374.

2. Pavela R, Sajfrtová M, Sovová H, Bárnet M, Karban J. The insecticidal activity of *Tanacetum parthenium* (L.) Schultz Bip. extracts obtained by supercritical fluid extraction and hydrodistillation. Ind Crops Prod, 2010;31(3):449-454. Doi: 10.1016/j.indcrop.2010.01.003

3. Maggi F. Nonvitamin and nonmineral nutritional supplements. Feverfew (*Tanacetum parthenium* (L.) Sch.Bip.). Academic Press; 2019. p. 223-225.

4. Recinella L, Chiavaroli A, di Giacomo V, Antolini MD, Acquaviva A, Leona S, et al. Anti-Inflammatory and Neuromodulatory Effects Induced by *Tanacetum parthenium* Water Extract: Results from *in silico, in vitro* and *ex vivo* Studies. Molecules, 2021;26(22). Doi: 10.3390/molecules26010022

5. Jain NK, Kulkarni SK. Antinociceptive and Anti-Inflammatory Effects of *Tanacetum parthenium* L. Extract in Mice and Rats. J Ethnopharmacol, 1999;68(1-3):251-259. Doi: 10.1016/s0378-8741(99)00115-4

6. Tiuman TS, Ueda-Nakamura T, Garcia Cortez DA, Dias Filho BP, Morgado-Diaz JA, de Souza W, et al. Antileishmanial activity of parthenolide, a sesquiterpene lactone isolated from Tanacetum parthenium. Antimicrob Agents Chemother, 2005;49(1):176-182. Doi: 10.1128/AAC.49.11.176-182.2005

7. da Silva BP, Cortez DA, Violin TY, Dias Filho BP, Nakamura CV, Ueda-Nakamura, et al. Antileishmanial activity of a guaianolide from *Tanacetum parthenium* (L.) Schultz Bip. Parasitol Int, 2010;59(4):643-646. Doi: 10.1016/j.parint.2010.08.005

8. Rabito MF, Britta EA, Pelegrini BL, Scariot DB, Almeida MB, Nixdorf SL, et al. *In vitro and in vivo* antileishmania activity of sesquiterpene lactone-rich dichloromethane fraction obtained from *Tanacetum parthenium* (L.) Schultz-Bip. Exp Parasitol, 2014;143(1):18-23. Doi: 10.1016/j.exppara.2014.04.014

9. Wu C, Chen F, Wang X, Kim HY, He GG, Haley-Zitlin V, et al. Antioxidant constituents in feverfew (*Tanacetum parthenium*) extract and their chromatographic quantification. Food Chem, 2006;96(2):220-227. Doi: 10.1016/j.foodchem.2005.02.024

10. Cogo J, Caleare ADO, Ueda-Nakamura T, Filho BPD, Ferreira ICP, Nakamura CV. Trypanocidal activity of guaianolide obtained from *Tanacetum parthenium* (L.) Schultz-Bip. and its combinational effect with benznidazole. Phytomedicine, 2012;20(1):59-66. Doi: 10.1016/j.phymed.2012.09.011

11. de Almeida LMS, Carvalho LSA De, Gazolla MC, Pinto PLS, da Silva MPN, de Moraes J, et al. Flavonoids and Sesquiterpene Lactones from *Artemisia absinthium* and *Tanacetum parthenium* against *Schistosoma mansoni* Worms. eCAM, 2016;2016. Doi: 10.1155/2016/9521349

12. Zengin G, Cvetanović A, Gašić U, Stupar A, Bulut G, Şenkardeş I, et al. Modern and traditional extraction techniques affect chemical composition and bioactivity of *Tanacetum parthenium* (L.) Sch.Bip. Ind Crops Prod, 2020;146. Doi: 10.1016/j.indcrop.2020.112202

13. Benassi-Zanqueta É, Marques CF, Valone LM, Pellegrini BL, Bauermeister A, Ferreira ICP, et al. Evaluation of anti-HSV-1 activity and toxicity of hydroethanolic extract of *Tanacetum parthenium* (L.) Sch.Bip. (Asteraceae). Phytomedicine, 2019;55:249-254. Doi: 10.1016/j.phymed.2018.06.040

14. Pfaffenrath V, Diener H, Fischer M, Friede M, Henneicke-von Zepelin HH. The efficacy and safety of *Tanacetum parthenium* (feverfew) in migraine prophylaxis-a double-blind, multicentre, randomized placebo-controlled dose-response study on behalf of the Investigators. Cephalalgia, 2002;22(7):523-532. Doi: 10.1046/j.1468-2982.2002.00396.x.

15. Tassorelli C, Greco R, Morazzoni P, Riva A, Sandrini G, Nappi G. Parthenolide is the component of tanacetum parthenium that inhibits nitroglycerin-induced Fos activation: Studies in an animal model of migraine. Cephalalgia, 2005;25(8):612-621. Doi: 10.1111/j.1468-2982.2005.00915.x

16. Pahwa R, Goyal A, Jialal I. Chronic Inflammation. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK493173/

17. Lončarić M, Strelec I, Moslavac T, Šubarić D, Pavić V, Molnar M. Lipoxygenase inhibition by plant extracts. Biomolecules, 2021;11(2):1-17. Doi: 10.3390/biom11020152

18. Methods for Identification of Herbal Drugs and Herbal Drug Preparations, HPTLC Association, 2019. Available from: https://www.hptlc-association.org/methods/methods_for_identification_of_herbals.cfm%20

19. Baylac S, Racine P. Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts. Int J Aromather, 2003;12(2-3):138-142. Doi: 10.1016/S0962-4562(03)00083-3

20. Biltekin SN, Karadağ AE, Demirci F, Demirci B. In Vitro Anti-Inflammatory and Anticancer Evaluation of *Mentha spicata* L. and *Matricaria chamomilla* L. Essential Oils. ACS Omega, 2023;8(19):17143-17150. Doi: 10.1021/acsomega.3c01501

21. Polatoğlu K, Demirci F, Demirci B, Gören N, Başer KHC. Antibacterial Activity and the Variation of *Tanacetum parthenium* (L.) Schultz Bip. Essential Oils from Turkey. J Oleo Sci, 2010;59(4):177-184. Doi: 10.5650/jos.59.177

22. Long C, Sauleau P, David B, Lavaud C, Cassabois V, Ausseil F, et al. Bioactive flavonoids of *Tanacetum parthenium* revisited. Phytochemistry, 2003;64(2):567-569. Doi: 10.1016/S0031-9422(03)00208-5

23. Siddiqui NA, Alam P, Alrehaily AJ, Alqahtani AS, Akhtar A, Alhowiriny TA, et al. Optimization of ultrasound-assisted parthenolide extraction from Tarchonanthus camphoratus leaves using response surface methodology: HPTLC and cytotoxicity analysis. Arab J Chem, 2021;14(6). Doi: 10.1016/j.arabjc.2021.103194

24. Sur R, Martin K, Liebel F, Lyte P, Shapiro S, Southall M. Anti-inflammatory activity of parthenolide-depleted feverfew (*Tanacetum parthenium*). Inflammopharmacology, 2009;17(1):42-49. Doi: 10.1007/s10787-008-8040-9

25. Sumner H, Salan U, Knight DW, Hoult JRS. Inhibition of 5-lipoxygenase and cyclooxygenase in leukocytes by feverfew. Biochem Pharmacol, 1992;43(11):2313-2320. Doi: 10.1016/0006-2952(92)90308-6

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Dietary habits, physical activity, sleep duration, and their association with overweight and obesity among children aged 6-10

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ABSTRACT

This study aimed to determine dietary habits, the time spent on sleeping and physical activity among primary school children and to compare the responses given by the children and their parents about their dietary habits. This cross-sectional study was conducted on 282 children. A questionnaire including demographic characteristics, dietary habits, and some anthropometric measurements were performed. Physical activity and sleep duration were statistically different between overweight/obese and normal-weight children groups. The consumption of breakfast, lunch, fresh vegetables, dessert with dairy products, meat and meat products, chocolates, wafer, instant cake, pastry, and fast food was statistically higher in the overweight/obese group. Additionally, a statistical difference was found between "lunch consumption", "junk food consumption" and "the child finishes all food on his/her plate" according to the responses of children and parents. It is important to raise awareness of healthy eating, exercise, and sleeping habits among primary school children.

Keywords: dietary habits, physical activity, sleep, children, body mass index

INTRODUCTION

Overweight and obesity are an increasing public health concern among chil-

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dren worldwide[1]. In Turkey, the prevalence of childhood obesity has grown in the past few decades. According to the World Health Organization (WHO) European Childhood Obesity Surveillance Initiative-2013, 22.5% of 7-8-year olds in the country were obese or overweight (14.2% overweight, 8.3% obese). In November 2017, the Turkish Minister of Health stated that the prevalence of obesity had grown to around 10%[2]. Childhood obesity considered by the WHO to be one of the most serious problems of the 21st century - is a well-known cause of non-communicable diseases (such as cardiovascular diseases, diabetes mellitus, and certain types of cancer) in adults[3]. In order to prevent childhood obesity, interventions should be made to gain healthy nutrition behaviors, increase the level of physical activity, and regulate sleep duration and hours[4,5].

Nutritional behaviors have considerably changed in recent years, and the consumption of high-energy-density and processed foods has increased[6]. According to a Turkish study, it was found that primary school children consume chocolate with added sugar at least once or twice a week, or even more. Additionally, most of them consumed crisps-potato and sugary drinks (such as cola)[7]. Appropriate dietary habits provide a basis for the healthy growth and development of children[8]. Since it is difficult to reshape eating habits in adulthood, it is important to provide healthy dietary habits to children, especially due to negative health effects on emotional and social life and irregular development[9,10]. Therefore, understanding the relationship between dietary and lifestyle habits is necessary for the treatment of overweight and obesity.

In recent years, the spent time on outdoor activities or sports has dramatically decreased among children, and the spent time on screen activities such as playing video games, watching TV, or using electronic devices has increased[11]. Specifically, positive associations were found between watching TV and various indices of adiposity in children in the literature[12]. The reasons for that are increasing consumption of snack foods while watching TV, and increased demand for high-energy and processed foods advertised on TV[13,14]. The fact that children spend more time with electronic devices prevents them from engaging in outdoor activities and sports activities[11].

Sleep plays a role in growth and development as well as health status in children, due to the control over the circadian rhythm, which is related to energy homeostasis[15]. Changes in the circadian clock are associated with temporal alterations in feeding behavior and increased weight gain[16]. A few studies showed that short sleep duration is related to childhood obesity[17,18].

For all these reasons, it is important to determine the actual prevalence of overweight and obesity in children and determine the relative contribution

of lifestyle habits (dietary habits, physical activity, and sleep duration). The present study aimed to determine dietary habits, the time spent sleeping and physical activity, and anthropometric measurements in primary school children. Additionally, the responses of both children and parents to questions about children's dietary habits were compared.

METHODOLOGY

Study design and participants

This cross-sectional study was conducted among children aged 6-10 years primary school students in Avcılar, Istanbul between October and December 2017. We used power analysis for sample selection. The prevalence was calculated as 20%, type 1 error rate α 0.05%, type 2 error rate β 0.05%, and test power 1-β = 0.95%. According to the calculation we included a total of 300 students (40.7% boys and 59.3% girls) in this study. However, since 18 parents did not complete the survey, a total of 282 children were included in the study.

The public primary schools were randomly selected from the institutional registry list of the Ministry of Education using online resources. Avcılar is a district on the European side of Istanbul. There are a total of 67,905 students studying in 53 primary schools (49,043 students) and 35 secondary schools (18,862)[19]. We randomly selected 19 primary schools located in the center of Avcılar. Also, children from these schools were randomly selected using a random list of the classes. Both children, parents, and school authorities gave their informed written consent for the study and approved the protocol. The Local Education Authority granted permission for the study.

A questionnaire including some demographic characteristics (gender, age, disease, time spent on physical activity, time spent on electronic devices, time spent on sleeping, and dietary habits was performed on the children. Before the study, expert opinion was obtained from 5 academicians specializing in nutrition and exercise for the survey questions. Some necessary changes were made to the questions by conducting a pilot study on a group of 20 students. Additionally, some anthropometric measurements were taken by a trained researcher to eliminate the bias.

Dietary habits

Dietary habits were determined using a questionnaire adapted version of the "Turkey Childhood Obesity Survey" (COSI TUR) study[20]. "WHO European Childhood Obesity Surveillance Initiative – COSI" study is a study initiated by WHO in 2007-2008. Turkey was included in the COSI program for the first time

in the 3rd round. The study was carried out in cooperation with the Turkey Ministry of Health, the Ministry of National Education, and Hacettepe University within the framework of the criteria and protocol determined by the WHO[20].

Questions about dietary habits were the number of meals, frequency, and place of main meals and snacks. Furthermore, questionnaire included 19 food items including fresh fruits (grown in season such as mandarin, orange, pomegranate, apple, pear, etc.), fresh vegetables and salads (grown in season such as spinach, leek, carrot, beet, radish, etc.), fruit juice (packaged, sold at the grocery store), fruit juice (freshly squeezed), soft drinks containing sugar (such as cola, ice tea, fanta, etc.), diet soft drinks (such as cola with zero sugar, diet cola, sprite with zero sugar, etc.), unflavored milk, flavored milk (such as cacao, fruit, etc.), cheese, yoghurt/ayran, dessert with dairy products (such as rice pudding, pudding, chicken breast), red meat/poultry, fish, egg, legumes (such as haricot, chickpeas, lentils, etc.), nuts (such as hazelnuts, peanuts, almonds, walnuts, etc.), bread/rice/pasta/bulgur, junk foods (such as chip, popcorn, chocolate, instant cake, pastry, etc.), and fast foods (such as sandwiches, pitas, hamburgers, fried chicken, french fries, chicken nuggets, pizza, hot dogs). Children reported how many times per day, week, or month they consumed these specific food items during the last month. The frequency of food consumption was recorded in five categories: never, 1-2 times per month, 1-3 times per week, 4-6 times per week, and every day. Additionally, a questionnaire including the same dietary habits of children was used for the parents, and the responses given by the children and parents were compared.

Anthropometric measurements

Anthropometric measurements (height, waist circumference, and hip circumference in centimeters) of the children were taken according to the standard procedures[21]. The height of patients was taken using a stadiometer (brand name: Tanita) with the nearest 0.1 cm, while each participant was standing erect against the wall with heels together touching the wall, without shoes. Waist circumference was measured using a non-stretch plastic tape within 1 mm after normal exhalation, at the umbilicus level, and without clothing in the area. The hip circumference was measured using a non-stretch plastic tape from the widest point between the waist and the thigh. Body composition in terms of weight (kg), body mass index (kg/m^2), fat (%), and skeletal muscle mass (kg) was measured using the Inbody 120 system (Inbody 120, Inbody, South Korea). Body mass index (BMI) <5 percentile indicates thinness, and BMI ≥85 overweight and obesity. BMI values between 5 and 85 percentiles refers to normal weight. The prevalence of wasted and short stature in children was also determined by

using weight-for-height percentile values by the WHO[22]. According to the WHO classification, <3 percentile refers to wasted, 3 and 15 percentiles as short, 15 and 85 percentiles as normal height, and ≥ 85 percentile as tall.

Sleep duration

To determine sleep duration, both weekdays and weekend mornings were asked to wake up and sleep time of the children. Total sleep time was calculated in hours as the difference between bedtime and wake-up time for weekdays and weekend days and as the mean duration of sleeping using the equation: (weekday time × 5 + weekend day time × 2)/7[5].

According to the National Sleep Foundation recommendations for school-age children, sleep duration was classified as low if less than 9 h mean per night, recommended if between 9 and 11 h per night or high if more than 11 h per night[23].

Physical activity

We asked about the time spent on physical activity such as outdoor activities or sports, on both weekdays and weekends for determining the physical activity behaviors. Total physical activity time was calculated in hours for weekdays and weekend days and as the mean duration of physical activity using the same equation for sleep duration: (weekday time × 5 + weekend day time × 2)/7[5].

According to the WHO and Turkey Physical Activity Guideline recommendations for children, physical activity duration was classified as low if less than 1 h per day, normal if equal to 1 h per day or high if more than 1 h per day[24,25].

Statistical analysis

Data were evaluated with the statistical program SPSS 23.0 (Statistical Package for the Social Sciences, Inc.; Chicago, Illinois, United States). Categorical data were expressed as the frequency (percentage), and differences were analyzed using the chi-square test. Quantitative variables were expressed as the mean ± SD (standard deviation), and the Kolmogorov-Smirnov test was used to assess whether the data were normally distributed. We also analyzed the homogeneity of the variables. Differences in quantitative variables were analyzed by the student's t-test or Mann-Whitney U-test, as appropriate. For all statistical tests, a p-value of ≤0.05 was considered statistically significant.

RESULTS and DISCUSSION

282 children (58.5% girls, 41.5% boys, mean age 8.34 ± 1.15 years) and their parents completed the study. The most three popular physical activity types were football (28.0%), running (25.3%), and basketball (16.0%) among chil-

dren. According to the height classification, the majority of children (52.5%) were in the normal range. Additionally, the majority of children (55.7%) were normal weight, 23.4% were overweight, and 12.0% were obese (Table 1).

Table 1. Characteristics of children (n=282)

Characteristics	Boys (n=117)	Girls (n=165)	Total (n=282)
Age (year)*	8.42 ± 1.04	8.46 ± 1.13	8.34 ± 1.15
Time spent on watching TV, playing video games, and using electronic devices (mean hour)*			
Weekdays	1.72 ± 1.51	1.50 ± 1.43	1.64 ± 1.43
Weekend days	2.47 ± 2.16	1.90 ± 1.56	1.99 ± 1.80
Time spent on physical activity (mean hour)*			
Weekdays	1.94 ± 0.69	1.76 ± 0.49	1.83 ± 0.51
Weekend days	1.64 ± 1.24	1.09 ± 0.51	1.42 ± 1.06
Type of physical activity**			
Football	42 (35.9)	-	42 (28.0)
Running	10 (13.5)	28 (36.8)	38 (25.3)
Basketball	14 (18.9)	10 (13.2)	24 (16.0)
Sleep duration (mean hour)*			
Weekdays	10.07 ± 2.00	10.22 ± 2.42	10.71 ± 3.05
Weekend days	11.37 ± 4.05	10.97 ± 2.46	11.79 ± 5.32
Body weight (kg)*	30.29 ± 8.47	30.12 ± 7.59	30.19 ± 7.94
Height (cm)*	129.96 ± 7.79	129.62 ± 8.38	129.76 ± 8.12
BMI (kg/m^2)*	17.74 ± 3.73	17.72 ± 3.29	17.73 ± 3.47
Classification of height			
Wasted	11 (9.4)	21 (12.6)	32 (11.3)
Short	31 (26.5)	47 (28.4)	78 (27.7)
Normal	66 (56.4)	82 (50.0)	148 (52.5)
Tall	9 (7.7)	15 (9.0)	24 (8.5)
Classification of BMI			
<5 percentile	9 (7.6)	16 (9.7)	25 (8.9)
5-85 percentile	68 (58.1)	89 (53.9)	157 (55.7)
≥85 percentile	22 (18.8)	44 (26.7)	66 (23.4)
≥95 percentile	18 (15.4)	16 (9.7)	34 (12.0)
Waist circumference (cm)*	61.60 ± 12.74	58.08 ± 12.69	59.53 ± 12.79
Hip circumference (cm)*	69.92 ± 12.36	68.48 ± 15.24	69.07 ± 14.10
Body muscle weight (kg)*	11.71 ± 2.27	11.80 ± 6.98	11.76 ± 5.54
Body fat percentage (%)*	22.04 ± 9.91	24.63 ± 8.61	23.56 ± 9.22

*Mean± standard deviation, **the first 3 most popular types of physical activity; BMI, Body Mass Index.

Classification of physical activity and sleep duration were statistically different between the two BMI groups ($p<0.05$). Additionally, the sleep duration in children with normal BMI was higher than the overweight and obese children, and similar results were found considering only weekdays or weekend days (Table 2).

Table 2. Classification of physical activity, sleep behaviors, and gender by BMI.

	5-85 BMI percentile (n = 157) n (%)	≥85 BMI percentile (n = 100) n (%)	Total (n = 257) n (%)	p value
Gender				0.78
Boy	68 (63.0)	40 (27.0)	108 (42.0)	
Girl	89 (59.7)	60 (40.3)	149 (58.0)	
Mean physical activity level	1.99 ± 0.96	1.93 ± 0.72	1.95 ± 1.75	0.06*
Weekdays	1.95 ± 1.75	1.60 ± 1.17	1.83 ± 1.58	0.90
Weekend days	2.31 ± 1.78	2.36 ± 2.01	2.33 ± 1.86	0.18
Classification of physical activity				0.04*
Low	67 (42.6)	34 (24.0)	101 (39.2)	
Normal	-	2 (2.0)	2 (0.8)	
High	90 (57.4)	64 (64.0)	154 (60.0)	
Sleep duration				
Mean sleep duration	10.46 ± 2.27	10.42 ± 1.81	10.44 ± 1.98	0.04*
Weekdays	10.23 ± 1.80	10.12 ± 2.93	10.16 ± 2.25	0.02*
Weekend days	11.19 ± 3.03	11.04 ± 3.54	11.13 ± 3.21	0.04*
Classification of sleep duration				0.03*
Low	4 (2.5)	4 (4.0)	8 (3.2)	
Recommended	78 (49.7)	47 (47.0)	125 (48.6)	
High	75 (47.8)	49 (49.0)	124 (48.2)	

*$p<0.05$, differences between the two BMI groups; p-values were computed with chi-square test for gender, classification of physical activity, sleep duration, and BMI groups; Student's T-test for mean sleep duration and BMI groups; Mann-Whitney U-test for mean physical activity and BMI groups; BMI, Body Mass Index.

According to the frequency of specific food groups, it was found that consumption of breakfast, lunch, fresh vegetables, dessert with dairy products, meat and meat products, chocolates, wafer, instant cake, pastry, and fast foods was statistically different between normal weight and overweight/obese groups ($p<0.05$) (Table 3).

Table 3. Frequency of children's consumption of certain foods by BMI

Foods	Everyday	4-6 per week	1-3 per week	1-2 per month	Never	p value
	n (%)	n (%)	n (%)	n (%)	n (%)	
Breakfast						
Normal weight	133 (84.8)	1 (0.6)	20 (12.7)	-	3 (1.9)	0.04*
Overweight and obese	60 (60.0)	6 (6.0)	22 (22.0)	9 (9.0)	3 (3.0)	
Lunch						
Normal weight	107 (68.2)	15 (9.5)	30 (19.1)	2 (1.3)	3 (1.9)	0.04*
Overweight and obese	64 (64.0)	7 (7.0)	11 (11.0)	8 (8.0)	10 (10.0)	
Dinner						
Normal weight	137 (87.3)	12 (7.6)	6 (3.8)	-	2 (1.3)	0.85
Overweight and obese	84 (84.0)	6 (6.0)	3 (3.0)	5 (5.0)	2 (2.0)	
Fresh fruit						
Normal weight	2 (1.3)	2 (1.3)	30 (19.1)	47 (29.9)	76 (48.4)	0.32
Overweight and obese	-	2 (2.0)	28 (28.0)	24 (24.0)	46 (46.0)	
Fresh vegetables						
Normal weight	15 (9.5)	9 (5.7)	40 (25.5)	40 (25.5)	53 (33.8)	0.04*
Overweight and obese	11 (11.0)	5 (5.0)	41 (41.0)	20 (20.0)	23 (23.0)	
Soft drinks containing sugar						
Normal weight	77 (49.0)	30 (19.1)	38 (24.2)	10 (6.4)	2 (1.3)	0.44
Overweight and obese	50 (50.0)	16 (16.0)	23 (23.0)	7 (7.0)	4 (4.0)	
Diet soft drinks						
Normal weight	127 (80.9)	15 (9.5)	9 (5.8)	4 (2.5)	2 (1.3)	0.27
Overweight and obese	89 (89.0)	9 (9.0)	1 (1.0)	-	1 (1.0)	
Milk						
Normal weight	22 (14.0)	2 (1.3)	36 (22.9)	26 (16.6)	71 (45.2)	0.36
Overweight and obese	10 (10.0)	4 (4.0)	21 (21.0)	18 (18.0)	47 (47.0)	
Flavored milk						
Normal weight	35 (22.3)	14 (8.9)	50 (31.8)	32 (20.4)	26 (16.6)	0.19
Overweight and obese	38 (38.0)	4 (4.0)	30 (30.0)	14 (14.0)	14 (14.0)	
Cheese						
Normal weight	16 (10.3)	3 (1.9)	31 (19.7)	28 (17.8)	79 (50.3)	0.10
Overweight and obese	6 (6.0)	5 (5.0)	18 (18.0)	23 (23.0)	48 (48.0)	
Yoghurt, ayran						
Normal weight	1 (0.6)	7 (4.5)	46 (29.3)	40 (25.5)	63 (40.1)	0.24
Overweight and obese	3 (3.0)	2 (2.0)	31 (31.0)	16 (16.0)	48 (48.0)	
Dessert with dairy products						
Normal weight	26 (16.6)	50 (31.8)	56 (35.6)	18 (11.5)	7 (4.5)	0.04*
Overweight and obese	26 (26.0)	40 (40.0)	22 (22.0)	4 (4.0)	8 (8.0)	
Meat and meat products						
Normal weight	15 (9.5)	21 (13.4)	70 (44.6)	30 (19.1)	21 (13.4)	0.02*
Overweight and obese	13 (13.0)	17 (17.0)	54 (54.0)	14 (14.0)	2 (2.0)	

Fish						
Normal weight	18 (11.5)	50 (31.8)	62 (39.5)	13 (8.3)	14 (8.9)	0.27
Overweight and obese	14 (14.0)	38 (38.0)	31 (31.0)	7 (7.0)	10 (10.0)	
Egg						
Normal weight	12 (7.6)	7 (4.5)	37 (23.6)	36 (22.9)	65 (41.4)	0.46
Overweight and obese	6 (6.0)	3 (3.0)	30 (30.0)	17 (17.0)	44 (44.0)	
Legumes						
Normal weight	21 (13.4)	20 (12.7)	58 (37.0)	30 (19.1)	28 (17.8)	0.39
Overweight and obese	7 (7.0)	11 (11.0)	49 (49.0)	15 (15.0)	18 (18.0)	
Nuts						
Normal weight	8 (5.1)	28 (17.8)	63 (40.1)	30 (19.2)	28 (17.8)	0.62
Overweight and obese	4 (4.0)	15 (15.0)	39 (39.0)	13 (13.0)	29 (29.0)	
Bread, rice, pasta, bulgur						
Normal weight	-	8 (5.1)	20 (12.7)	41 (26.1)	88 (56.1)	0.19
Overweight and obese	10 (10.0)	4 (4.0)	21 (21.0)	19 (19.0)	46 (46.0)	
Chips and Popcorn						
Normal weight	26 (16.5)	40 (25.5)	62 (39.5)	18 (11.5)	11 (7.0)	0.91
Overweight and obese	28 (28.0)	27 (27.0)	32 (32.0)	7 (7.0)	6 (6.0)	
Chocolate, wafer, instant cake, pastry						
Normal weight	1 (0.6)	49 (31.3)	61 (38.9)	23 (14.6)	23 (14.6)	0.06*
Overweight and obese	11 (11.0)	35 (35.0)	37 (37.0)	15 (15.0)	2 (2.0)	
Fast foods						
Normal weight	10 (6.4)	50 (31.8)	50 (31.8)	19 (12.2)	28 (17.8)	0.001*
Overweight and obese	26 (26.0)	38 (38.0)	31 (31.0)	3 (3.0)	2 (2.0)	

*p<0.05, differences between the two BMI groups; p values were computed by chi-square test; BMI, Body Mass Index. Normal weight children (n= 157), overweight and obese children (n=100)

In the responses of children and parents about dietary habits, a statistical difference was found between “lunch consumption”, “junk food consumption” and “the child finishes all food on his/her plate” (p<0.05). Parents said more when children said they consume less junk food; whereas parents said less when children said they finish more all food on his/her plate. Additionally, “the child continues to eat his/her favorite food even if he/she is full” was statistically different between the two BMI groups (p<0.05). Because overweight and obese children had a higher prevalence of continuing to eat “his/her favorite food even if he/she is full” than children of normal weight (not shown in table) (Table 4).

Table 4. Differences between children's and parents' responses to children's dietary habits

	Every day	4-6 per week	1-3 per week	1-2 per month	Never	p value*	p value**
Breakfast consumption						0.15	0.04**
Children	196 (69.5)	20 (7.1)	51 (18.1)	9 (3.2)	6 (2.1)		
Parents	181 (64.2)	37 (13.1)	29 (10.3)	21 (7.4)	14 (5.0)		
Lunch consumption						<0.001*	0.04**
Children	188 (66.7)	25 (8.9)	43 (15.2)	11 (3.9)	15 (5.3)		
Parents	211 (74.6)	38 (13.5)	15 (5.3)	11 (3.9)	7 (2.5)		
Dinner consumption						0.46	0.85
Children	235 (83.3)	20 (7.1)	17 (6.0)	5 (1.8)	5 (1.8)		
Parents	248 (88.0)	17 (6.0)	8 (2.8)	6 (2.1)	3 (1.1)		
Junk food consumption						0.004*	0.62
Children	21 (7.5)	31 (11.0)	127 (45.0)	60 (21.3)	43 (15.2)		
Parents	51 (18.1)	46 (16.3)	84 (29.8)	66 (23.4)	35 (12.4)		
The child continues to eat his/her favorite food even if he/she is full						0.62	0.01**
Children	40 (14.2)	32 (11.3)	56 (19.9)	33 (11.7)	121 (42.9)		
Parents	59 (21.0)	27 (9.6)	41 (14.5)	39 (13.8)	116 (41.1)		
The child consumes fast foods when he/she does not like the food						0.31	0.80
Children	9 (3.2)	12 (4.3)	37 (13.1)	31 (11.0)	193 (68.4)		
Parents	25 (8.9)	14 (5.0)	33 (11.7)	39 (13.8)	171 (60.6)		
The child finishes all food on his/her plate						0.001*	0.20
Children	122 (43.2)	38 (13.5)	70 (24.8)	27 (9.6)	25 (8.9)		
Parents	82 (29.1)	63 (22.3)	86 (30.5)	29 (10.3)	22 (7.8)		
The child likes to eat fast foods more than home-made meals						0.35	0.84
Children	26 (9.2)	16 (5.7)	29 (10.3)	44 (15.6)	167 (59.2)		
Parents	37 (13.1)	19 (6.7)	37 (13.1)	52 (18.5)	137 (48.6)		

*p<0.05, the difference between children and parents, **p<0.05, the difference between the two BMI groups; p values were computed by chi-square test; BMI, Body Mass Index.

The childhood obesity rate is increasing worldwide and has become a major global burden, particularly in developed countries.[1,4] Our findings showed that 23.4% of children were overweight and 12.0% were obese. Classification of physical activity and sleep duration were statistically different between the normal and overweight/obese groups. Additionally, the consumption of fresh vegetables, desserts with dairy products, meat and meat products, chocolate, wafer, instant cake, pastry, and fast foods were statistically different between the two BMI groups. We also compared the responses of children and parents about dietary habits and found that "lunch consumption", "junk food consumption" and "the child finishes all food on his/her plate" were statistically

different. Since parents said more when children said they consume less junk food; whereas parents said less when children said they finish more all food on his/her plate.

Obesity is a disease of modern life, and childhood obesity is one of the most serious global public health challenges of the 21st century according to the WHO[26]. Its prevalence has doubled in more than 70 countries since 1980.[27] Turkey, with a 10% increase in childhood obesity from 2013 to 2017, is one of these countries.[2] Our results showed that a total of 8.9% of children were underweight, 55.7% normal, 23.4% overweight and 12.0% obese according to the BMI classification. Also, the prevalence of overweight and obesity was found to be higher in girls than in boys (36.4% of girls and 34.2% of boys were overweight and obese). However, we did not find statistical differences between BMI classification and gender (p: 0.30). Considering that the prevalence of overweight and obesity is gradually increasing and may adversely affect, it is urgent to take measures to reduce obesity.

Regular consumption of main meals is an important process in weight control[28]. Breakfast is the most important dietary habit due to individuals who eat breakfast regularly are less likely to be obese[4]. According to the literature, it was found that the percentage of children eating breakfast daily decreased, and they skipped breakfast more frequently than other meals[4,29]. Additionally, studies showed that obese children tend to skip meals, particularly breakfast, more often than children with normal BMIs.[4,30] Our results showed that 28.4% of children skip breakfast, also 2.1% never had breakfast. Similarly to previous studies, we found that overweight and obese children are more tend to skip breakfast compared to normal-weight children (40.0%, and 15.2%, respectively). Furthermore, lunch meal supplies about 30% of the daily energy and represents the highest proportion of protein, fat, and carbohydrate intake[28]. According to a study, 53.9% of overweight and obese children skipped lunch[8]. In this study, the prevalence of overweight and obese children who skipped lunch (36.0%) was higher compared to normal-weight children (31.8%). This may be the result of not having time for lunch at school or a false belief that skipping meals is a weight loss control.

The WHO recommends a diet must be poor in fat, sugar, and salt, and rich in fruits and vegetables[31]. It has been reported that fruit and vegetable consumption reduces the risks of all causes of mortality and morbidity from cardiovascular disease, stroke, diabetes mellitus, metabolic syndrome, non-alcoholic fatty liver disease, and some types of cancer[32,33]. We found that 11.0% of overweight and obese children eat fresh vegetables every day, and there was a statistical difference between overweight/obese and normal-weight children. Additionally, the prevalence of both overweight/obese children who did not consume

fresh vegetables at 33.8% was higher compared to normal-weight children at 23.0%. Given that foods with lower energy density, such as fresh vegetables, can help with weight management when eaten in place of high-calorie foods, it is important that school-based nutrition intervention programs attempt to increase children's fresh vegetable intake[34].

Studies indicated that children were the main consumers of fast foods and junk foods[7,35]. The consumption of these types of foods will result in increased weight gain which, in turn, may cause many diseases such as diabetes mellitus, cardiovascular diseases, etc.[4,8] In the present study, a statistical difference was found between overweight/obese and normal-weight children in the consumption of desserts with dairy products, junk foods (such as chocolate, wafer, instant cake, and pastry), and fast foods. A total of 26.0% of obese children consumed fast food every day. Additionally, chocolate, wafer, instant cake, pastry, and dessert with dairy products consumption every day was 11.0% and 26.0%, respectively.

Furthermore, we analyzed the responses of children and parents about their dietary habits of children. A statistical difference was found between "lunch consumption", "junk food consumption" and "the child finishes all food on his/her plate" due to parents said more when children said they consume less lunch and junk food; whereas parents said less when children said they finish more all food on his/her plate. The reason for this difference may be that when the study was carried out, the children knew that the experts would ask questions about dietary habits, and they gave different responses from their parents in order to give the correct response according to them. Also, the fact that the education level of most of the parents on nutrition was generally low may have led to this difference. Moreover, the parents might have had inadequate observations of their children's nutritional status, and their failure to follow what children ate played a role.

The association between obesity and physical activity in children has been widely investigated[12,36,37]. According to the WHO and national guide recommendations for children, physical activity duration was classified as low if less than 1 h per day, normal if equal to 1 h per day, or high if more than 1 h per day[24,25]. We found that the mean hours of physical activity were 1.99 ± 0.96 h for normal weight and 1.93 ± 0.72 h for overweight and obese children, these values were at the recommended hours. Although most of the children were classified as having high physical activity (60.0%), it was found that the physical activity levels of overweight and obese children were lower and this value was statistically different. Physical inactivity is a major contributor to childhood obesity and health disparities throughout life[37]. Overweight and obese children might have passive hobbies (music, computer games, etc.) contrary to

normal weight/underweight counterparts who could be involved in more active hobbies such as basketball, football, athletics, etc.[29] Therefore, it is important to take measures to increase the level of physical activity at an early age.

Studies showed that short sleep duration is associated with inappropriate dietary habits[16,38]. Therefore, it may be related to childhood obesity.[5,17,18] For example, a study found that children with obesity had lower sleep duration compared to children with normal weight (7.48 h versus 7.52 h)[39]. A recent meta-analysis showed that short sleep duration was significantly associated with obesity in preliminary school children[40]. According to the National Sleep Foundation recommendations for school-age children, sleep duration was recommended between 9 and 11 h per night[23]. In our study, a total of 48.6% of children were in the recommended classification group. However, there was a statistical difference between normal weight and overweight/obese groups, the mean sleep duration of overweight and obese children was lower than normal-weight children. The possible mechanism of the relationship between sleep duration and obesity is that circadian clock changes are linked to temporal changes in feeding behavior and increased weight gain[26].

The present study had some limitations. First, this was a cross-sectional study. Therefore, a generalization based on the cause-effect relationship cannot be made. Second, we collected data on dietary habits, physical activity, and sleep duration by using a questionnaire. Additionally, our study was conducted among public primary schools only. Thus, we did not take into the socio-economic factors of children's dietary habits. However, the strength of the study is that it evaluated the responses of both children and parents in the face of childhood obesity, which is increasing day by day in our country.

Our study indicated that the prevalence of overweight and obesity among primary school children was high. Therefore, some strategies should be taken to decrease the prevalence of obesity. The prevention of childhood obesity requires a multidisciplinary approach. In addition to diet and physical activity, it is thought that the evaluation of sleep duration will be beneficial in the prevention of childhood obesity. Therefore, future studies are needed.

STATEMENT OF ETHICS

The ethics committee of Istanbul Gelisim University Non-Interventional Clinical Research Ethics Committee (Number:2017-19, date: 29.09.2017) and Provincial Directorate of National Education (Number:59090411-44E.18445641, date: 03.11.2017) approved the study, which followed the principles of the Declaration of Helsinki.

CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

AUTHOR CONTRIBUTIONS

The authors contributed equally.

REFERENCES

1. NCD Risk Factor Collaboration. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. The Lancet, 2017;390(10113):2627-2642. Doi: 10.1016/S0140-6736(17)32129-3

2. World Health Organization. WHO contributes to new plans to tackle childhood obesity in Turkey. Geneva 2019. Available from: https://www.euro.who.int/en/countries/turkey/news/news/2018/3/who-contributes-to-new-plans-to-tackle-childhood-obesity-in-turkey

3. World Health Organization. Noncommunicable diseases: childhood overweight and obesity, 2020. Available from: https://www.who.int/news-room/q-a-detail/noncommunicable-diseases-childhood-overweight-and-obesity

4. Koca T, Akcam M, Serdaroglu F, Dereci S. Breakfast habits, dairy product consumption, physical activity, and their associations with body mass index in children aged 6–18. Eur J Pediatr, 2020;176(9):1251-1257. Doi: 10.1007/s00431-017-2976-y

5. Rosi A, Calestani MV, Parrino L, Milioli G, Palla L, Volta E, et al. Weight status is related with gender and sleep duration but not with dietary habits and physical activity in primary school Italian children. Nutrients, 2017;9(6):579. Doi: 10.3390/nu9060579

6. World Health Organization, Food and Agriculture Organization of the United Nation Expert Consultation. Diet, nutrition and the prevention of chronic diseases. *World Health Organ Tech Rep Ser*. 2003;916(i-viii):1-149.

7. Kutlu R, Çivi S. The assessment of nutritional habits and body mass indexes of the students attending a private primary school. Fırat Medical Journal, 2009;14(1):18-24.

8. Zalewska M, Maciorkowska E. Selected nutritional habits of teenagers associated with overweight and obesity. PeerJ, 2017;5:e3681. Doi: 10.7717/peerj.3681

9. Sahoo K, Sahoo B, Choudhury AK, Sofi NY, Kumar R, Bhadoria AS. Childhood obesity: causes and consequences. Fam Med Prim Care Rev, 2015;4(2):187-192. Doi: 10.4103/2249-4863.154628

10. Todd AS, Street SJ, Ziviani J, Byrne NM, Hills AP. Overweight and obese adolescent girls: the importance of promoting sensible eating and activity behaviors from the start of the adolescent period. Int J Environ Res Public Health, 2015;12(2):2306-2329. Doi: 10.3390/ijerph120202306

11. Granich J, Rosenberg M, Knuiman M, Timperio A. Understanding children's sedentary behaviour: a qualitative study of the family home environment. Health Educ Res, 2010;25(2):199-210. Doi: 10.1093/her/cyn025

12. Boyland EJ, Harrold JA, Kirkham TC, Halford JC. The extent of food advertising to children on UK television in 2008. Int J Pediatr Obes, 2011;6(5-6):455-461. Doi: 10.3109/17477166.2011.608801

13. Van den Bulck J, Van Mierlo J. Energy intake associated with television viewing in adolescents, a cross sectional study. Appetite, 2004;43(2):181-184. Doi: 10.1016/j.appet.2004.04.007

14. Halford JC, Gillespie J, Brown V, Pontin EE, Dovey TM. Effect of television advertisements for foods on food consumption in children. Appetite, 2004;42(2):221-225. Doi: 10.1016/j.appet.2003.11.006

15. Seo SH, Shim YS. Association of sleep duration with obesity and cardiometabolic risk factors in children and adolescents: a population-based study. Sci Rep, 2019;9:9463. Doi: 10.1038/s41598-019-45951-0

16. St-Onge MP. The role of sleep duration in the regulation of energy balance: effects on energy intakes and expenditure. J Clin Sleep Med, 2013;9(1):73-80. Doi: 10.5664/jcsm.2348

17. Chaput JP, Brunet M, Tremblay A. Relationship between short sleeping hours and childhood overweight/obesity: results from the 'Quebec en Forme' Project. IJO, 2006;30(7):1080-1085. Doi: 10.1038/sj.ijo.0803291

18. Touchette É, Petit D, Tremblay RE, Boivin M, Falissard B, Genolini C, et al. Associations between sleep duration patterns and overweight/obesity at age 6. Sleep, 2008;31(11):1507-1514. Doi: 10.1093/sleep/31.11.1507

19. Istanbul Provincial National Education Statistics. 2017. Available from: https://istanbul.meb.gov.tr/meb_iys_dosyalar/2017_04/12163411_2016-2017_Ystatistik_KitabY_Taslak.pdf

20. Turkish Ministry of Health. Turkish Childhood (Primary School Second Grade Students) Obesity Survey COSI-TUR 2016. 2017. Available from: https://hsgm.saglik.gov.tr/depo/birimler/saglikli-beslenme-ve-hareketli-hayat-db/Dokumanlar/Ingilizce_Yayinlar/Turkey_Childhood_Obesity_Cosi_tur_2016.pdf

21. Pekcan G. Beslenme durumunun saptanmasi. Diyet El Kitabi, 2008;726:67-141.

22. World Health Organization. Growth reference data for 5-19 years. Geneva, 2007. Available from: https://www.who.int/tools/growth-reference-data-for-5to19-years

23. Hirshkowitz M, Whiton K, Albert SM, Alessi C, Bruni O, DonCarlos L, et al. National Sleep Foundation's updated sleep duration recommendations. Sleep Health, 2015;1(4):233-243. Doi: 10.1016/j.sleh.2014.12.010

24. Chaput JP, Willumsen J, Bull F, Chou R, Ekelund U, Firth J, et al. 2020 WHO guidelines on physical activity and sedentary behaviour for children and adolescents aged 5–17 years: summary of the evidence. Int J Behav Nutr Phys Act, 2020;17(141). Doi: 10.1186/s12966-020-01037-z

25. Turkish Ministry of Health Turkey Physical Activity Guide. 2014. Available from: https://hsgm.saglik.gov.tr/depo/birimler/saglikli-beslenme-hareketli-hayat-db/Fiziksel_Aktivite_Rehberi/Turkiye_Fiziksel_Aktivite_Rehberi.pdf

26. World Health Organization. Taking action on childhood obesity. Geneva, 2018. Available from: https://apps.who.int/iris/bitstream/handle/10665/274792/WHO-NMH-PND-ECHO-18.1-eng.pdf

27. Weihrauch-Blüher S, Wiegand S. Risk factors and implications of childhood obesity. Curr Obes Rep, 2018;7(4):254-259. Doi: 10.1007/s13679-018-0320-0

28. Akbarzade Z, Djafarian K, Saeidifard N, Majd SA, Garousi N, Samadi F, et al. The association between lunch composition and obesity in Iranian adults. BJN, 2022;127(10):1517-1527. Doi: 10.1017/S0007114521002543

29. Smetanina N, Albaviciute E, Babinska V, Karinauskiene L, Albertsson-Wikland K, Petrauskiene A, et al. Prevalence of overweight/obesity in relation to dietary habits and lifestyle among 7–17 years old children and adolescents in Lithuania. BMC Public Health, 2015;15:1001. Doi: 10.1186/s12889-015-2340-y

30. Baldinger N, Krebs A, Müller R, Aeberli I. Swiss children consuming breakfast regularly have better motor functional skills and are less overweight than breakfast skippers. J Am Coll Nutr, 2012;31(2):87-93. Doi: 10.1080/07315724.2012.10720013

31. World Health Organization. Global strategy on diet, physical activity and health. Geneva, 2004. Available from: https://www.who.int/dietphysicalactivity/strategy/eb11344/strategy_english_web.pdf

32. Cooper AJ, Forouhi NG, Buijsse B, Arriola L, Balkau B, Barricarte A, et al. Fruit and vegetable intake and type 2 diabetes: EPIC-InterAct prospective study and meta-analysis. Eur J Clin Nutr, 2012;66(10):1082-1092. Doi: 10.1038/ejcn.2012.85

33. Liu J, Wang J, Leng Y, Lv C. Intake of fruit and vegetables and risk of esophageal squamous cell carcinoma: a meta-analysis of observational studies. Int J Cancer, 2013;133(2):473-485. Doi: 10.1002/ijc.28024

34. Folkvord F, Naderer B, Coates A, Boyland E. Promoting Fruit and Vegetable Consumption for Childhood Obesity Prevention. Nutrients, 2021;14(1):157. Doi: 10.3390/nu14010157

35. Poti JM, Duffey KJ, Popkin BM. The association of fast food consumption with poor dietary outcomes and obesity among children: is it the fast food or the remainder of the diet? Am J Clin Nutr, 2014;99(1):162-171. Doi: 10.3945/ajcn.113.071928

36. Viner RM, Cole TJ. Television viewing in early childhood predicts adult body mass index. J Pediatr, 2005;147(4):429-435. Doi: 0.1016/j.jpeds.2005.05.005

37. Hubbard K, Economos CD, Bakun P, Boulos R, Chui K, Mueller MP, et al. Disparities in moderate-to-vigorous physical activity among girls and overweight and obese schoolchildren during school-and out-of-school time. Int J Behav Nutr Phys Act, 2016;13:39. Doi: 10.1186/s12966-016-0358-x

38. St-Onge MP, Shechter A. Sleep restriction in adolescents: forging the path towards obesity and diabetes? Sleep, 2013;36(6):813-814. Doi: 10.5665/sleep.2694

39. Skjåkødegård HF, Danielsen YS, Frisk B, Hystad SW, Roelants M, Pallesen S, et al. Beyond sleep duration: Sleep timing as a risk factor for childhood obesity. Pediatr Obes, 2021;16(1):e12698. Doi: 10.1111/ijpo.12698

40. Guerlich K, Gruszfeld D, Czech-Kowalska J, Ferre N, Closa-Monasterolo R, Martin F, et al. Sleep duration and problem behaviour in 8-year-old children in the Childhood Obesity Project. Eur Child Adolesc Psychiatry, 2022;31(3):519-527. Doi: 10.1007/s00787-021-01731-8

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The relationship between healthy living-style behaviors and type-2 diabetes risk of students of health sciences

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ABSTRACT

The aim of this study was to determine the relationship between healthy life-style behaviors and risk of Type 2 Diabetes Mellitus of students, and also to compare the sub-dimensions of Healthy Living-Style Behaviors Scale-II (HLBS-II) with the anthropometry and general characteristics. Socio-demographic form, HLBS-II and The Finnish Diabetes Risk Score (FINDRISC) were used and anthropometric measurements were taken. With the increase in waist/height ratio, physical activity sub-dimension of HLBS-II was affected ($p<0.05$). The medical check-up status effected every sub-dimension and the total score of HLBS-II ($p<0.001$). With the increase in waist/hip ratio of female students, FINDRISC also increased ($p<0.001$). As the waist/height ratio increased, the mean scores of FINDRISC also increased ($p<0.001$). Students with a BMI value ≥30 had higher FINDRISC scores ($p<0.001$). There is a negative relationship between HLBS-II total score, nutrition, self-actualization, and stress management, which are sub-dimensions of HLBS-II, and FINDRISC scores of students of health sciences.

Keywords: Type 2 Diabetes, FINDRISC, HLBS II, health behavior, university students

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INTRODUCTION

Diabetes is defined as a metabolic disease with a chronic course that occurs as a result of insufficiency in insulin secretion or in the use of insulin. This metabolic disease is based on the constant high level of sugar in the blood[1]. According to TURDEP I and TURDEP II studies conducted on approximately 25.000 people in 1997 and 2010 in Turkey, diabetes prevalence increased from 7.2% to 13.7% in a 12-year period[2,3]. It is important for individuals to be able to understand health-related information and maintain their health, because diabetes is a disease that can be prevented and/or controlled before it occurs. Creating the correct perception and increasing awareness about the disease shows that it is possible to prevent the rate of diabetes increase and all related complications[4]. The main goal of the treatment of diabetic individuals should be to provide glycemic control. In addition, other known risk factors such as blood pressure and weight gain of patients should be monitored[5]. In order to bring diabetic individuals blood glucose levels to the reference levels and to optimize their daily life activities, they should receive a medical therapy, medical nutrition therapy and increase their physical activity[6].

The basis of healthy lifestyle choices and behaviors exhibited in adulthood is laid in childhood and adolescence[7]. In this period, when young individuals start university life, which also includes adolescence, they try to get used to many changes that also affects their habits in adulthood. Individuals' in this period, leaving the family home, tending towards eating behaviors independent of the family, preferring food such as fast food rather than healthy food, inactivity, trying to get used to the university life, meeting new people and wanting to resemble their peers, increasing the tendency to use tobacco and tobacco products may pave the way for the emergence of many chronic diseases such as diabetes in the future, as well as causing many changes in individuals' private life and healthy lifestyle behaviors[8-10]. Some of the important causes of diabetes are social environment, lack of information and motivation of individuals and an understanding of unhealthy lifestyle[11]. The fact that university students are in the young age group may reduce the risk of diabetes, but the increase in obesity in recent years due to the sedentary life of the students and the rapid life causes the Type 2 Diabetes Mellitus (T2DM) risk prevalence of university students to increase[12,13]. Students are expected to reflect these behaviors to their lives with the education they receive so that they can gain healthy eating habits, recognize changeable risk factors of diabetes such as increasing physical activity, and make healthy lifestyle behaviors a habit.

Health sciences students' application of healthy lifestyle behaviors to their lives affects the lives of other people in terms of both increasing their quality of life and being a role model for the society they live in[8,14,15]. With this study, it was aimed to determine the relationship between healthy lifestyle behaviors of health science students, who will have a key role in the future both in the society and health institutions, and their risk of developing T2DM.

METHODOLOGY

Study design and sampling

This cross-sectional study was conducted at Marmara University Faculty of Health Sciences between November 2019 and May 2020.

The sample size was calculated using the EpiInfo program. In this calculation, the incidence of the event was 50%, the error level was 5%, and the pattern effect was taken as 2, and the sample size was determined as 648. For the losses that may arise during the research process, it was planned to invite 730 students to the study.

The inclusion criteria for this study were: To be a registered student of the Faculty of Health Sciences at the duration of the study. The exclusion criteria were: Pregnant and lactating women, students that were diagnosed as Type 1 or Type 2 Diabetes Mellitus prior to the study.

Measures

The data was collected by the researchers during face-to-face interviews. Participants of the study completed a socio-demographic form, The Healthy Living-Style Behaviors Scale II (HLBS II) and The Finnish Diabetes Risk Score (FINDRISC) form.

The Healthy Living-Style Behaviors Scale II: HLBS II was prepared by Walker et al. in 1987 and renewed in 1996[16]. The scale measures health-promoting behaviors, such as healthy eating, regular physical activity, positive relationships and reducing stress, associated with an individual's healthy lifestyle. The scale consists of 52 items in total and has 6 sub-factors. Subgroups are health responsibility, physical activity, nutrition, self-actualization, interpersonal support and stress management. The overall score of the scale gives the healthy lifestyle behaviors score. All items of the scale are positive. The rating is in the form of a 4-point Likert; never (1), sometimes (2), often (3), regularly (4). The lowest score for the entire scale is 52, the highest score is 208 and higher scores are interpreted as good healthy lifestyle behavior of the individuals. In our country, a validity and reliability study were carried out by Bahar and col-

leagues; the Cronbach Alpha coefficient of the scale is 0.92 and it has a high degree of reliability. The reliability coefficients of the sub-dimensions of the scale are; Health responsibility 0.77, Physical Activity 0.79, Nutrition 0.68, Self-Actualization 0.79, Interpersonal Support 0.80, Stress Management 0.64[17].

The Finnish Diabetes Risk Score: FINDRISC was developed in 2003 by Lindström and Tuomilehto to measure the 10-year risk of developing T2DM in Finland[18]. FINDRISC is also used by the International Diabetes Federation, and its Turkish translation has been made by Turkey Endocrinology and Metabolism Society in our country. It is recommended to be used for research on risk of developing diabetes in the following 10-years. FINDRISC consists of 8 questions. When the scores obtained to determine the diabetes risk of individuals are added together, those who score less than 7 are considered to have "low risk", 7-11 points have "mild risk", 12-14 points have "medium risk", 15-20 points have "high risk" and more than 20 points are considered to have "very high risk"[6].

Evaluation of anthropometric measurements

All anthropometric measurements were carried out by the researchers at the faculty. The height of the students was measured with a fixed height meter that had 0.5 cm intervals; the measurements were taken without shoes. For body weight, a bioelectric impedance analysis device (Inbody 270 portable) was used. Students were asked to remove all heavy clothing and shoes before stepping on the device. The device was set to -1.0 kg for the remaining clothes. Waist circumference (WC) was measured after normal exhalation, with an inflexible tape at the umbilicus level and without clothes in the area[19], and hip circumference were measured around the largest part of hips and the distance was noted.

Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared and classified into four groups according to World Health Organization. The BMI was considered underweight if it was <18.5, normal if it was 18.5-24.9 kg/m^2, overweight if the BMI was 25.0-29.9 kg/m^2, obese if the BMI was ≥30.0[20].

Statistical analysis

The data were evaluated statistically using the SPSS (Statistical Package for the Social Sciences) 28.0 package program. The Kolmogorov Smirnov Z test was used to determine whether the mean scores of the scale were compatible with the normal distribution. Spearman correlation for determining the relationship between scale scores (sub-dimensions of HBLS and FINDRISC); para-

metric (Independent t-test, One-way ANOVA test), or non-parametric tests (Man Whitney U test, Kruskal Wallis test) were used to compare scale scores with independent variables. Statistical significance was accepted as $p<0.05$ in all analyzes.

RESULTS and DISCUSSION

From the 730 students that were invited for the study, 9 students were excluded for reasons such as not meeting inclusion criteria, and with 721 students the study was started. Five students were excluded from the study due to missing data. Overall, 716 (98.1%) students in 2nd, 3rd and 4th grades from the Department of Nutrition and Dietetics, Physiotherapy and Rehabilitation, Midwifery, Health Management and Nursing completed the study (Figure 1).

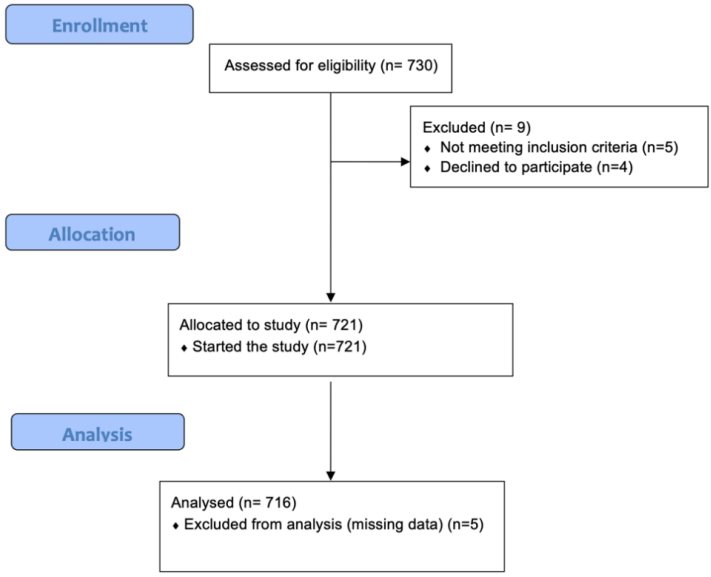

Figure 1. Modified CONSORT flow diagram for a single-arm, nonrandomized study

General characteristics of students were shown in Table 1. Of all students, 99.03% were single, most of the students (43.44%) lived with their family and only 9.93% were employed. The median age of students was 21.0 (19.0-33.0), BMI was 21.3 (15.8-38.5), the median waist circumference measurement was 71.6 cm (58-122) and the median hip circumference measurement was 96.0 cm (69.0-130.0). The median of total scores of HLBS II was 129.0 (64.0-185.0) (not shown in table).

Table 1. General characteristics and anthropometric measurements of students (n=716)

Variable	Number (n)	Percent (%)
Gender		
Female	607	84.78
Male	109	15.22
Department		
Nutrition and Dietetics	127	17.73
Midwifery	101	14.10
Physiotherapy & Rehabilitation	147	20.54
Nursing	283	39.53
Health Management	58	8.10
Class		
2nd grade	234	32.68
3rd grade	253	35.34
4th grade	229	31.98
Body Mass Index		
Underweight (<18.5)	85	11.87
Normal (18.50-24.99)	549	76.68
Overweight (≥25)	67	9.35
Obese (≥30)	15	2.09
Number of Main Meals		
<3 meals	340	47.49
3 meals	372	51.96
>3 meals	4	0.55
Meal Skipping Status		
Yes	578	80.73
No	138	19.27
Physical Activity Level		
Very light	68	9.49
Light	226	31.57
Moderate	379	52.94
Vigorous	40	5.59
Maximal	3	0.41
Medical Problems		
Yes	63	8.79
No	653	91.21
Medical Check-ups		
Yes	247	34.49
No	469	65.51
FINDRISC Categories		
Low Risk	615	85.9
Mild Risk	79	11.0
Medium Risk	17	2.4
High Risk	5	0.7
Very High Risk	0	0.0

Considering the risk of developing T2DM in the next 10 years, it was seen that majority (85.9%) of the students participating in the study were in the low-risk group and only a few of them (0.7%) was in the high-risk group. In a study in which Çolak used FINDRISC, it was observed that 72% of university students

had low risk of T2DM, 24.7% had mild risk, 2.8% had moderate risk and 0.5% had high risk, and these results were similar to our findings[21].

The items of the FINDRISC scale and the distribution of students according to these items were shown in Table 2. Since all the students were under the age of 45, they received 0 points from this item. Only 2.1% of the students had a BMI above 30 and 3.3% had higher waist circumference than reference values and got 3 points in these categories (see Table 1 for the FINDRISC category distribution of students).

Table 2. Distribution of FINDRISC Type 2 Diabetes Risk Factors (n=716)

Variables	Category		FINDRISC Scores	Number (n)	Percent (%)
Age	<45		0	716	100
Family history of diabetes	No		0	289	40.4
	Yes, 1st degree relative		3	303	42.3
	Yes, 2nd degree relative		5	124	17.3
Waist Circumference (cm)	Female	Male			
	<80	<94	0	614	85.8
	80-88	94-102	3	78	10.9
	>88	>102	4	24	3.3
30 minutes exercise per day	Yes		0	678	94.7
	No		2	38	5.3
Daily consumption of vegetables and fruits	Yes		0	364	50.8
	No		1	352	49.2
Use of blood pressure medication	No		0	695	97.1
	Yes		2	21	2.9
History of high blood glucose	No		0	669	93.4
	Yes		5	47	6.6
BMI	<25		0	628	87.7
	25-30		1	73	10.2
	>30		3	15	2.1

According to the data obtained by comparing the anthropometric measurements and the FINDRISC scores presented in Table 3; statistically significant differences were found between T2DM risk scores and waist circumference of both female students' ($p<0.001$) and male students' ($p=0.01$). It was observed that students with a BMI value of 30 and above had statistically higher FINDRISC scores ($p<0.001$).

Table 3. Comparison of anthropometric measurements and FINDRISC Type 2 Diabetes Risk Scores (n=716)

Variables	FINDRISC Score (Mean±SD)	Statistics* Post Hoc**

Waist Circumference of Females (cm)		
<80	3.07±2.39[a]	F= 198.03 p<0.001
80-88	6.03±3.73[b]	c>b>a
>88	9.26±4.00[c]	
Waist Circumference of Males (cm)		
<94	3.57±2.92[a]	F= 6.586 p=0.010
94-102	9.92±4.38[b]	b>a
>102	11.60±2.07[c]	
Waist/Hip Ratio of Females		
<0.85	3.55±2.96	Z=-5.750 p<0.001
>0.85	6.62±4.10	
Waist/Height Ratio		F=109.672 p<0.001
<0.4	3.04±2.30[a]	c>a
0.4-<0.5	3.20±2.54[b]	d>a
0.5-0.6	8.53±3.62[c]	c>b
>0.6	11.85±1.77[d]	d>b
		d>c
BMI		F=101.968 p<0.001
Underweight (<18.5)	3.23±2.31[a]	c>a
Normal (18.50-24.99)	3.14±2.47[b]	d>a
Overweight (≥25)	7.49±3.99[c]	c>b
Obese (≥30)	11.73±2.18[d]	d>b
		d>c

*Z= Mann Whitney U test, F= One-Way ANOVA test **PostHoc = Scheffe Test, Tamhane's T2

Recent studies on waist/height ratio emphasize that this ratio is a better measure for determining cardiometabolic risk and T2DM risk than BMI, waist circumference and waist/hip ratio[22-24]. In this study, a statistically significant difference was found between the waist/height ratio of the students and their diabetes risk scores. When the data obtained were evaluated, it was determined that when waist/height ratio were increased, the averages of FINDRISC scores were also increased.

In Gezer's study to determine the risk of diabetes with nursing students between the ages of 19-24, the rate of female students in the low-risk group for T2DM was found to be 65.5%, while the rate of male students in the same risk group was found to be 77.0%[22]. In our study, no relationship was found between the gender of the students and their diabetes risk scores.

Shown in Table 4, the relationship between the general characteristics of the participants and their HLBS II scores was examined. The average of health re-

sponsibility sub-dimension was higher in female students whose waist circumference was higher than 88 cm and the average score of interpersonal support sub-dimension was higher in those with a waist circumference lower than 80 cm (respectively, p=0.001; p=0.037). The average score of the physical activity sub-dimension of the nursing students was higher than the other departments (p=0.021) and nutrition and dietetics students' average score for the nutrition sub-dimension was higher than the other departments (p<0.001). Also, the mean score of the nutrition sub-dimension of third grade students was found to be statistically higher than other grades (p=0.042) (not shown in table).

Table 4. Comparison of general characteristics and anthropometric measurements of students and sub-dimensions of the Healthy Living-Style Behaviors Scale (n=716)

Variables		Health Responsibility		Physical Activity		Nutrition		Self-actualization		Interpersonal Support		Stress Management		HLBS II Total	
	n	Min.-Max.	Median	Min.-Max.	Median	Min.-Max.	Median	Min.-Max.	Median	Min.-Max.	Median	Min.-Max.	Median	Mean	±SD
Gender															
Female	607	9-35	21	8-32	16	10-34	20	11-36	26	14-36	26	9-29	19	128.6	16.7
Male	109	10-34	20	8-32	18	9-34	20	11-36	26	13-36	26	9-29	19	128.8	20.7
		p=0.125*		p<0.001*		p=0.413*		p=0.974*		p=0.139*		p=0.867*		p=0.914**	
Waist/Height Ratio															
<0.4	180	9-34	20	8-28	16	11-29	19.5	14-36	26	13-36	26	11-29	19	126.5	16.8
0.4-<0.5	462	9-35	21	8-32	17	9-34	20	11-36	27	14-36	26	9-29	19	129.8	17.8
0.5-0.6	67	11-34	21	8-30	17	12-28	20	16-35	26	14-33	26	11-26	19	127.7	15.9
>0.6	7	19-25	23	8-25	15	18-24	19	19-30	25	18-27	24	13-22	16	120	15.6
		p=0.284***		p=0.026***		p=0.103***		p=0.117***		p=0.318***		p=0.051***		p=0.103****	
BMI															
Underweight (<18.5)	85	11-33	20	8-29	16	11-26	19	14-3	26	15-36	26	11-29	18	124.5	15.8
Normal (18.50-24.99)	549	9-35	21	8-32	17	10-34	20	11-36	26	13-36	26	9-29	19	129.4	17.5
Overweight (≥25)	67	10-34	21	8-28	17	9-29	20	11-36	26	16-36	26	9-27	19	128.1	17.6
Obese (≥30)	15	17-34	23	8-30	15	15-28	19	19-34	26	18-33	27	12-26	18	127.8	20.6
		p=0.029***		p=0.012***		p=0.003***		p=0.536***		p=0.970***		p=0.541***		p=0.114****	
Main Meals															
<3 meals	340	9-34	20	8-32	17	9-32	19	11-36	26	14-36	25	9-29	19	126.1	17.1
3 meals	372	11-35	21	8-32	17	12-34	21	13-36	27	13-36	26.5	11-27	19	131.1	16.7
>3 meals	4	22-24	22	15-21	18	16-26	20	21-31	29	19-30	27	14-24	19	133.2	18.7
		p=0.006***		p=0.699***		p<0.001***		p=0.020***		p=0.020***		p=0.105***		p=0.001****	

Table 4. Comparison of general characteristics and anthropometric measurements of students and sub-dimensions of the Healthy Living-Style Behaviors Scale (n=716)

Variables		Health Responsibility		Physical Activity		Nutrition		Self-actualization		Interpersonal Support		Stress Management		HLBS II Total	
	n	Min.-Max.	Median	Min.-Max.	Median	Min.-Max.	Median	Min.-Max.	Median	Min.-Max.	Median	Min.-Max.	Median	Mean	±SD
Mothers' Educational Status															
Not Literate	57	10-28	19	8-24	16	9-26	18	11-34	25	16-36	24	9-26	18	118.7	20.2
Literate	52	10-27	20	8-30	16	12-26	20	18-36	26	14-34	25	12-29	19	125.9	15.2
Primary education	425	9-35	21	8-32	17	10-34	20	11-36	27	14-36	26	9-29	19	129.3	16.6
High school	136	11-34	21	8-29	16	12-34	21	13-36	26	13-36	26	11-28	19	130.3	18.1
University and above	46	14-39	22	8-31	17	12-32	21	20-34	27	20-34	27	14-28	19	133.5	16.3
		p<0.001***		p=0.157***		p=0.001***		p=0.045***		p=0.001***		p=0.168***		p<0.001****	
Fathers' Educational Status															
Not Literate	10	11-18	18	8-20	14	9-24	17.5	11-34	24.5	16-36	23.5	9-24	16.5	117.1	26.6
Literate	22	14-26	19	8-25	16.5	12-26	20	18-34	25.5	15-34	23.5	13-23	19	123.6	17.6
Primary education	343	9-33	21	8-32	17	11-33	20	11-36	26	14-36	26	9-29	19	128.3	17.1
High school	233	9-35	20	8-32	17	10-34	20	11-36	26	13-36	26	9-29	19	128.7	17.6
University and above	108	13-34	21	8-38	16	12-29	21	17-36	26	19-36	27	12-28	19	131.9	16.3
		p=0.088***		p=0.650***		p=0.016***		p=0.503***		p=0.027***		p=0.643***		p=0.038****	
Medical Check-ups															
Yes	247	9-35	23	8-32	18	10-34	21	13-36	27	18-36	27	12-29	20	136.5	16.2
No	469	9-33	19	8-32	16	9-34	19	11-36	26	13-36	25	9-28	19	124.5	16.6
		p<0.001*		p<0.001*		p<0.001*		p<0.001*		p<0.001*		p<0.001*		p<0.001**	

*Mann Whitney U test **Independent Sample T test ***Kruskal Wallis test

****One-Way ANOVA test SD: Standard Deviation

Table 5. Relationship between sub-dimensions of Healthy Living-Style Behaviors Scale and FINDRISC Type 2 Diabetes Risk Assessment (n=716)

	Health Responsibility		Physical Activity		Nutrition		Self-actualization		Interpersonal Support		Stress Management		Total HLBS II Score	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p
FINDRISC Total Score	0.015	0.680	-0.034	0.361	-0.078	**0.037**	-0.085	**0.022**	-0.061	0.103	-0.127	**0.001**	-0.087	**0.020**

*Spearman Correlation test

The correlations between the sub-dimensions of HLBS II and FINDRISC scores were shown in Table 5.

In a study conducted with only female university students, the score of physical activity of sub-dimension of HLBS II were found to be the lowest of all sub-dimensions[25]. In another study, it was found that male university students' physical activity and stress management sub-dimensions of HLBS II were significantly higher than female students[26]. Similar to this study, we found the physical activity sub-dimension scores of male university students statistically higher than the scores of female students.

In a study it was found that the average scores of self-actualization, physical activity, nutrition, interpersonal support and total HLBS II scores of the group with normal waist-to-height ratio (0.4-0.5) to be significantly higher than students with waist-to-height ratio lower than 0.4[25]. Similarly in our study we found that physical activity sub-dimension of HLBS II scores were statistically higher in students with normal waist-to height ratio (0.4-0.6). While some studies could not find any difference between the nutrition sub-dimension and BMI[15,27], Alkan et al. found that students with normal BMI had higher scores in nutrition sub-dimension than underweight students[25]. In our study we found that nutrition sub-dimension score was significantly higher in students that were in the normal and overweight BMI range.

In current study, statistically significant differences were found between the mothers' educational status of the students and the health responsibility, nutrition, self-actualization and interpersonal support. Also, statistically significant differences were found between the fathers' educational status of the students and the sub-dimension of HLBS II; nutrition, interpersonal support and total score of HLBS II. In a study conducted in Mexico, it was observed that as the mothers' educational level increased, the mean scores in nutrition, physical activity, stress management, interpersonal support subscales and the total score

of HLBS II increased significantly[28]. In the study of Tuğut and Bekar, when the health perception and healthy lifestyle behaviors of university students were examined, it was seen that educational status of mothers and fathers was effective in terms of health perception on university students[29]. These results support our findings.

In similar studies it was stated that students mostly had three main meals[30,31]. In the study conducted by Mazıcıoğlu and Öztürk with third and fourth grades of university students, it was found that 48.9% consumed three meals a day, 24.8% consumed less than three meals and 26.1% consumed more than three meals a day[32]. In our study, 51.96% of the students had three main meals, while 47.49% had less than 3 meals and 0.55% had more than 3 meals a day. Significant differences were found between students' main meal consumption status and subscales of HLBS II; health responsibility, nutrition, self-actualization, interpersonal support and total HLBS II scores. Accordingly, it was seen that the average HLBS II score of those who consume more than 3 meals is higher than those who consume 3 meals or less. The reason of majority of the students participating in this study consuming 3 or more meals may be due to the fact that the study was conducted in the faculty of health sciences and the awareness on this issue was high.

In our study, statistically significant differences were found between students' medical check-up status and HLBS II sub-dimensions; health responsibility, physical activity, nutrition, self-actualization, interpersonal support, stress management and HLBS II total score. Accordingly, the average HLBS II score of the students who had medical check-ups was found to be higher than the students who did not. In the study conducted by Cihangiroglu and Deveci with health school students, it was determined that as the students' evaluation of their health status increased in the "good" direction, the total score of the HLBS II scale and the mean scores of health responsibility, physical activity and stress management also increased[15]. Similarly, in the study of Ayaz and colleagues, it was reported that there was a positive significant relationship between the importance of health and self-actualization, nutrition, stress management and HLBS II scale scores[33]. The students' fulfillment of these attitudes and behaviors and their high scores suggested that they care about their health, taking responsibility for their own care, monitoring their own health, having regular medical check-ups, paying attention to the frequency and order of medical controls, and their behaviors in maintaining and improving health were sufficient.

The fact that the study was conducted in a single university and the female gender was very high compared to the males can be shown among the limitations of the study. In addition, since the health awareness of the students studying in health-related departments is high, it is necessary to conduct similar studies with students from other departments.

In conclusion, this student-based study has various results that healthy living-style behaviors have an important impact on the risk of type 2 diabetes mellitus. Students' BMIs, waist/height ratio, waist to hip ratio, waist circumferences have effects on their FINDRISC scores. Also, genders, the educational levels of parents, numbers of main meals and getting medical check-ups affect their HLBS II scores. Moreover, the sub-dimensions of HLBS II (especially, nutrition, self-actualization, and stress management) can affect the FINDRISC total scores. When all our findings are considered together, the risk of developing T2DM may be low but still present in the students of health sciences, especially in terms of anthropometric measurements and socio-demographic characteristics.

STATEMENT OF ETHICS

This study was approved ethically by the Marmara University Faculty of Health Sciences Non-Invasive Clinical Studies Ethics Committee with the protocol no: 31.10.2019/103 and the research was conducted following the principles stated in the Helsinki Declaration.

CONFLICT OF INTEREST STATEMENT

No conflict of interest was declared by the authors.

AUTHOR CONTRIBUTIONS

Design: AHİ, FEG; Acquisition of data: GS, MA, BA; Analysis of data: AHİ, GS, MA, ZMÇ; Drafting of the manuscript: AHİ, ZMÇ; Critical revision of the manuscript: AHİ, FEG; Statistical analysis: AHİ, ZMÇ; Supervision: AHİ, FEG.

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REFERENCES

1. Rosenbek Minet LK. Self-Management in Diabetes Care. The importance of self-care management intervention in chronically ill patients diagnosed with diabetes. Syddansk Universitet. Det Sundhedsvidenskabelige Fakultet, 2011.

2. Satman I, Yilmaz T, Sengül A, Salman S, Salman F, Uygur S, et al. Population-based study of diabetes and risk characteristics in Turkey: results of the Turkish diabetes epidemiology study (TURDEP). Diabetes Care, 2002;25(9):1551-1556. Doi: 10.2337/diacare.25.9.1551

3. Satman I, Imamoğlu S, Yilmaz C, Ayvaz G, Colekci A. Diabetes in Turkey and in the World. 16th edition. Turk J Endocrinol Metab, 2012;24-27.

4. Atmaca HU, Akbaş F, Şak T, Şak DU, Acar Ş, Niyazoğlu M. Consciousness level and disease awareness among patients with diabetes. Istanb Med J, 2015;16(3):101-104. Doi: 10.5152/imj.2015.57625

5. Erol Ö. Endocrine system diseases and care, internal medicine nursing. Istanbul: Akademi Press; 2013. p. 251-278.

6. Society of Endocrinology and Metabolism of Turkey. Manual of Diagnosis, Management and Follow-up of Diabetes Mellitus and Its Complications. 2019.

7. Kelly SA, Melynk BM, Jacobson DL. Correlates among healthy lifestyle cognitive beliefs, healthy lifestyle choices, social support and healthy behaviors in adolescents: implications for behavioral change strategies and future research. J Pediatr Health Care, 2011;25(4):216-223. Doi: 10.1016/j.pedhc.2010.03.002

8. Bozlar V, Arslanoğlu C. Healthy life style behaviors of university students of school of physical education and sports in terms of body mass index and other variables. Univers. J Educ Res, 2016;4(5):1189-1195. Doi: 10.13189/ujer.2016.040532

9. Kadıoğlu M, Ergün A. The eating attitudes of the university students, self-efficacy and affecting factors. Clin Exp Health Sci, 2015;5(2):96-104. Doi: 10.5455/musbed.20150309011008

10. Jones JM, Bennett S, Olmsted MP, Lawson ML, Rodin G. Disordered eating attitudes and behaviours in teenaged girls: a school-based study. Can Med Assoc J, 2001;165(5):547-552.

11. Metintas S, Kalyoncu C, Arıkan İ. Two distinct training methods for a doctrine of life with healthy heart in a low socioeconomic society model. Int J Environ Health Res, 2009;6(11):2883-2897. Doi: 10.3390/ijerph6112883

12. Lima ACS, Araújo MFM, de Freitas RWJF, Zanetti ML, de Almeida PC, Damasceno MMC. Risk factors for Type 2 Diabetes Mellitus in College Students: Association with Sociodemographic Variables. Rev Latino-Am, 2014;22:484-490. Doi: 10.1590/0104-1169.3053.2441

13. Sealey-Potts C, Reyes-Velazquez W. Perceived and Actual Risks of College Students for Developing Type 2 Diabetes. Austin J Nutr Metab, 2014;1:1-5.

14. Topbaş E. Type 2 diabetes Mellitus DM risk in university students and related factors. Acıbadem Üniv Sağlık Bilim Derg, 2019;10(4):616-620. Doi: 10.31067/0.2019.192

15. Cihangiroğlu Z, Deveci SE. Healthy life style behaviours and related influencing factors of the students of Elazig High School of Health Sciences of Fırat University. Fırat Medical Journal, 2011;16(2):78-83.

16. Walker SN, Hill-Polerecky DM. Psychometric evaluation of the health-promoting lifestyle profile II. Unpublished manuscript, University of Nebraska Medical Center, 1996;13:120-126.

17. Bahar Z, Beşer A, Gördes N, Ersen F, Kıssal A. Validity and Reliability Study of the Healthy Lifestyle Behaviors Scale II. *Journal of Cumhuriyet University School of Nursing*. 2008;12(1):1-13.

18. Lindström J, Tuomilehto J. A practical tool to predict type 2 diabetes risk. Diabetes Care, 2003;26(3):725-731. Doi: 10.2337/diacare.26.3.725

19. Saghafi-Asl M, Pirouzpanah S, Ebrahimi-Mameghani M, Asghari-Jafarabadi M, Aliashrafi S, Sadein B. Lipid profile in relation to anthropometric indices and insulin resistance in overweight women with polycystic ovary syndrome. Health Promot Perspect, 2013;3:206-216. Doi: 10.5681/hpp.2013.024

20. World Health Organization. Body mass index classification. https://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi. Accessed 22 October 2022.

21. Çolak M. Physical Activity Levels and Type 2 Diabetes Risk Scores of University Students. Stud Ethno Med, 2015;(3):401-409. Doi: 10.1080/09735070.2015.11905458

22. Gezer C. Evaluation of the relationship between waist/height ratio and type 2 diabetes risk in nursing students. Food and Health. 2017;3(4):141-149. Doi: 10.3153/JFHS17017

23. Bohr AD, Laurson K, McQueen MB. A novel cutoff for the waist-to-height ratio predicting metabolic syndrome in young American adults. BMC Public Health, 2016;16:295-303. Doi: 10.1186/s12889-016-2964-6

24. Ashwell M, Gunn P, Gibson S. Waist-to-height ration is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: systematic review and meta-analysis. Obes Rev, 2012;13:275-286. Doi: 10.1111/j.1467-789X.2011.00952.x

25. Alkan S, Ersoy N, Eskici G, Ersoy, G. Assessment of eating attitudes and healthy lifestyle behaviors of females students studying nutrition and dietetics. Indian J Nutr Diet, 2017;54(1):1-13. DOI:10.21048/ijnd.2017.54.1.10841

26. Oral B, Cetinkaya F. Health perceptions and healthy lifestyle behaviors of Erciyes University students. Medicine, 2020;9(4):829-836. DOI: 10.5455/medscience.2020.05.076

27. Bozhuyuk A. Assessing the healthy lifestyle behaviors of the health science students in Cukurova University. In: Faculty of Medicine. Cukurova University, Adana. 2010.

28. Ulla Díez SM, Pérez-Fortis A. Socio-demographic predictors of health behaviors in Mexican college students. Health Promot Int, 2010;25(1):85-93. Doi: 10.1093/heapro/dap047

29. Tuğut N, Bekar M. The relationship between university students' health perceptions and healthy lifestyle behaviors. Atatürk Üniversitesi Hemşirelik Yüksekokul Dergisi, 2008;11(3).

30. Saygın M, Öngel K, Çalışkan S, et al. Nutritional habits of Süleyman Demirel University students. SDÜ Tıp Fakültesi Dergisi. 2011;18(2),43-47.

31. Orak S, Akgün S, Orhan H. Investigation of nutritional habits of Süleyman Demirel University students. SDÜ Tıp Fakültesi Dergisi, 2006;13(2):5-11.

32. Mazıcıoğlu MM, Öztürk A. Dietary habits and influencing factors in university students at 3rd and 4th grades. Erciyes Tıp Dergisi, 2003;25(4):172-178.

33. Ayaz S, Tezcan S, Akıncı F. Health promotion behaviors of nursing school students. Cumhuriyet Üniversitesi Hemşirelik Yüksek Okulu Dergisi, 2005;9:26-34.

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Monitoring the charge variant profile of antibody-tomaymycin conjugates by icIEF method

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ABSTRACT

This study aimed to evaluate the charge variant profile of antibody-tomaymycin conjugates using an imaged capillary isoelectric focusing method (icIEF). Two unconjugated antibodies were examined, along with two conjugation methods involving cleavable and non-cleavable linkers. The cleavable linker contained one amine group. mAb-1 demonstrated greater homogeneity with a single main peak at a p*I* of 8.5, whereas mAb-2 exhibited two main peaks at p*I* values of 8.95 and 9.00. Conjugation with tomaymycin molecules resulted in an increased charge heterogeneity of both cleavable and non-cleavable conjugates. The non-cleavable conjugates displayed a higher number of additional acidic variants (p*I* range: 7.4 to 8.4 for mAb-1 and 8.2 to 8.9 for mAb-2) compared to the cleavable conjugates (p*I* range: 7.8 to 8.4 for mAb-1 and 8.4 to 8.9 for mAb-2). The linker nature had a lesser influence on the charge heterogeneity of the antibody-tomaymycin conjugates compared to the nature of the unconjugated antibody.

Keywords: antibody, charge isoforms, conjugates, linker

INTRODUCTION

Monoclonal antibodies (mAbs) constitute a very important therapeutic class developed to treat various diseases, including cancer[1]. Conjugation of antibod-

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ies with chemotherapeutic drugs has been extensively studied to improve their antitumor activity[2-4]. To date, the FDA has approved twelve antibody drug conjugates (ADCs) for marketing[5].

ADCs are composed of cytotoxic drugs covalently attached to monoclonal antibodies via a chemical linker. The conjugation process of drug molecules often involves cysteine (Cys) and lysine (Lys) residues on the monoclonal antibodies[6].

The choice of chemical linkers plays a critical role in determining the efficacy of investigated ADCs[5-9]. The linkers must ensure the chemical stability of the ADC in the bloodstream and facilitate the rapid release of cytotoxic agents to target cells. ADC linkers in ADCs can be classified into two groups: cleavable and non-cleavable linkers[5-8]. Over 80% of approved ADCs employ cleavable linkers[9].

Cleavable linkers efficiently release the active agents from ADCs within targeted cells through chemical mechanisms such as pH changes and redox reactions (hydrazone or disulfide bonds) or enzymatic activity (peptide-based or phosphate-based linkers)[7-9]. Non-cleavable linkers (thioether and maleimidocaproyl) are chemically and enzymatically stable[7-9].

The physicochemical properties of ADCs significantly influence their therapeutic effectiveness, emphasizing the importance of evaluating ADC homogeneity. Homogeneous drug conjugation is crucial for producing effective anticancer ADCs[6,10]. Therefore, assessing the charge heterogeneity of ADCs using relevant methods is essential[11-14].

Monitoring the charge variants forms of mAbs or ADCs provides valuable information on protein stability, batch to batch purity, degradation pathways, and more. Various chromatographic and electrophoretic methods have been employed to evaluate the charge variant profile of ADCs[15-20]. Whole-column imaging-detection capillary isoelectric focusing (icIEF) have been utilized for monitoring the charge heterogeneity of mAbs and ADCs due to its advantages such as higher resolution, speed, and quantitative analysis[20-21].

Tomaymycin is an anti-cancer antibiotic produced by Streptomyces achromogenes[22]. In this study, tomaymycin molecules were linked to two monoclonal antibodies through non-cleavable or cleavable linkers. The objective of this work was to study the impact of the nature of the antibody and the linker on the charge distribution profile of the tomaymycin conjugates using icIEF method developed in another study[23].

METHODOLOGY

Chemicals

The ICE280 chemical test kit, ICE280 electrolyte solution Kit, 1% and 0.5% methylcellulose, and p*I* markers (6.61, 7.05, 8.18 and 9.5) were obtained from Convergent Bioscience. The Pharmalyte solutions (3-10 and 8-10.5) were obtained from GE Healthcare. Urea, sucrose, histidine, and phosphoric acid were purchased from Sigma.

mAbs and ADCs

Two monoclonal antibodies, namely mAb-1 (anti-EphA2) and mAb-2 (anti-CD19), were investigated in this study. The naked antibody solutions of both mAbs were prepared in a phosphate buffer at a pH of 6.5 with an approximate concentration of 10mg/mL.

The two mAbs were conjugated to tomaymycin molecules via two types of linkers: cleavable and non-cleavable linker. These ADCs were formulated in a buffer containing 10mM histidine, 10% sucrose, and N-methyl-2-pyrrolidone (NMPs%) at a pH of 6.5 with an concentration of approximately 2mg/mL.

Sample preparation

The protein sample was prepared by diluting to the desired final concentration in a solution containing 0.35% methylcellulose, 4% pharmalytes (3–10) and pharmalytes (8–10.5) in a 1:1 ratio, 2M urea and p*I* markers (6.61, 8.81, 7.05, and 9.5 Following the preparation, the sample was centrifuged at 6000rpm for 3minutes to remove any precipitates. Subsequently, the clarified sample was transferred to a glass autosampler vial and centrifuged again to eliminate any remaining bubbles. Finally, the prepared sample was placed in the autosampler carousel for subsequent analysis.

icIEF instrument

The icIEF analysis was performed using an iCE280 instrument equipped with a PrinCE autosampler, manufactured by Convergent Bioscience. The capillary column utilized in the analysis had dimensions of 50mm length, 100µm inner diameter (ID), and 200µm outer diameter (OD). This transparent capillary column was embedded into a glass cartridge, and its inner surface was coated with a fluorocarbon material to minimize electroosmotic flow. For the analysis of both the ADCs and mAbs, a cathodic solution consisting of 100mM NaOH and 0.1% methylcellulose, as well as an anodic solution containing 80mM H_3PO_4 and 0.1% methylcellulose, were employed. The protein focusing time

was carried out for either 7 or 10minutes at a voltage of 3000V. Detection of the focused proteins was achieved using a CCD camera operating at a wavelength of 280nm.

RESULTS and DISCUSSION

mAbs are protein molecules that often exhibit a significant level of charge heterogeneity. This heterogeneity can arise from modifications that occur during various stages from production to storage[13]. Characterizing the charge variant profile of therapeutic mAbs is crucial for ensuring purity and batch-to-batch consistency[11-14].

In this study, two specific monoclonal antibodies were investigated: mAb-1, an anti-CD19 antibody targeting the CD19 cell surface antigen, and mAb-2, an anti-EphA2 antibody designed to recognize and bind to EphA2.

The charge heterogeneity profiles of these mAbs were analyzed using icIEF, and the results are presented in (Figure 1). It was observed that mAb-1 exhibited greater homogeneity and had a more acidic nature compared to mAb-2.

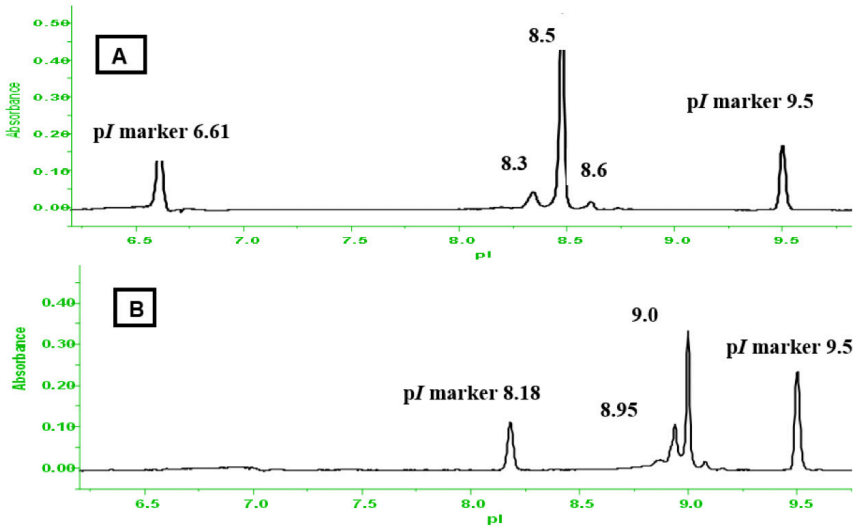

Figure 1. Analysis of unconjugated antibodies by icIEF: (A) mAb-1 and (B) mAb-2. Experimental conditions; final concentration 0.2 mg/mL in 0.35% methyl cellulose, 2% 3–10 pharmalytes and 2% 8–10.5 pharmalytes (1:1 ratio) and 2M urea. pI markers: 6.61, 8.18, 9.50. Focusing time: 7min at 3000 V. Detection I: 280 nm.

Table 1 provides further details regarding the charge variants of the two mAbs. For mAb-1, a single main peak corresponding to a specific charge variant with a p*I* value of 8.5 was observed, accounting for 85% of the total. On the other hand, mAb-2 displayed two distinct charge variants, with p*I* values of 9.00 (60%) and 8.95 (30%). The presence of the more acidic charge variant in mAb-2 (p*I* 8.95) suggests the possibility of deamidation occurring at one or two asparagine (Asn) residues, leading to a shift in the p*I* value compared to the main charge variant (p*I* 9.00) [24]. This observation aligns with previous studies that have reported similar p*I* values for charge variants of anti-EphA2 mAbs, albeit with higher percentages (9.0 at 64% and 8.9 at 36%)[19].

Table 1. Charge variant profile of unconjugated antibodies (mAb-1 and mAb-2), including charge variant number, p*I* range, Δ p*I*, And % Area of major species.

Unconjugated antibody	Charge variant Number	p*I* range	Δ p*I*	p*I* and % Area of major species
mAb-1	3	8.3-8.6	0.3	8.5: 85%
mAb-2	4	8.9-9.1	0.2	8.95: 30%, 9.00: 60%

Overall, both mAbs exhibited an acceptable level of charge homogeneity, and the observed degree of charge heterogeneity is consistent with what is typically observed in therapeutic monoclonal antibodies in previous studies[6, 16, 19, 25-27].

Antibody-tomaymycin conjugate charge profile

Two monoclonal antibodies (mAb-1 and mAb-2) that were characterized using icIEF were conjugated to tomaymycin molecules using either non-cleavable or cleavable linkers. The effects of the linker type on the charge variant profile of the resulting ADCs were evaluated using the icIEF method.

Cleavable conjugates

The tomaymycin molecules were conjugated to the amino groups of the mAb Lys residues using an optimized cleavable linker that contained a hindered disulfide bond (Figure 2). Conjugating the Lys residues of the mAb can reduce the solubility of the mAb conjugates due to the decreased net charge of the resulting conjugate[14]. To improve the solubility of the resulting ADCs, the cleavable linker incorporates an amino group. This amino group is expected to introduce additional positive charges to the antibody-tomaymycin conjugates and potentially alter their charge profile. The charge variant profiles of the cleavable antibody-tomaymycin conjugates are depicted in (Figure 3).

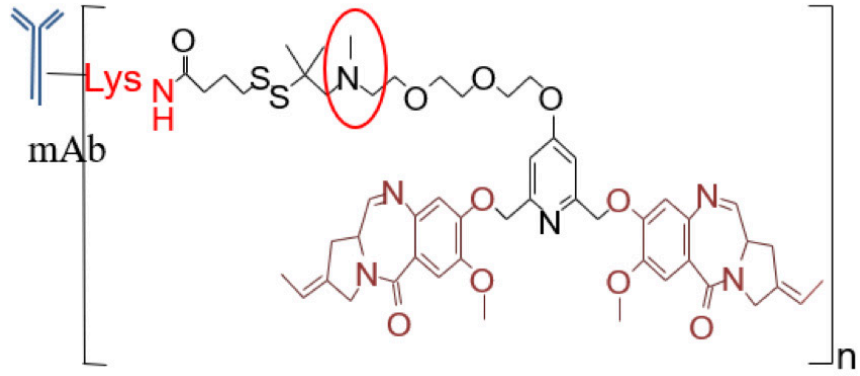

Figure 2. Chemical structure of tomaymycin molecules conjugated to mAbs Lys residues through cleavable linker. The cleavable linker contains a hindered disulfide bond and an amine group.

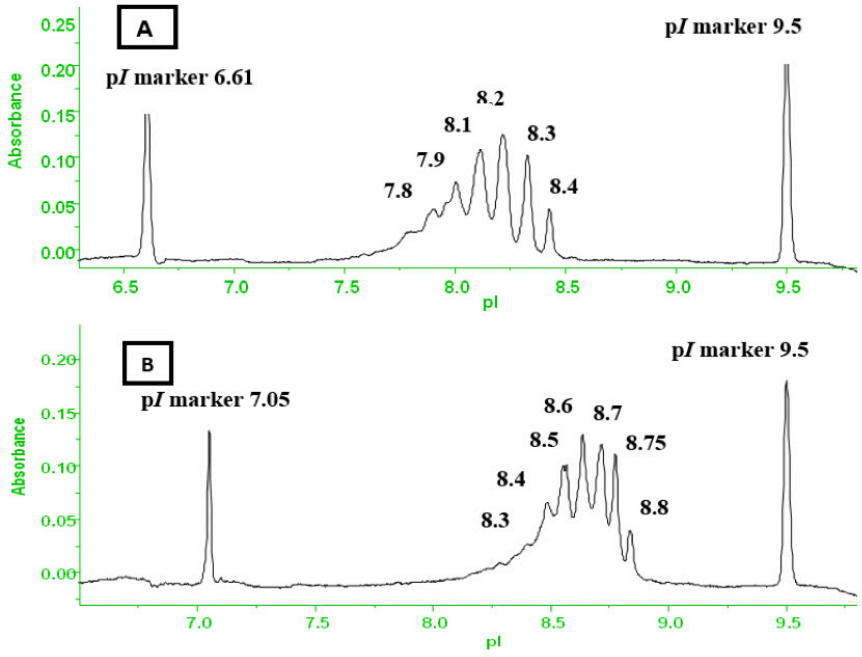

Figure 3. Analysis using icIEF of (a) cleavable mAb-1 tomaymycin conjugate, (b) cleavable mAb-2 tomaymycin conjugate. Experimental conditions; final concentration 0.5 mg/mL in 0.35% methyl cellulose, 3–10 pharmalytes (2%) and 8–10.5 pharmalytes (2%) in 1:1 ratio and 2M urea. pI markers: 6.61, 7. 05, 9.50. Focusing time: 10 min at 3000 V. Detection I: 280 nm.

As anticipated, the cleavable antibody-tomaymycin conjugates exhibited greater heterogeneity compared to the corresponding unconjugated antibodies[11]. While the unconjugated antibodies had a narrow p*I* range (Δp*I*: 0.2 for mAb-1 and Δp*I*: 0.3 for mAb-2) (Table 1), the cleavable antibody-tomaymycin conjugates displayed a wider p*I* range (Δp*I*: approximately 0.5) and the same number of charge variants (7) (Table 2). This charge heterogeneity in the ADCs is due to the covalent binding of the tomaymycin-linker to the free amino groups of the mAb Lys residues. Typically, mAbs contain up to 80 Lys residues[6], resulting in a heterogeneous mixture of unconjugated mAbs and mAbs conjugated with varying numbers of drugs in random combinations at different sites on the antibodies[6,7].

Table 2. Charge variant profiles of cleavable tomaymycin conjugated mAb-1 and mAb-2, including charge variant number, p*I* range, Δ p*I*, and % area of major species

cleavable tomaymycin conjugates	Charge variant Number	p*I* range	Δ p*I*	p*I* and % Area of major species
mAb-1	7	7.8-8.4	0.6	8.1: 21%, 8.2: 22%, 8.3: 18%, 8.4: 6%
mAb-2	7	8.4-8.9	0.5	8.5:21%, 8.6:21%, 8.75: 13%, 8.8: 4.8%

The cleavable antibody-tomaymycin conjugates exhibited increased acidity (p*I* range for tomaymycin-conjugated mAb-1: 7.8 to 8.4 and mAb-2: 8.4 to 8.9) compared to their unconjugated counterparts (p*I* range for mAb-1: 8.3 to 8.6 and mAb-2: 8.9 to 9.1). The decrease in the p*I* range of the cleavable ADCs is attributed to the reduction in positive charges when the tomaymycin-linker is attached to the mAb Lys residues. The number of Lys amino groups conjugated to the tomaymycin-linker affects the p*I* values of the resulting charge variants, with an increase in modified amino groups leading to more acidic variants. Previous studies have demonstrated that chemical conjugation of mAbs with various drugs using Lys residues can alter the electrostatic properties of the mAb surface and decrease the p*I* values[11-13,28].

The findings regarding the charge variant profiles of the investigated cleavable ADCs align with previous study on cleavable maytansinoid antibody conjugates, which exhibited higher heterogeneity and acidity compared to the unconjugated monoclonal antibodies[19]. Similar observations were made by Baylon et al., where the chemical conjugation of IgG1 Fc with Alexa Fluor 350 via Lys residues resulted in a decrease in the p*I* of the conjugated species compared to the unconjugated species[29].

The p*I* values of the charge variants in the cleavable mAb-1-tomaymycin conjugates were lower than those in the cleavable mAb-2-tomaymycin conjugates. This can be attributed to the fact that mAb-1 is inherently more acidic than mAb-2. Consequently, the p*I* values of the charge variants in the mAb-1-tomaymycin conjugates shifted towards lower values compared to the charge variants in the mAb-2-tomaymycin conjugates.

Evaluating the level of unconjugated antibodies in the ADC conjugates is crucial, as residual unconjugated antibody can directly impact the efficacy of the ADC[11,30]. Table 2 demonstrates that the percentage of charge isoforms corresponding to unconjugated antibodies was approximately 0% in the ADC, indicating the successful conjugation process of the mAb with tomaymycin.

Non-cleavable conjugates

The tomaymycin molecules were conjugated to the amino groups of Lys (Lys) residues in the two monoclonal antibodies under investigation using non-cleavable linkers, as depicted in (Figure 4). The charge heterogeneity profiles of the non-cleavable tomaymycin-conjugated antibodies are presented in (Figure 5). Detailed information on the charge profiles of the non-cleavable monoclonal antibody tomaymycin conjugates, including the number of isoforms, p*I* range, Δp*I*, and p*I* and % area of major species, can be found in (Table 3).

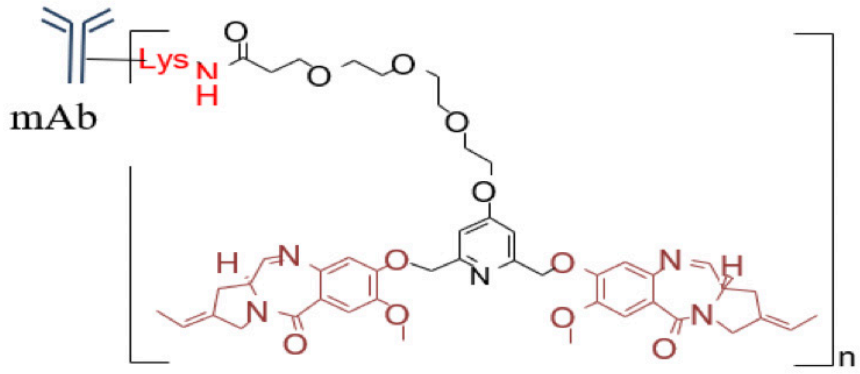

Figure 4. Chemical structure of tomaymycin molecules conjugated to mAbs Lys residues through non-cleavable linker.

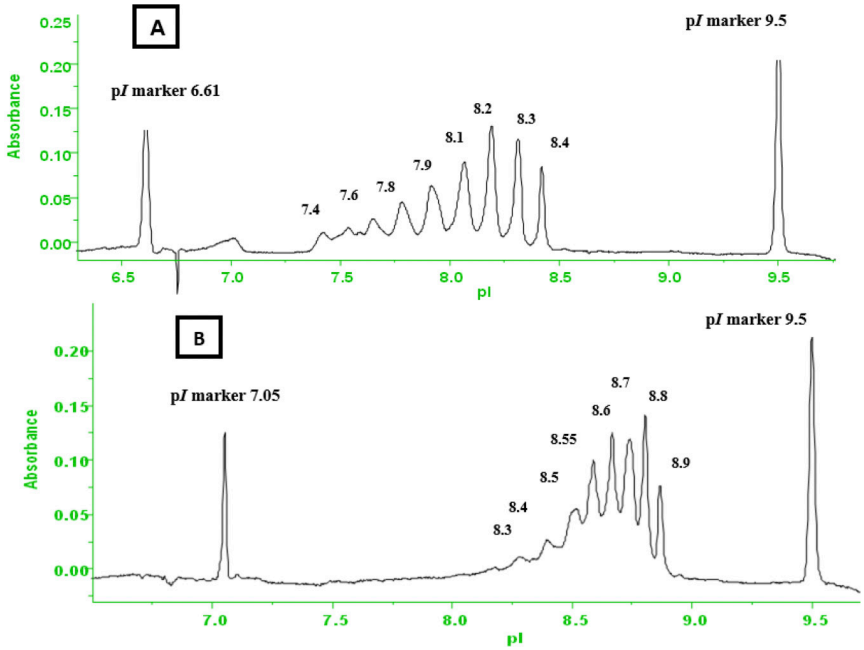

Figure 5. Analysis using icIEF of (a) non-cleavable tomaymycin mAb-1 conjugate, (b) noncleavable tomaymycin mAb-2 conjugate. Experimental conditions; final concentration 0.5 mg/mL in 0.35% methyl cellulose, 2% 3–10 pharmalyte and 2% 8–10.5 pharmalyte in 1:1 ratio and 2M urea. p*I* markers: 6.61, 7.05, 9.50. Focusing time 10 min at 3000 V. Detection I: 280 nm.

Table 3. Charge variant profiles of non-cleavable tomaymycin conjugated mAb-1 and mAb-2, including charge variant number, p*I* range, Δ p*I*, And % Area of major species

Non-cleavable tomaymycin conjugate	Charge variant number	pI range	Δ p*I*	p*I* and % area of major species
mAb-1	9	7.4-8.4	1	8.1: 27%, 8.2: 27%, 8.3: 17%, 8.4: 10%
mAb-2	8	8.2-8.9	0.7	8.6:20%, 8.7: 22%, 8.8:17%, 8.9: 7.5%

Similarly for the cleavable tomaymycin conjugated antibodies, it was observed that the non-cleavable conjugates exhibited greater heterogeneity and acidity compared to their corresponding unconjugated antibodies. The pI ranges for the mAb-1 and mAb-2 tomaymycin conjugates with non-cleavable linkers were 7.4 to 8.4 (Δp*I*: 1) and 8.2 to 8.9 (Δp*I*: 0.7), respectively. The increase in charge heterogeneity of the non-cleavable conjugates (wide Δp*I*) and the decrease of the isoforms p*I* values was were attributed to the number of drugs attached to the free amino groups of Lys residues in the antibodies. The percentage of

charge isoforms corresponding to the naked antibodies was approximately 0% for non-cleavable conjugates.

Comparison of charge profiles of cleavable and non-cleavable conjugates

When comparing the charge profiles of cleavable and non-cleavable conjugates, it was observed that the non-cleavable mAb-1 tomaymycin conjugates exhibited higher heterogeneity (Δp*I*: 1) compared to the cleavable conjugates (Δp*I*: 0.6), while the non-cleavable mAb-2 conjugates showed a slight increase in charge heterogeneity (Δp*I*: 0.6) compared to the cleavable conjugates (Δp*I*: 0.5).

The number of charge variants was higher for the non-cleavable conjugates (9 for mAb-1 and 8 for mAb-2) compared to the cleavable conjugates (7 for both mAb-1 and mAb-2).

The non-cleavable conjugates exhibited additional acidic variants (p*I* range: 7.4 to 8.4 for mAb-1 conjugates and 8.2 to 8.9 for mAb-2 conjugates) compared to the cleavable conjugates (p*I* range: 7.8 to 8.4 for mAb-1 conjugates and 8.4 to 8.9 for mAb-2 conjugates). These additional acidic isoforms suggested an increase in the number of drug-linker attached to the amino groups of Lys residues. Similar findings were reported by Lin et al., where the icIEF charge profile of mAb-DM4 conjugates exhibited a shift in p*I* values towards acidic values due to an increase in the number of conjugated DM4 molecules[31].

As mentioned earlier, the cleavable linker contained a basic group (amino group). Previous studies by Stan et al. indicated that conjugation of drugs to mAb carbohydrates did not affect the protein charge, but when the drug-linker possessed a charge, there was a change in the p*I* values of the resulting conjugates[32]. Therefore, it was expected that the additional amino groups in the cleavable linker would increase the p*I* of the charge variants in the cleavable conjugates. Interestingly, the percentages of the most basic species (p*I* 8.4 for mAb-1 and p*I* 8.9 for mAb-2 conjugates) were higher for the non-cleavable conjugates compared to the cleavable conjugates. These results indicate that the additional amino group in the cleavable linker did not significantly impact the p*I* of the charge variants in the conjugates.

Two monoclonal antibodies, designated as mAb-1 and mAb-2, were conjugated to tomaymycin molecules through either a non-cleavable or cleavable linker. The results obtained from icIEF demonstrated that mAb-1 exhibited greater homogeneity compared to mAb-2. Conjugation process led to a decrease in the p*I* values and an increase in charge heterogeneity of the tomaymycin-antibody

conjugate when compared to the unconjugated antibody. The impact of the unconjugated antibody's characteristics on the p*I* isoform profile of the antibody-drug conjugate (ADC) appears to be more significant than the nature of the linker (cleavable *vs* non-cleavable).

STATEMENT OF ETHICS

Ethical approval was not required to perform this study.

CONFLICT OF INTEREST STATEMENT

The author declares that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Ayat Abbood carried out the analysis and wrote the article.

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Not Applicable

REFERENCES

1. Alejandra WP, Miriam Irene JP, Fabio Antonio GS, Patricia RR, Elizabeth TA, Aleman-Aguilar JP. et al. Production of monoclonal antibodies for therapeutic purposes: a review. Int Immunopharmacol, 2023;120:110376. Doi: 10.1016/j.intimp.2023.110376

2. Khongorzul P, Ling CJ, Khan FU, Ihsan AU, Zhang J. Antibody-drug conjugates: a comprehensive review. Mol Cancer Res, 2020;18(1):3-19. Doi : 10.1158/1541-7786.MCR-19-0582

3. Dumontet C, Reichert JM, Senter PD, Lambert J, Beck A. Antibody–drug conjugates come of age in oncology. Nat Rev Drug Discov, 2023;(22):641-661.
Doi: 10.1038/s41573-023-00709-2

4. Fu Z, Li S, Han S, Shi C, Zhang Y. Antibody drug conjugate: the «biological missile» for targeted cancer therapy. Signal Transduct Target Ther, 2022;7(1):93. Doi: 10.1038/s41392-022-00947-7

5. Sheyi R, de la Torre BG, Albericio F. Linkers: an assurance for controlled delivery of antibody-drug conjugate. Pharmaceutics, 2022;14(2):396. Doi : 10.3390/pharmaceutics14020396

6. Tsuchikama K, An Z. Antibody-drug conjugates: recent advances in conjugation and linker chemistries. Protein Cell, 2018;9:33-46. Doi : 10.1007/s13238-016-0323-0

7. Su Z, Xiao D, Fei Xie, Liu L, Wang Y, Fan S, et al. Antibody–drug conjugates: recent advances in linker chemistry. Acta Pharm Sin B, 2021;11(12):3889-3907. Doi : 10.1016/j.apsb.2021.03.042

8. Jain N, Smith SW, Ghone S, Tomczuk B. Current ADC linker chemistry. Pharm Res, 2015;32(11):3526-3540. Doi: 10.1007/s11095-015-1657-7

9. Lyon R. Drawing lessons from the clinical development of antibody-drug conjugates. Drug Discov Today Technol, 2018;30:105-109. Doi : 10.1016/j.ddtec.2018.10.001

10. Anami Y, Otani Y, Xiong W, Ha SYY, Yamaguchi A, Rivera-Caraballo KA, et al. Homogeneity of antibody-drug conjugates critically impacts the therapeutic efficacy in brain tumors. Cell Rep, 2022;39(8):10839. Doi: 10.1016/j.celrep.2022.110839

11. Wagh A, Song H, Zeng M, Tao L, Das TK. Challenges and new frontiers in analytical characterization of antibody-drug conjugates. Mabs, 2018;1(2):222-243. Doi : 10.1080/19420862.2017.1412025

11. Cauchon NS, Oghamian S, Hassanpour S, Abernathy M. Innovation in chemistry, manufacturing, and controls-a regulatory perspective from industry. J Pharm Sci, 2019;108(7):207–2237. Doi :10.1016/j.xphs.2019.02.007

12. Torkashvand F, Vaziri B. Main Quality attributes of monoclonal antibodies and effect of cell culture components. *Iran Biomed J*. 2017;21(3):131-141. Doi: 10.18869/acadpub.ibj.21.3.131

13. Wakankar A, Chen Y, Gokarn Y, Jacobson FS. Analytical methods for physicochemical characterization of antibody drug conjugates. *Mabs*. 2011;3(2):161-172. Doi : 10.4161/mabs.3.2.14960

14. Zhu X, Huo S, Xue C, An B, Qu J. Current LC-MS-based strategies for characterization and quantification of antibody-drug conjugates. J Pharm Sci, 2020;10(3):209-220. Doi : 10.1016/j.jpha.2020.05.008

15. Thallaj N, Abbood A. Comparison between chromatofocusing and icIEF charge variant profiles of unconjugated monoclonal antibodies and their drug conjugates. Tishreen University Journal-Medical Sciences Series, 2023;45(2):47-57.

16. Kaur H, Beckman J, Zhang Y, Li ZJ, Szigeti M, Guttman A. Capillary electrophoresis and the biopharmaceutical industry: therapeutic protein analysis and characterization. TrAC Trends Anal Chem, 2021;144:116407. Doi : 10.1016/j.trac.2021.116407

17. Chen T, Chen Y, Stella C, Medley CD, Gruenhagen JA, Zhang K. Antibody-drug conjugate characterization by chromatographic and electrophoretic techniques. J Chromatogr B Analyt Technol Biomed Life Sci, 2016;1032:39-50. Doi : 10.1016/j.jchromb.2016.07.023

18. Abbood, A. Study of formulation effects on the charge variant profile of antibody maytansine conjugates by icIEF method. Acta Pharm Sci, 2023. Doi: 10.1155/2023/8150143

19. Mack S, Cruzado-Park I, Chapman J, Ratnayake C, Vigh G. A systematic study in CIEF: defining and optimizing experimental parameters critical to method reproducibility and robustness. Electrophoresis, 2009;30(23):4049-4058. Doi : 10.1002/elps.200800690

20. Salas-Solano O, Kennel B, Park SS, Roby K, Sosic Z, Boumajny B, et al. Robustness of iCIEF methodology for the analysis of monoclonal antibodies: an interlaboratory study. J Sep Sci, 2012;35(22):3124-3129. Doi : 10.1002/jssc.201200633

21. Arima K, Kosaka M, Tamura G, Imanaka H, Sakai H. Studies on tomaymycin, a new antibiotic. I. Isolation and properties of tomaymycin. J Antibiot (Tokyo), 1972;25(8):437-444. Doi : 10.7164/antibiotics.25.437

22. Abbood A. Optimization of the Imaged cIEF Method for Monitoring the Charge Heterogeneity of Antibody-Maytansine Conjugate. J Anal Methods Chem, 2023;8150143. Doi: 10.1155/2023/8150143.

23. Wakankar AA, Borchardt RT. Formulation considerations for proteins susceptible to asparagine deamidation and aspartate isomerization. J Pharm Sci, 2006;95(11):2321-2336. Doi : 10.1002/jps.20740

24. Füssl F, Trappe A, Cook K, Scheffler K, Fitzgerald O, Bones J. Comprehensive characterisation of the heterogeneity of adalimumab via charge variant analysis hyphenated online to native high resolution Orbitrap mass spectrometry. Mabs, 2019;11(1):116-128. Doi : 10.1080/19420862.2018.1531664

25. Füssl F, Cook K, Scheffler K, Farrell A, Mittermayr S, Bones J. Charge Variant Analysis of Monoclonal Antibodies Using Direct Coupled pH Gradient Cation Exchange Chromatography to High-Resolution Native Mass Spectrometry. Anal Chem, 2018;90(7):4669-4676. Doi: 10.1021/acs.analchem.7b05241

26. Gahoual R, Beck A, Leize-Wagner E, François YN. Cutting-edge capillary electrophoresis characterization of monoclonal antibodies and related products. J Chromatogr B Analyt Technol Biomed Life Sci, 2016;1032:61-78. Doi: 10.1016/j.jchromb.2016.05.028

27. Lechner A, Giorgetti J, Gahoual R, Beck A, Leize-Wagner E, François YN. Insights from capillary electrophoresis approaches for characterization of monoclonal antibodies and antibody drug conjugates in the period 2016-2018. J Chromatogr B Analyt Technol Biomed Life Sci, 2019;1122-1123:1-17. Doi: 10.1016/j.jchromb.2019.05.014

28. Boylan NJ, Zhou W, Proos RJ, Tolbert TJ, Wolfe JL, Laurence JS. Conjugation site heterogeneity causes variable electrostatic properties in Fc conjugates. Bioconjug Chem, 2013;24(6):1008-1016. Doi : 10.1021/bc4000564

29. Chen L, Wang L, Shion H, Yu C, Yu YQ, Zhu L, et al. In-depth structural characterization of Kadcyla® (ado-trastuzumab emtansine) and its biosimilar candidate. Mabs, 2016;8(7):1210-1223. Doi: 10.1080/19420862.2016.1204502.

30. Lin J, Lazar AC. Determination of charge heterogeneity and level of unconjugated anti-

body by imaged cIEF. Methods Mol Biol, 2013;1045:295-302. Doi: 10.1007/978-1-62703-541-5_19

31. Stan AC, Radu DL, Casares S, Bona CA, Brumeanu TD. Antineoplastic efficacy of doxorubicin enzymatically assembled on galactose residues of a monoclonal antibody specific for the carcinoembryonic antigen. Cancer Res, 1999;59(1):115-121.