e-ISSN: 2621 - 4814

Borneo Journal of PHARMACY

Volume 7 Issue 2 May 2024

Accredited at SINTA 2 until February 2025
by Ministry of Research and Technology / National Research and Innovation Agency, Indonesia
No: 148/M/KPT/2020.

Institute for Research and Community Services
Universitas Muhammadiyah Palangkaraya

BORNEO JOURNAL OF PHARMACY

Borneo J Pharm
e-ISSN: 2621-4814

Volume 7 Issue 2 May 2024

Department of Pharmacy
Faculty of Health Sciences
Universitas Muhammadiyah Palangkaraya

PUBLISHED BY

Institute for Research and Community Services
Universitas Muhammadiyah Palangkaraya
RTA Milono St. Km. 1,5 Palangka Raya 73111
bjop@umpr.ac.id
https://journal.umpr.ac.id/index.php/bjop

EDITORIAL WORDS

apt. Mohammad Rizki Fadhil Pratama, S.Farm., M.Si.

Editor in Chief
Borneo J Pharm

Assalamu'alaikum Wr. Wb.

Alhamdulillahirabbil 'alamin. The next edition of **Borneo Journal of Pharmacy** (*Borneo J Pharm*), has been published at May 2024. This edition contains ten articles: Pharmacology-Toxicology, Pharmacognosy-Phytochemistry, Pharmaceutical, Analytical Pharmacy-Medicinal Chemistry, Microbiology Pharmacy, Natural Product Development, Clinical-Community Pharmacy, and Management Pharmacy. This edition includes writings from five countries: Indonesia, Malaysia, Nigeria, Sierra Leone, and Uganda. The authors come from several institutions, including Institut Teknologi Bandung, Universitas Darussalam Gontor, Universitas Koperasi Indonesia, Universitas Lambung Mangkurat, Universitas Muhammadiyah Prof. DR. HAMKA, Institut Pertanian Bogor, Biomatics Indonesia, Indonesian Institute of Bioinformatics, Universitas Nasional, University of Ilorin, Summit University, Ambrose Alli University, Mbarara University of Science and Technology, Kampala International University, Premium Medical Services, Kebbi State University of Science and Technology, Clarke International University, Universitas Muhammadiyah Yogyakarta, International Islamic University Malaysia, Universitas Mahasaraswati Denpasar, Universitas Indonesia, National Research and Innovation Agency of the Republic of Indonesia, Menur National Mental Hospital, and East Java Provincial Health Service.

Editorial boards are fully aware that there are still room for improvement in this edition, hence with all humility willing to accept constructive suggestions and feedback for improvements to the publication for the next editions. The editorial board would like to thank all editors and reviewers, and contributors of the scientific articles who have provided the repetoire in this issue. We hope that all parties, especially the contributors, could re-participate for the publication in the next edition on August 2024.

Wassalamu'alaikum Wr. Wb.

Palangka Raya, May 2024

Editor-in-Chief

Table of Contents

BORNEO JOURNAL OF PHARMACY

Borneo J Pharm – e-ISSN: 2621-4814

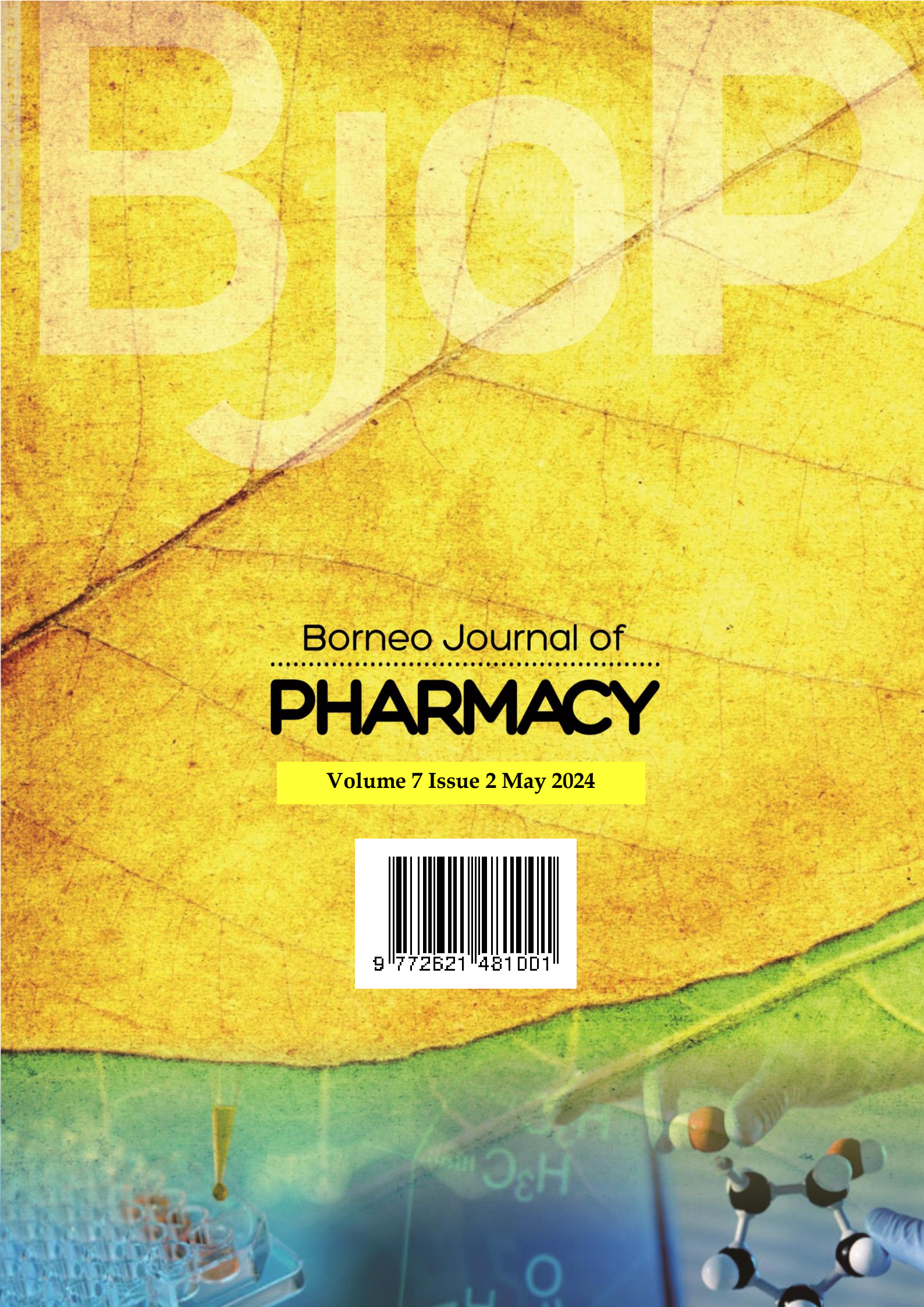
Volume 7 Issue 2 May 2024

Author Guidelines

BORNEO JOURNAL OF PHARMACY

Borneo J Pharm – e-ISSN: 2621-4814

1. Writing is the result of research or review of critical analysis study in the field but not limited to Pharmacology-Toxicology, Pharmacognosy-Phytochemistry, Pharmaceutical, Analytical Pharmacy-Medicinal Chemistry, Natural Product Development, Clinical-Community Pharmacy, Management Pharmacy, and other Pharmacy aspects which have never been published in other scientific publication media.
2. Manuscripts that have been published in the form of a preprint on several platforms such as arXiv, INA-RXiv, SSRN, and ResearchGate are welcome to publish.
3. The manuscript is written in English with Book Antiqua font 10 pt, two-column, density 1.15 spaces, on A4 paper with a top-down margin of 2.5 cm and right-left margins 1.5 cm.
4. The manuscript is typed with MS-Word program using the provided template and saved in .doc or .docx format.
5. The manuscript is typed strictly with the following rules:
   a. The title, short and clear, written in English. The title length does not exceed 20 words.
   b. Authors, listed without mention of title or degree, under the name including the affiliation and complete address, and email of the corresponding author. The author's name, which consists of one word, must be repeated twice for first and last names. To author with names of more than two words, the author's last name consists of only one word, the rest is written as the first name.
   c. Abstract, written in English which is a summary of the article. The abstract is created in one paragraph and a maximum of 250 words with Book Antiqua font 9 pt, single column, and space 1. Points that must be listed in the abstract include the research objectives, methods, results, conclusions, and keywords. The number of keywords is between three and five keywords.
   d. Introduction, contain background, reasons for the importance of underlying research or hypotheses, general approaches and objectives of the research and relevant literature review. The number of paragraphs not restricted but the contents must include background, objectives, positions on previous research, as well as the novelty of the research. The little theoretical basis which indirectly related to the contents of the article could be included without sub-chapters required. Citation is written in occurrence number on the manuscript (Vancouver style).
   e. Methodology, Research method consists of 2 sub-chapters including materials and methods. If data analysis is specific, or the way of analysis is novel, then data analysis subchapter could be added. The sub-chapters are written without numbering or bullet.
   f. Results and Discussion, at least contain three subjects (1) description of results, (2) interpretation or explanation of results, and (3) comparative of results with previous studies results. If the subchapters very length could be made sub-sub-chapters with Arabic numbering. Description of the results can be in the form of tables and figures with a serial number (Table uses a sequence of Roman numerals and placed at the top, while figures using Arabic numeric sequence and placed in the below).
   g. Conclusion, made in a single paragraph without the citation contains the final conclusion and suggestions for advanced research.
   h. References, a list of recently selected topics is published last five years (the minimum percentage and a minimum number of references are different for each type of manuscript). The bibliography is written in chronological order with the serial number (Vancouver style) and suggested using a reference management application such as EndNote, Mendeley, Zotero, and other applications.
6. Authors whose manuscripts are published are free of charge for both Publication cost and article processing charge.
7. Authors will get full access to the article published on e-journal of Universitas Muhammadiyah Palangkaraya at the website https://journal.umpr.ac.id/index.php/bjop.


BJOP
Borneo Journal of
PHARMACY
Volume 7 Issue 2 May 2024
9 772621 481001

Borneo Journal of Pharmacy
Vol 7 Issue 2 May 2024
Pages 126 – 135

https://journal.umpr.ac.id/index.php/bjop/article/view/6590
DOI: https://doi.org/10.33084/bjop.v7i2.6590
e-ISSN: 2621-4814

Research Article

Computer-Assisted Histopathological Calculation Analysis of the Sciatic Nerve of Diabetic Neuropathy Rat Model

Indah Tri Lestari [1,2*]

Kusnandar Anggadiredja [3]

Afrillia Nuryanti Garmana [4]

Sevi Nurafni [5]

[1] Doctoral Program of Pharmacy, Institut Teknologi Bandung, Bandung, West Java, Indonesia
[2] Department of Pharmacy, Universitas Darussalam Gontor, Ponorogo, East Java, Indonesia
[3] Department of Pharmacology and Clinical Pharmacy, Institut Teknologi Bandung, Bandung, West Java, Indonesia
[4] Department of Pharmacy, Institut Teknologi Bandung, Bandung, West Java, Indonesia
[5] Department of Data Science, Universitas Koperasi Indonesia, Sumedang, West Java, Indonesia

*email: indahtrilestari94@gmail.com; phone: +6288809456318

Keywords:
Gaussian adaptive threshold
Histopathology digitization
ImageJ plugin
Sciatic nerve

Abstract

Histopathology is the science that studies the signs of disease by studying the structural and functional changes that occur in cells using certain types of dyes such as hematoxylin and eosin (H&E). Traditionally histopathological testing is carried out using semi-quantitative methods. A more advanced method is done by taking photos digitally, and then digital photos are quantified with the help of software such as ImageJ using plug-in tools. Recent advances in digital pathology require the development of more efficient computerized image analysis such as the Gaussian adaptive threshold method. This research aims to compare the calculation results of computer-assisted digitalization of histopathology using the ImageJ plugin manual method with automatic calculations using Gaussian adaptive threshold to quantify the amount of sciatic nerve cell damage in the Diabetic peripheral neuropathy (DPN) rat model. In this study, two image analysis methods were used to test their ability to measure the amount of cell damage in the sciatic nerve of normal rats using a model of diabetic neuropathy. The first method uses the ImageJ plugin manual. The second method is the Gaussian adaptive threshold method. The ImageJ plugin manual method obtained a cell abnormality value of 213 cells. Meanwhile, with the Gaussian adaptive threshold method, a value of 204 cells was obtained. The calculation results of the two methods show an insignificant difference between the methods $p > 0.05$. This study presents a computerized morphometric image analysis method with the potential for pathology digitalization applications.

Received: January 15th, 2024
1st Revised: May 21st, 2024
Accepted: May 22nd, 2024
Published: May 30th, 2024

INTRODUCTION

Histopathology is the science that studies the signs of disease by studying the structural and functional changes that occur in cells and tissues. Visualization of differences in tissue components under a microscope is carried out by processing tissue biopsies or specimens and mounting them on glass slides, then the tissue is stained in such a way as to provide color contrast between the cells. Several types of dyes such as hematoxylin-eosin, immunohistochemistry, or immunofluorescence labeling have been widely used[1].

Hematoxylin and eosin (H&E) staining is the most commonly used light microscope staining method in histopathology laboratories and has been used by pathologists for over one hundred years. The H&E staining allows increasing contrast and distinguishing between nuclei and cytoplasm in tissues. Hematoxylin colors the cell nucleus blue, formed from aluminum ions and oxidized hematoxylin, while eosin colors the cytoplasm and connective tissue pink. Due to H&E's long history, established methods, and a large amount of data and publications, there is a strong belief among many pathologists that the practice of H&E will continue for the next 50 years[2]. Traditionally, histopathological testing is carried out by a

How to cite: Lestari IT, Anggadiredja K, Garmana AN, Nurafni S. Computer-Assisted Histopathological Calculation Analysis of the Sciatic Nerve of Diabetic Neuropathy Rat Model. Borneo J Pharm. 2024;7(2):126-35. doi:10.33084/bjop.v7i2.6590

pathologist using the eye to directly observe whether there are damages, differences, or cancer cells in a tissue sample after staining under a microscope using the semi-cutification method. This method takes time and effort. In addition, traditional detection methods are subjective and lack quantitative feature parameters as a reference, resulting in different recognition accuracies[3].

A more advanced method involves taking photos digitally with the help of software such as ImageJ and calculating manually using plug-in tools. Cell Counter Plug-in from the ImageJ platform (https://imagej.net/ij/index.html) was used to precisely count cell nuclei. ImageJ is a Java-based public domain image analysis program. ImageJ has become the image processing tool in many laboratories. However, counting nuclei one by one is laborious and time-consuming. Therefore, it becomes less effective in laboratories with a large number of cases for diagnosis, so automatic calculations of cellular nuclei using software began to be developed[4]. Therefore, efficient quantitative, stable, and accurate analysis detection, as well as cell image identification are the focus of many research works[3].

As technology develops, computer-aided disease diagnosis (CAD) plays a very important role and has become a major research subject in histopathological imaging and diagnostics. Computer-assisted histopathological studies have been performed for various breast cancer detection and assessment applications[4-6], colon[7,8], lung[9], prostate[10], skin[11,12], and other cancers. However, histopathological detection of other diseases is still very limited. One of the diseases that may be observed with computer-assisted histopathological studies is damage to the sciatic nerve tissue in diabetic neuropathy. Diabetic neuropathy is a rapidly growing pathology and should be of global concern. In most cases, diabetic neuropathy begins as a small fiber neuropathy affected by lower serum glucose levels and blood flow[13]. Several human and animal studies have shown that hyperglycemia-related damage to unmyelinated C fibers, small myelinated Aδ fibers, and large myelinated Aβ fibers contribute to the development of diabetic peripheral neuropathy (DPN)[14].

Over the past few years, studies in animal models have begun to yield important insights into the mechanisms of pain in DPN. The streptozotocin (STZ)-induced rat model is the most widely used experimental model in diabetes models similar to humans. Induction of inflammation has been associated with microvascular tissue and nerve damage in human diabetes and rodent models of diabetes. The observed morphological damage could provoke functional deficits of the sciatic nerve in untreated diabetic rats. The presence of abnormal fibers in the sciatic nerve with axonal degeneration and myelin damage is one of the symptoms of streptozotocin-induced diabetes in rats[15]. The large number of abnormal cells in the sciatic nerve can indicate the severity of diabetic neuropathy. Therefore, it is hoped that quantification of abnormal cells can be the basis for diagnosing the severity of diabetic neuropathy in the future. Recent advances in digital pathology have required the development of quantitative computerized image analysis and automated algorithms to assist pathologists interpreting large numbers of histopathological digital images[16].

In this study, a comparative method of computer-assisted histopathological digital image analysis was carried out on the sciatic nerve from an animal model of diabetic neuropathy using manual calculations using the ImageJ plugin and Gaussian adaptive threshold to quantify observable cell abnormalities. This histological assessment will provide important insight into the phenotypic properties of the tissue microenvironment. This is in line with the development of pharmaceutical research which often relies on visual assessment of tissue morphology, either for characterization of *in vivo* experiments, pharmacodynamics, and mechanisms of action of drugs, as well as toxicological assessments or as criteria for clinical trials. Automatic computational analysis of histopathology data is expected to speed up tissue analysis work and provide more objective quantitation with rapidly developing technology, thereby increasing the effectiveness and performance of researchers.

MATERIALS AND METHODS

Materials

The materials used in this study included a sciatic pain rat model, CO_2 gas, 10% formalin, paraffin wax blocks, 95% ethanol, eosin, hematoxylin, xylene, and distilled water. The instrument and software used included a microscope, ImageJ, and Gaussian analysis program.

Methods

The H&E staining and image digitization

Diabetic peripheral neuropathy rats were euthanized by placing them in a closed container with a flow of CO_2 gas. Sciatic nerve tissue was taken and fixed in 10% buffered formalin. After fixation, the tissue was dehydrated in gradient ethanol (70-95%). The tissue was then cleaned using xylene. Tissue was embedded in paraffin wax blocks for sectioning and sectioned to a thickness of 5 µm using a microtome. Tissue samples were placed on microscope slides and deparaffinized using xylene (100%), then rehydrated with absolute ethanol, gradient ethanol, and finally distilled water. The slides were then washed using distilled water and soaked in hematoxylin for 3-8 minutes. The slides were then cleaned with running water for 20 minutes and then rehydrated with ethanol 70-95%. The slides were then stained with eosin for 30 seconds, rinsed again with gradient ethanol immediately, and finally dried. Xylene was added to clear the tissue. The slide was then covered with a coverslip. The slides were analyzed microscopically and photographs were taken under a microscope at 4x and 10x magnification[17]. This study has been approved by the Animal Ethic Committee of Institut Teknologi Bandung with approved ID 08/KEPHP-ITB/3-2022.

Manual analysis with ImageJ plugin

Cell counting was performed using ImageJ software (https://imagej.net/ij/download.html) according to the instructions[18]. To count the cells, ImageJ was first installed. Then, the Cell Counter plugin was accessed by navigating to Plugins → Analyze → Cell Counter. This plugin is compatible with single grayscale, single-color, or multicolor images. Grayscale images were identified by "8-bit" or "16-bit" at the top, while color images were labeled "RGB". Cells were counted by clicking on them in the image. Each click added a colored box around the cell and updated the count. If an object was mistakenly included, it could be removed using Edit → Undo (limited to one undo per action). After finishing the counting process, the total cell count was obtained by clicking Analyze → Measurements in the Results window. Results could be filtered, copied and pasted, or saved as an Excel .xls spreadsheet. Saving tagged images required using the Print Screen key and saving the capture as a .tif or .jpg file.

Analysis with Gaussian adaptive threshold

The first image (Image 0) was created to show the original image. The program first read the image named "Image.jpeg" and saved it in the variable 'im'. The image was then displayed. Next, the program converted the image in 'im' to grayscale and stored it in the variable 'Gray1'. The grayscale image was then smoothed using a Gaussian filter with a kernel of (5,5) and stored in the variable 'grey'. Variables were then configured for adaptive threshold processing: -maxValue = 255, -adaptiveMethod = cv2. ADAPTIVE_THRESH_GAUSSIAN_C, -thresholdType = cv2.THRESH_BINARY, -blockSize = 3, -C = 2. The adaptive threshold process was carried out on the 'grey' image using the configured variables. The results of the process were stored in the variable 'im_threshold'. The connected objects in the image 'im_threshold' were calculated using the 'label' function in the SciPy library. The image labels were stored in the variable 'label_array'. The number of connected objects in the variable 'particle_count' was displayed in a comment line.

Data analysis

Analysis data was collected from three different areas of the image and then averaged to obtain the results. The resulting data was analyzed using paired T-test statistics, with a p-value of <0.05.

RESULTS AND DISCUSSION

Visualizing and annotating histopathology images is crucial for pharmaceutical research and clinical trials[19]. This study employed an automatic testing procedure with the Gaussian adaptive method to quantify histopathological features. The results showed no significant difference between the automatic method and manual calculations. Diabetic neuropathy involves oxidative damage that alters nerve structure. These changes, including axonal degeneration, segmental demyelination, and Schwann cell apoptosis, lead to damage or loss of myelin and unmyelinated fibers in patients. These histopathological changes manifest as distinct image features and colors between the normal and DPN groups, forming the

basis for the Gaussian method calculations. The study utilized a neuropathic diabetic pain model with nerve injury to induce hyperalgesia and allodynia in rodents, mimicking aspects of human neuropathic pain.

In histopathological testing, the first step involves preparing tissue slides. To better visualize specific structures within the tissue, such as the nucleus and cytoplasm, staining is necessary. The (H&E) staining is the most common method used for histopathological examination of the sciatic nerve. Hematoxylin selectively binds to DNA, staining the nucleus blue or purple, while eosin stains other cellular components pink, allowing for clear differentiation of various structures[20].

Following histopathological staining, the slides are examined under a microscope and digitized using a camera. Representative areas were captured as digital images using a smartphone camera. The images were taken at a magnification of 40x objective lens and 10x eyepiece lens. Since magnification affects the level of detail visible, adjusting the image threshold might be necessary for optimal analysis. Three images were chosen for cell analysis. Due to their large file size, captured RGB images are typically compressed using JPEG or JPEG 2000 formats[21].

Several factors can introduce challenges in creating optimal image designs for automated analysis of histopathology slides. These challenges arise from variations that can occur during tissue preparation, staining, and slide digitization[22]. Improper fixation, for example, can alter tissue morphology, leading to inaccurate results from image analysis software. Differences in protocols and the appearance of staining reagents can vary considerably between laboratories. Even within a single lab, staining results may differ due to pre-analytical factors like fixation delays or incompatible staining conditions. Finally, variations in slide scanners' optics, photodetectors, and light sources can contribute to display inconsistencies. The histopathological findings in this study reveal a loss of regularity in the nerve fibers. This is attributed to nerve fiber degeneration, impaired myelin density, Schwann cell degeneration, and endometrial edema, as shown in **Figure 1**.

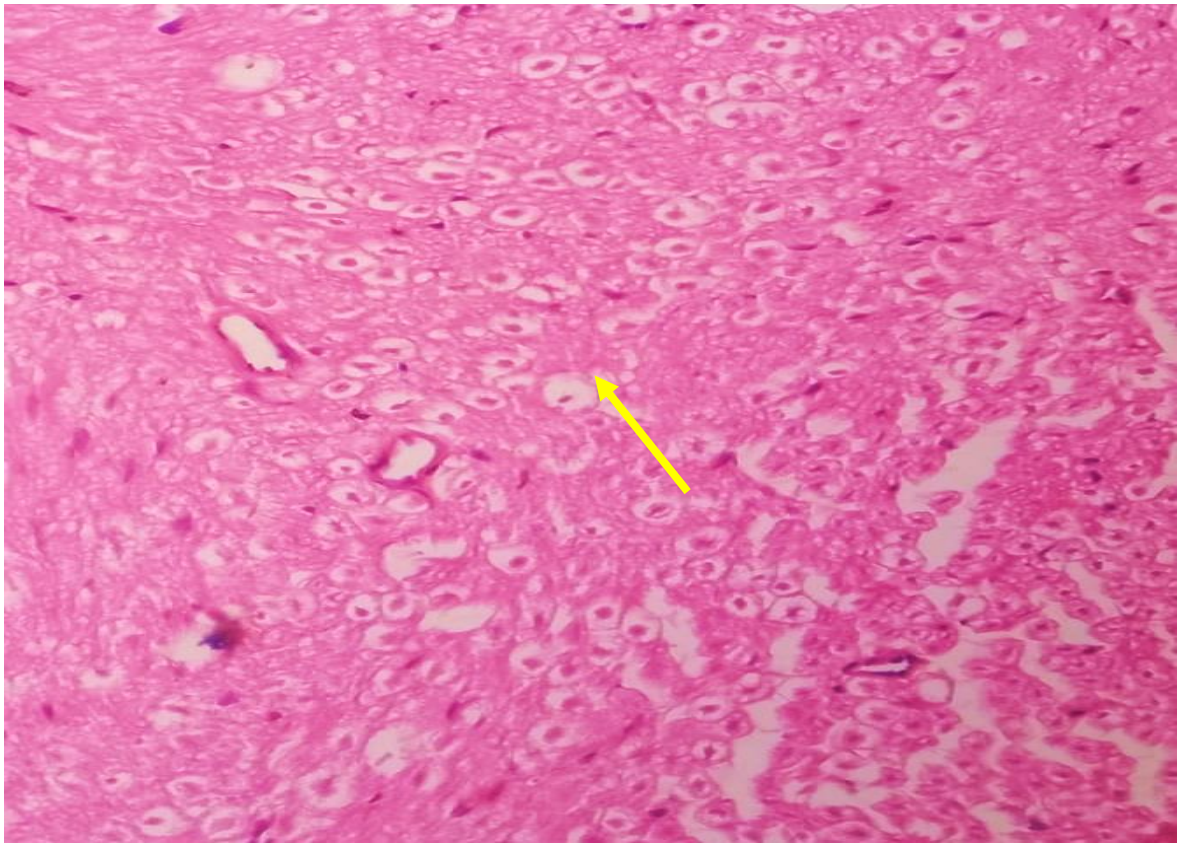


Figure 1. Histopathology results of the sciatic nerve in diabetic rat neuropathology, the yellow arrow indicates damaged cells included in the calculation.

ImageJ software, with its plugin feature, was used to analyze the digitized images. Plugins are functionalities available on all ImageJ versions, including the original (version 1), ImageJ2, and Fiji. These plugins appear in the plugin menu (or a submenu) upon starting ImageJ. They enable various tasks beyond cell damage analysis, such as image rendering, user interface extensions, processing single-image microscopy data, and result analysis. This even includes finding and adjusting image points for reconstruction[23]. The results of image analysis using the ImageJ plugin can be seen in **Figure 2**.

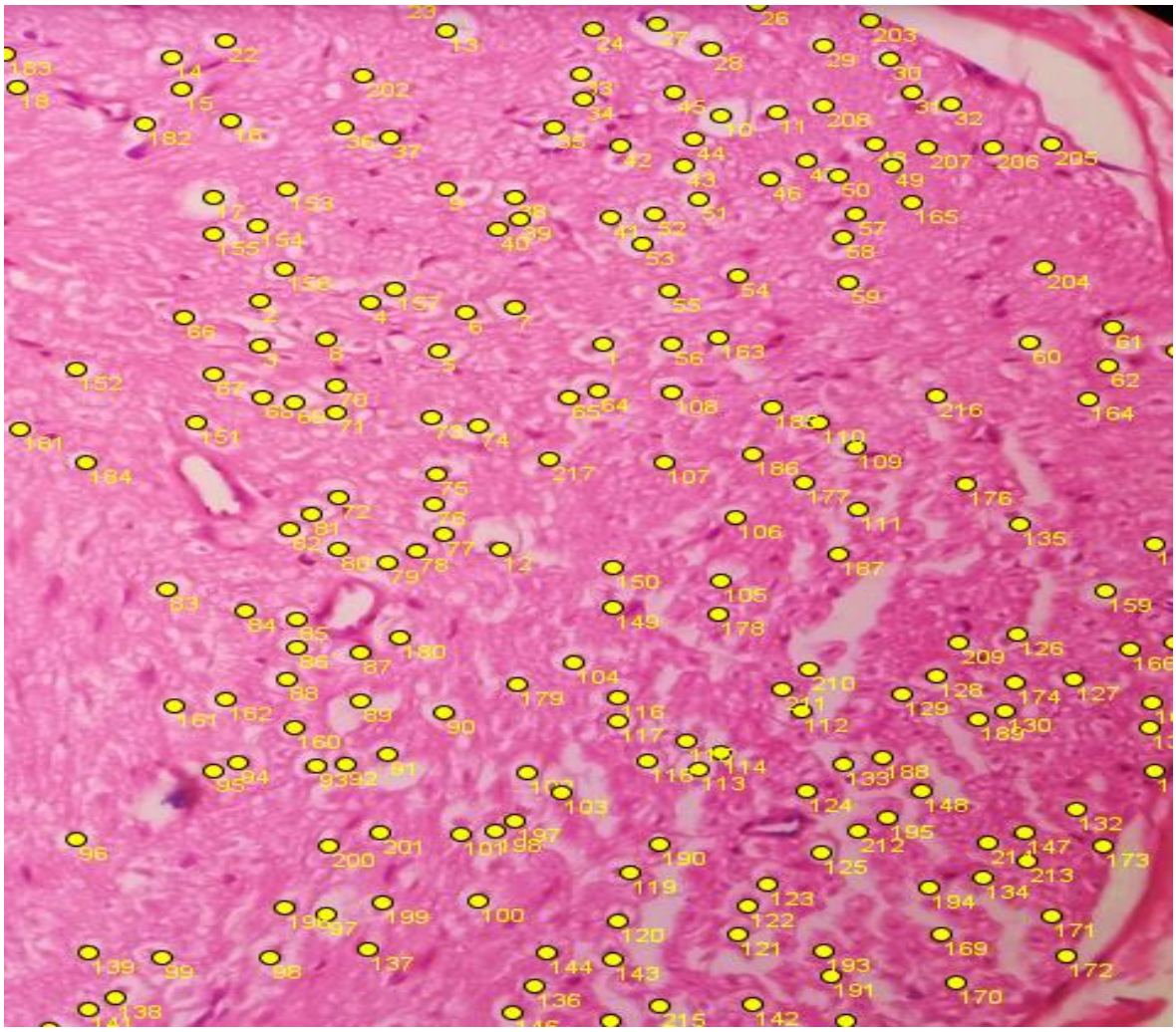


Figure 2. Presentation of manual calculations using ImageJ, yellow dots indicate counted cells.

For comparison purposes, the digitized images were also analyzed using the Gaussian adaptive thresholding method. This method divides the image into smaller regions, and calculates a specific threshold value for each region. This approach helps retain more information compared to simpler thresholding techniques. The threshold values are determined using a Gaussian function. Adaptive thresholding is particularly suitable for images with relatively uniform intensity levels[24].

The Gaussian adaptive thresholding method involves several steps. First, the color microscope image is converted to grayscale. Then, adaptive threshold segmentation is applied to identify background pixels. This technique classifies pixels based on their intensity: pixels brighter than a certain threshold are considered background, while darker pixels are considered foreground objects. The key factor in segmentation is the threshold value. In this method, the threshold for each pixel is calculated adaptively as the average intensity of its surrounding pixels within a 3 x 3 square region. Finally, binary segmentation creates a new image where each pixel is assigned a value of 0 (background) or 1 (object). Binarization essentially converts grayscale images into black and white (foreground and background). Gaussian adaptive threshold is particularly useful for images with uneven lighting or variations in pixel intensity[25].

The Gaussian adaptive thresholding method uses an odd-sized block to analyze the image. This allows each block to have a unique threshold value that adapts to local variations in pixel intensity. A constant value (C) is then subtracted from the average intensity within each block to achieve the desired segmentation[26]. This technique is particularly effective at isolating individual nuclei, even those with faint or blurred boundaries, from the surrounding tissue[27]. The results of this image processing step are shown in **Figure 3**.

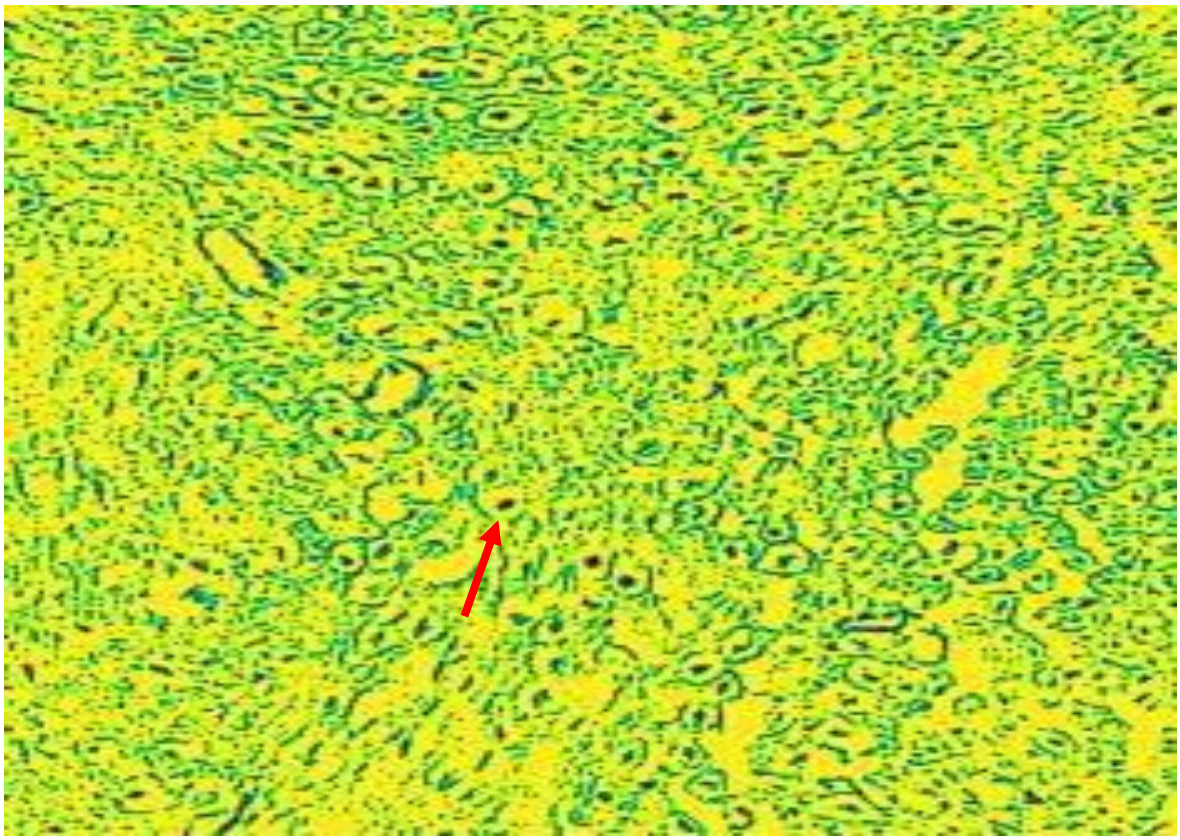


Figure 3. Presentation of calculation results with Gaussian adaptive threshold after normalization. Red arrows show examples of counted cells. In Gaussian the value is shown automatically.

To obtain quantitative data, measurements were taken from three distinct regions of the sciatic nerve in a single slide. This approach ensures a comprehensive analysis of the sample. The measurements were then averaged to produce mean values, which are presented in a graph in **Figure 4**. This visual representation facilitates data interpretation and comparison. **Figure 4** shows that the average cell count obtained using ImageJ (213.00 ± 19.31 cells) is higher compared to the average obtained using Gaussian adaptive thresholding (204.67 ± 5.03 cells). This difference can be attributed to the underlying calculation principles. Manual analysis with ImageJ involves manually counting damaged nerve cells one by one. In contrast, Gaussian adaptive thresholding automates the process. By selecting an appropriate threshold, it converts the histopathology image into a binary image, preserving only essential information about the size and shape of the nuclear regions. This simplification reduces image complexity and facilitates feature extraction and classification[28]. Our findings align with previous study comparing manual and automated cell counting methods, which showed no significant differences in the average cell counts. The observed difference in standard deviation in this study might be due to a sample size of less than 100 slides, which is considered insufficient for statistically robust estimation of standard deviation[29].

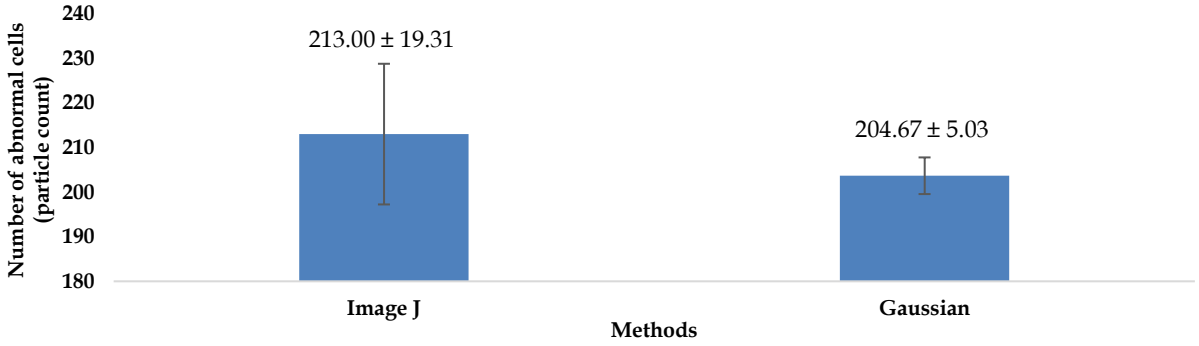


Figure 4. Comparison of abnormal cell calculations with ImageJ and Gaussian.

This study explores the use of Gaussian adaptive thresholding for cell analysis in histopathology images. However, the method has limitations. Closely spaced cell nuclei might be misidentified as a single object, and inflammatory cells with similar color intensity may also be misinterpreted. Additionally, variations in background color intensity can affect results. Manual cell counting using software like ImageJ offers an alternative approach, but it suffers from limitations such as time consumption, high workload, and potential subjectivity. The digitization of biological data opens doors for computer-aided diagnosis[30]. While this study has not yet evaluated the accuracy of machine learning methods for this purpose, and the sample size remains relatively small, the precise calculation results obtained here suggest promise for developing a robust analytical method in the future.

The ever-growing use of computers, coupled with the development of advanced image analysis algorithms, has fueled the development of computer-assisted approaches for analyzing biomedical data. Manual interpretation of tissue slides is a laborious, expensive process prone to human error and inconsistency. Automated image analysis offers a faster, more reproducible method for generating additional insights, aiding pathologists in reaching accurate diagnoses. However, challenges remain in computer-assisted diagnosis. One hurdle is achieving the necessary accuracy and speed to provide truly useful results, particularly when dealing with the large datasets generated by digital histology samples. Additionally, ensuring accessibility of these methods to the entire pathology community is another crucial hurdle to overcome[31].

CONCLUSION

The calculation results of the two methods show no significant differences between the methods. The test results show that the computerized morphometric image analysis method has the potential to be applied in the digitalization of pathology because it can provide an image-based environment for managing and interpreting information generated from images on glass slides into quantitative data. This digitalization also offers substantial results in improving the safety of pharmaceutical drugs in toxicology testing, preclinical pathology, and clinical trials.

ACKNOWLEDGMENT

This work was funded by the Centre for Education Services (*Pusat Layanan Pendidikan*) under the Ministry of Education, Culture, Research and Technology of the Republic of Indonesia. Research Funding scheme from the Indonesian Education Scholarship (*Beasiswa Pendidikan Indonesia*; BPI) and the Indonesia Endowment Funds for Education (*Lembaga Pengelola Dana Pendidikan*; LPDP) [Grant number: 202101121414].

AUTHORS' CONTRIBUTION

Conceptualization: Indah Tri Lestari
Data curation: Indah Tri Lestari, Sevi Nurafni
Formal analysis: Indah Tri Lestari, Sevi Nurafni
Funding acquisition: Indah Tri Lestari
Investigation: Indah Tri Lestari, Sevi Nurafni
Methodology: Sevi Nurafni
Project administration: Kusnandar Anggadiredja, Afrillia Nuryanti Garmana
Resources: Indah Tri Lestari, Sevi Nurafni
Software: Indah Tri Lestari, Sevi Nurafni
Supervision: Kusnandar Anggadiredja, Afrillia Nuryanti Garmana
Validation: Sevi Nurafni
Visualization: Indah Tri Lestari
Writing - original draft: Indah Tri Lestari

Writing - review & editing: Kusnandar Anggadiredja, Afrillia Nuryanti Garmana

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

REFERENCES

1. Jimenez-del-toro O, Otálora S, Andersson M, Eurén K, Hedlund M, Rousson M, et al. Chapter 10 - Analysis of Histopathology Images: From Traditional Machine Learning to Deep Learning. In: Depeursinge A, Al-Kadi OS, Mitchell JR, editors. Biomedical Texture Analysis: Fundamentals, Tools and Challenges. Cambridge (MA): Academic Press; 2018. p. 281–314. DOI: 10.1016/B978-0-12-812133-7.00010-7

2. Gurcan MN, Boucheron LE, Can A, Madabhushi A, Rajpoot NM, Yener B. Histopathological image analysis: a review. IEEE Rev Biomed Eng. 2009;2:147–71. DOI: 10.1109/rbme.2009.2034865; PMCID: PMC2910932; PMID: 20671804

3. He W, Liu T, Han Y, Ming W, Du J, Liu Y, et al. A review: The detection of cancer cells in histopathology based on machine vision. Comput Biol Med. 2022;146:105636. DOI: 10.1016/j.compbiomed.2022.105636; PMID: 35751182

4. Fulawka L, Halon A. Proliferation Index Evaluation in Breast Cancer Using ImageJ and ImmunoRatio Applications. Anticancer Res. 2016;36(8):3965–72. PMID: 27466501

5. da Silva LG, da Silva Monteiro WRS, de Aguiar Moreira TM, Rabelo MAE, de Assis EACP, de Souza GT. Fractal dimension analysis as an easy computational approach to improve breast cancer histopathological diagnosis. Appl Microsc. 2021;51(1):6. DOI: 10.1186/s42649-021-00055-w; PMCID: PMC8087740; PMID: 33929635

6. Burrai GP, Gabrieli A, Polinas M, Murgia C, Becchere MP, Demontis P, et al. Canine Mammary Tumor Histopathological Image Classification via Computer-Aided Pathology: An Available Dataset for Imaging Analysis. Animals. 2023;13(9):1563. DOI: 10.3390/ani13091563; PMCID: PMC10177203; PMID: 37174600

7. Martin B, Banner BM, Schäfer EM, Mayr P, Anthuber M, Schenkirsch G, et al. Tumor proportion in colon cancer: results from a semiautomatic image analysis approach. Virchows Arch. 2020;477(2):185–93. DOI: 10.1007/s00428-020-02764-1; PMCID: PMC7985049; PMID: 32076815

8. Wang KS, Yu G, Xu C, Meng XH, Zhou J, Zheng C, et al. Accurate diagnosis of colorectal cancer based on histopathology images using artificial intelligence. BMC Med. 2021;19(1):76. DOI: 10.1186/s12916-021-01942-5; PMCID: PMC7986569; PMID: 33752648

9. Bao J, Walliander M, Kovács F, Nagaraj AS, Hemmes A, Sarhadi VK, et al. Spa-RQ: an Image Analysis Tool to Visualise and Quantify Spatial Phenotypes Applied to Non-Small Cell Lung Cancer. Sci Rep. 2019;9(1):17613. DOI: 10.1038/s41598-019-54038-9; PMCID: PMC6879493; PMID: 31772293

10. Nagpal K, Foote D, Tan F, Liu Y, Chen PHC, Steiner DF, Manoj N, et al. Development and Validation of a Deep Learning Algorithm for Gleason Grading of Prostate Cancer from Biopsy Specimens. JAMA Oncol. 2020;6(9):1372-80. DOI: 10.1001/jamaoncol.2020.2485; PMCID: PMC7378872; PMID: 32701148

11. Kuiava VA, Kuiava EL, Chielle EO, Bittencourt FM De. Artificial intelligence algorithm for the histopathological diagnosis of skin cancer. Clin Biomed Res. 2020;40(4):218–22.

12. Li T, Xie P, Liu J, Chen M, Zhao S, Kang W, et al. Automated Diagnosis and Localization of Melanoma from Skin Histopathology Slides Using Deep Learning: A Multicenter Study. J Healthc Eng. 2021;2021:5972962. DOI: 10.1155/2021/5972962; PMCID: PMC8564171; PMID: 34745503

13. Raafat KM, El-Zahaby SA. Niosomes of active Fumaria officinalis phytochemicals: Antidiabetic, antineuropathic, anti-inflammatory, and possible mechanisms of action. Chinese Med. 2020;15:40. DOI: 10.1186/s13020-020-00321-1; PMCID: PMC7195756; PMID: 32377229

14. Shinouchi R, Shibata K, Hashimoto T, Jono S, Hasumi K, Nobe K. SMTP-44D improves diabetic neuropathy symptoms in mice through its antioxidant and anti-inflammatory activities. Pharmacol Res Perspect. 2020;8(6):e00648. DOI: 10.1002/prp2.648; PMCID: PMC7677968; PMID: 33215875

15. Sameni H, Panahi M. The Effect of Co-administration of 4-Methylcatechol and Progesterone on Sciatic Nerve Function and Neurohistological Alterations in Streptozotocin-Induced Diabetic Neuropathy in Rats. Cell J. 2011;13(1):31-8. PMCID: PMC3652538; PMID: 23671825

16. Moscalu M, Moscalu R, Dascălu CG, Țarcă V, Cojocaru E, Costin IM, et al. Histopathological Images Analysis and Predictive Modeling Implemented in Digital Pathology-Current Affairs and Perspectives. Diagnostics. 2023;13(14):2379. DOI: 10.3390/diagnostics13142379; PMCID: PMC10378281; PMID: 37510122

17. Khan A, Shal B, Khan AU, Ullah R, Baig MW, Ul Haq I, et al. Suppression of TRPV1/TRPM8/P2Y Nociceptors by Withametelin via Downregulating MAPK Signaling in Mouse Model of Vincristine-Induced Neuropathic Pain. Int J Mol Sci. 2021;22(11):6084. DOI: 10.3390/ijms22116084; PMCID: PMC8200233; PMID: 34199936

18. Labno C. Two Ways to Count Cells with ImageJ. Chicago (IL): Integrated Light Microscopy Core University of Chicago; 2014. p. 1–5. Available from: https://cpb-us-w2.wpmucdn.com/voices.uchicago.edu/dist/c/2275/files/2020/01/cell_counting_automated_and_manual.pdf

19. Lutnick B, Ramon AJ, Ginley B, Csiszer C, Kim A, Flament I, et al. Accelerating pharmaceutical R&D with a user-friendly AI system for histopathology image analysis. J Pathol Inform. 2023;14:100337. DOI: 10.1016/j.jpi.2023.100337; PMCID: PMC10582575; PMID: 37860714

20. Veta M, Pluim JPW, van Diest PJ, Viergever MA. Breast cancer histopathology image analysis: A review. IEEE Trans Biomed Eng. 2014;61(5):1400–11. DOI: 10.1109/tbme.2014.2303852; PMID: 24759275

21. Raghavan V, Rao KR. An ImageJ Based Semi-Automated Morphometric Assessment of Nuclei in Oncopathology. Int J Sci Study. 2015;3(7):189–94. DOI: 10.17354/ijss/2015/475

22. Aeffner F, Zarella MD, Buchbinder N, Bui MM, Goodman MR, Hartman DJ, et al. Introduction to Digital Image Analysis in Whole-slide Imaging: A White Paper from the Digital Pathology Association. J Pathol Inform. 2019;10:9. DOI: 10.4103/jpi.jpi_82_18; PMCID: PMC6437786; PMID: 30984469

23. Herbert A. Single Molecule Light Microscopy ImageJ Plugins. East Sussex (UK): University of Sussex; 2014. p. 1–156. Available from: http://www.sussex.ac.uk/gdsc/intranet/pdfs/SMLM.pdf

24. Anggraeni DT. Perbaikan Citra Dokumen Hasil Pindai Menggunakan Metode Simple, Adaptive-Gaussian, dan Otsu Binarization Thresholding. EXPERT J Manajemen Sistem Informasi Teknologi. 2021;11(2):71-7. DOI: 10.36448/expert.v11i2.2170

25. Korzynska A, Roszkowiak L, Lopez C, Bosch R, Witkowski L, Lejeune M. Validation of various adaptive threshold methods of segmentation applied to follicular lymphoma digital images stained with 3,3'-Diaminobenzidine&Haematoxylin. Diagn Pathol. 2013;8:48. DOI: 10.1186/1746-1596-8-48; PMCID: PMC3656801; PMID: 23531405

26. Rehman NA, Haroon F. Adaptive Gaussian and Double Thresholding for Contour Detection and Character Recognition of Two-Dimensional Area Using Computer Vision. Eng Proc. 2023;32(1):23. DOI: 10.3390/engproc2023032023

27. Kowal M, Filipczuk P, Obuchowicz A, Korbicz J. Computer-aided diagnosis of breast cancer based on fine needle biopsy microscopic images. Comput Biol Med. 2013;43(10):1563–72. DOI: 10.1016/j.compbiomed.2013.08.003; PMID: 24034748

28. Das A, Nair MS, Peter SD. Computer-Aided Histopathological Image Analysis Techniques for Automated Nuclear Atypia Scoring of Breast Cancer: a Review. J Digit Imaging. 2020;33(5):1091–121. DOI: 10.1007/s10278-019-00295-z; PMCID: PMC7573034; PMID: 31989390

29. Suarez-Arnedo A, Figueroa FT, Clavijo C, Arbeláez P, Cruz JC, Muñoz-Camargo C. An image J plugin for the high throughput image analysis of in vitro scratch wound healing assays. PLoS One. 2020;15(7):e0232565. DOI: 10.1371/journal.pone.0232565; PMCID: PMC7386569; PMID: 32722676

30. Chan HP, Hadjiiski LM, Samala RK. Computer-aided diagnosis in the era of deep learning. Med Phys. 2020;47(5):e218-27. DOI: 10.1002/mp.13764; PMCID: PMC7293164; PMID: 32418340

31. Brixtel R, Bougleux S, Lézoray O, Caillot Y, Lemoine B, Fontaine M, et al. Whole Slide Image Quality in Digital Pathology: Review and Perspectives. IEEE Access. 2022;10:131005-35. DOI: 10.1109/ACCESS.2022.3227437

Borneo Journal of Pharmacy
Vol 7 Issue 2 May 2024
Pages 136 – 146

https://journal.umpr.ac.id/index.php/bjop/article/view/6636
DOI: https://doi.org/10.33084/bjop.v7i2.6636
e-ISSN: 2621-4814

Research Article

Ethnobotanical Study of Medicinal Plants of Banjar and Java Tribes in Pandansari Village, South Kalimantan

Sutomo [1*]

Alif Vera Aprilianes [2]

Nani Kartinah [3]

Arnida [4]

Khoirunnisa Muslimawati [5]

Nabila Hadiah Akbar [5]

[1] Center for Study of Natural Medicine, Universitas Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia
[2] Undergraduate Program of Pharmacy, Universitas Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia
[3] Department of Pharmaceutical Technology, Universitas Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia
[4] Department of Pharmaceutical Biology, Universitas Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia
[5] Department of Pharmaceutical Chemistry, Universitas Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia

*email: sutomo01@ulm.ac.id; phone: +6285393089976

Keywords:
Banjar ethnicity
Ethnobotany
Javanese ethnicity
Medicinal plants

Abstract

In Pandansari village, South Kalimantan, Indonesia, residents from Banjar and Javanese communities traditionally utilize medicinal plants as alternatives to conventional medicine. This study aimed to explore the demographics, plant use practices, and preliminary phytochemical analysis of these plants. Using a qualitative, phenomenological approach, semi-structured interviews and questionnaires were conducted with four purposively selected informants. The findings revealed 52 medicinal plant species. Leaves were the most commonly used plant part (40%), followed by boiling as the preferred processing method (54%) and drinking as the primary route of administration (58%). Two unidentified plants, *asam sembelekan* and *daun malaysia*, were frequently mentioned. Phytochemical screening detected flavonoids, saponins, phenols, and terpenoids in *asam sembelekan*, while *daun malaysia* contained alkaloids, flavonoids, saponins, and terpenoids. These findings provide a foundation for further research on the efficacy and safety of these traditional medicinal plants in this community.

Received: January 23rd, 2024
1st Revised: May 23rd, 2024
Accepted: May 27th, 2024
Published: May 30th, 2024

INTRODUCTION

Indonesia, a megadiverse country, boasts a vast array of plant life[1]. This rich tapestry of plant species serves humanity in numerous ways, including as ingredients in traditional medicine—practices often passed down through generations[2]. Ethnobotanical studies delve specifically into this wealth of knowledge, exploring the potential medicinal properties of plants used by communities for extended periods[3].

South Kalimantan, a province in southeastern Borneo, encompasses both lowlands and highlands, including the Maratus Mountains. The Banjar tribe is the largest ethnic group in South Kalimantan, but others like the Javanese, Bugis, Batak, and Madurese also call this region home[4]. Both the Banjar and Javanese tribes have a rich heritage of using plants for medicinal purposes[5]. The Javanese tradition, known as *golek tombo* or *suwuk*, incorporates prayer and medicinal plants for healing[6].

How to cite: Sutomo, Aprilianes AV, Kartinah N, Arnida, Muslimawati K, Akbar NH. Ethnobotanical Study of Medicinal Plants of Banjar and Java Tribes in Pandansari Village, South Kalimantan. Borneo J Pharm. 2024;7(2):136-46. doi:10.33084/bjop.v7i2.6636

The Banjar tribe practices *betetamba*, which translates to treatment or shaman. The Banjar tribe beliefs associate *betetamba* rituals with the influence of nature and the surrounding environment[7,8].

Pandansari Village, located in Kintap District, covers an area of 50.00 km² and lies 9.31 km from the sub-district center. The village is home to a diverse population, including Banjar, Javanese, and other ethnicities. The majority of the community works in farming and gardening. Pandansari Village, Kintap District, boasts well-preserved natural resources. Despite the lack of prior research exploring its natural potential, this village presents a compelling opportunity for investigation. This ethnobotanical study aims to document the medicinal plants used in Pandansari Village. We collect comprehensive data on utilized plant parts, preparation methods, processing techniques, and application methods. Additionally, phytochemical screening was conducted on these plants. This study has the potential to provide valuable data and documentation of the plant species traditionally used by the community for medicinal purposes. Furthermore, it can pave the way for the development of these plants as future therapeutic agents.

MATERIALS AND METHODS

Materials

Medicinal plants were collected from Pandansari Village, South Kalimantan, Indonesia. Analytical grade chemicals and reagents were used throughout the study, including 70% ethanol, distilled water, $FeCl_3$, concentrated HCl, $CHCl_3$, NaOH, magnesium powder, gelatin, Dragendorff's reagent, and Lieberman-Burchard reagent. The following equipment was used for sample collection, preparation, and analysis: cutting tools, measuring tapes, test tubes, macerators, grinders, analytical balances, ovens, and water baths. Documentation of plant materials was facilitated by a digital camera.

Methods

Research design

This study employed a qualitative research approach with a phenomenological perspective[9]. In this case, phenomenology allowed us to delve into the experiences and meanings associated with the use of medicinal plants. Data collection involved semi-structured interviews and questionnaires administered to consenting participants from the village (informed consent was obtained from all participants prior to their involvement in the study). The research was conducted from December 2022 to February 2023 in Pandansari Village, Kintap District, Tanah Laut Regency, South Kalimantan, Indonesia. Additionally, laboratory analyses were performed at the Pharmacognosy-Phytochemistry Laboratory of the Faculty of Mathematics and Natural Sciences Universitas Lambung Mangkurat.

Respondents

Purposive sampling was employed to recruit participants for this study. We specifically targeted traditional healers from the Banjar and Javanese ethnicities residing in Pandansari Village. Inclusion criteria for participation were: (1) experience as a traditional healer, (2) willingness to participate in the study, and (3) knowledge and use of medicinal plants for treatment purposes.

Retrieval procedure of research data

Semi-structured interviews were conducted with consenting participants from the Pandansari Village community. Interviews were conducted in Indonesian or the participant's preferred regional language (e.g., Banjarese, Javanese) to ensure clear communication and minimize misunderstandings. The interview guide explored topics related to the traditional use of medicinal plants, including plant names and perceived benefits, utilized plant parts, processing and administration methods, and duration of use. Additionally, a questionnaire was administered to gather information on ownership of medicinal plants, purposes of traditional plant-based treatments, age demographics of plant users, source of knowledge regarding traditional medicine, and length of experience using medicinal plants for treatment.

Phytochemical screening

Phytochemical screening was performed on plant samples identified as potentially novel based on a lack of prior documentation in the scientific literature. These samples were subjected to a battery of tests using the tube method to detect

the presence of various secondary metabolites, including alkaloids, flavonoids, saponins, quinones, phenols, terpenoids, steroids, and tannins. Detailed descriptions of the specific methods employed for each phytochemical test can be found in **Table I**.

Table I. Phytochemical screening[10-12].

Test	Sample volume (mL)	Treatment	Positive result
Alkaloid	3	+ 3 drops of Dragendorff's reagent	Brick-red
Flavonoid	3	+ Mg powder and 3 mL HCl	Yellow, orange, red
Saponin	3	+ 3 mL hot distilled water, then shaken vigorously until foamy + a drop 2N HCl	Foam stabilized for ± 10 minutes
Quinones	3	+ 5 drops of NaOH through the tube wall	Red
Phenol	3	+ 3 drops of FeCl3	Blackish green or dark blue
Steroid	3	+ 3 drops of Lieberman-Burchard reagent	Turquoise
Terpenoid	3	+ 3 drops of Lieberman-Burchard reagent	Red or purple ring
Tannin	3	+ 3 drops of gelatin solution	White color sediment

Data analysis

Data obtained from interviews and questionnaires were analyzed to identify plant names, reported benefits, and utilized plant parts. Additionally, documented plants were examined for morphological characteristics to aid in classification. The results were presented in two main formats: qualitative and quantitative. Qualitative data, including narratives and tables, were detailed the traditional knowledge regarding medicinal plants. Quantitative data was presented in the form of a percentage diagram illustrating the prevalence of specific plant parts used, processing methods employed, and applications of medicinal plants within the Pandansari Village community. The formula used to calculate these percentages was provided in **Equation 1**[13].

$$\%\ Plant\ parts = \frac{\text{Parts of the plant used}}{\text{All parts of the plant used}} \text{x} 100\% \qquad [1]$$

RESULTS AND DISCUSSION

Informants for this study were recommended by the Pandansari Village administration, specifically traditional healers or *penenamba* known for treating patients within the community. Semi-structured interviews were employed to gather data from participants. This interview method allows for flexibility and exploration of new information that may arise during the conversation. Details regarding the research informants are presented in **Table II**.

Table II. Informant data.

Initial name	Age	Gender	Tribe	Residence	Job	Number of plants used	Duration of use (year)
DL	53	Woman	Banjar	Pandansari Village	Midwife	12	>10
AT	50	Man	Javanese	Pandansari Village	Traditional healer	20	>10
SR	51	Woman	Banjar	Pandansari Village	Traditional healer	5	>10
MK	68	Woman	Javanese	Pandansari Village	Midwife	15	>10

Interviews with Pandansari Village residents revealed 52 plant species traditionally used for medicinal purposes. Following identification efforts that included consulting both application practices and relevant literature, 50 plant species were successfully identified. However, two plant species remained unidentified. Collaborations with the Banua Botanical Garden, Banjarbaru, South Kalimantan, and the Indonesian Biology Generation Foundation, Gresik, East Java, are currently underway to identify these unknown species (details on these ongoing efforts can be provided upon request). Data on the identified medicinal plants used in Pandansari Village are presented in **Table III**. Analysis of data revealed nine distinct plant parts utilized for traditional medicine in Pandansari Village by the local healers or *penenamba*. These parts included root, leaf, stem, rhizome, sap, fruit, flower, tuber, and whole plant parts. Leaves emerged as the most frequently used plant part, as illustrated in the percentage diagram presented in **Figure 1**.

Table III. Medicinal plants in Pandansari Village.

Name	Species	Efficacy	Parts used	Administration method	Processing method
Betel palm	*Areca catechu*	Male stamina	Root	Oral	Boiled
Cogon grass	*Imperata cylindrica*	Stiffness/sore	Root	Oral	Boiled
Avocado	*Persea americana*	High blood pressure	Leaf	Oral	Boiled
Ti plant	*Cordyline fruticosa*	Dysentery	Root	Oral	Boiled
Asam sembelekan	-	Flu and cough	Leaf	Compressed on the forehead	Boiled
Mantangan	*Merremia peltata*	High blood pressure	Stem	Oral	Taken the stem liquid
Madeira vine	*Anredera cordifolia*	Scar	Leaf	Topical on open Wounds	Squeezed
Cotton candy berry	*Muntingia calabura*	Diabetic	Leaf	Oral	Boiled
Cutleaf groundcherry	*Physalis angulata*	All sort of sickness	Whole plant	Oral	Boiled
December tree	*Erythrina subumbrans*	Fever	Leaf	Compressed on the forehead	Squeezed
Daun malaysia	-	Cholesterol	Leaf	Oral	Boiled
Durian	*Durio zibethinus*	Fever	Bark	Steam bath	Boiled
Water willow	*Justicia gendarussa*	Headache	Leaf	Compressed on the forehead	Squeezed
Gale of the wind	*Phyllanthus niruri*	Stiffness/sore	Whole plant	Oral	Boiled
Ginger	*Zingiber officinale*	Cough	Rhizome	Oral	Boiled
Guava	*Psidium guajava*	Diarrhea	Leaf shoot	Oral	Squeezed
Cashew	*Anacardium occidentale*	Diarrhea	Leaf	Oral	Boiled
Purging nut	*Jatropha curcas*	Toothache	Sap	Dripped on cavities	Taken the leaf sap
Lime	*Citrus aurantifolia*	Cough	Fruit	Oral	Squeezed
Shortleaf spikesedge	*Kyllinga brevifolia*	Diarrhea	Whole plant	Oral	Boiled
Cape jasmine	*Gardenia jasminoides*	Fever and cholesterol	Leaf	Compressed on the forehead	Squeezed
Frangipani	*Plumeria acuminata*	Toothache	Sap	Dripped on cavities	Taken the flower sap
Senduduk	*Melastoma malabathhricum*	Wound	Leaf and flower	Topical on open wounds	Pounded
Papaya	*Carica papaya*	Improves bowel movements	Fruit	Oral	Without processing
Rodent tuber	*Typhonium flagelliforme*	Diabetic	Tuber	Oral	Boiled
Coconut	*Cocos nucifera*	Allergy	Water	Oral	Without processing
Moringa	*Moringa oleifera*	Cholesterol	Leaf	Oral	Boiled
Basil	*Ocimum basilicum*	Remove body odor	Leaf	Oral	Squeezed
Hibiscus	*Hibiscus* sp.	Fever	Leaf	Compressed on the forehead	Squeezed
Aromatic ginger	*Kampferia galanga*	Cough	Rhizome	Oral	Boiled
Jack in the bush	*Chromolaena odorata*	Scar	Leaf	Topical on open wounds	Squeezed
Java tea	*Orthosiphon aristatus*	Stiffness/sore	Root	Oral	Boiled
White turmeric	*Curcuma zedoaria*	Improve appetite	Rhizome	Oral	Grated and squeezed
Aloe vera	*Aloe vera*	Stomach acid	Stem	Oral	Taken the stem gel
Shoeblackplant	*Hibiscus rosa-sinensis*	Lowering blood pressure	Fruit	Oral	Dried
Indian mulberry	*Morinda citrifolia*	High blood pressure	Fruit	Oral	Boiled
White mulberry	*Morus alba*	Scar	Leaf	Topical on open wounds	Squezeed
Pineapple	*Ananas comosus*	High blood pressure	Fruit	Oral	Without processing
Cane-reed	*Costus speciosus*	Hemorrhoids	Rhizome	Oral	Soaked
Petawali	*Tinospora crispa*	All sort of sickness	Stem	Oral	Boiled
Stinking passionflower	*Passiflora foetida*	High blood pressure	Leaf	Oral	Boiled
Black pepper	*Piper nigrum*	Diarrhea	Fruit and seed	Oral	Boiled
Snakefruit	*Salacca zalacca*	Chest pain	Sap	Topical	Taken the fruit sap
Lemongrass	*Cymbopogon citratus*	Stiffness/sore	Stem	Oral	Boiled
Betel	*Piper betle*	Whiteness discharge	Leaf	Washing the feminine area	Boiled
Pepper elder	*Peperomia pellucida*	Gout	Whole plant	Oral	Boiled
Soursop	*Annona muricata*	Chest pain	Leaf	Topical	Squezeed
Cucha cara	*Elephantopus scaber*	Gout	Whole plant	Oral	Dried and brewed
Sugarcane	*Saccharum officinarum*	Diabetic	Stem	Oral	Squezeed
Curcuma	*Curcuma xanthorrhiza*	Improve appetite	Rhizome	Oral	Grated and squezeed
Cathedral bells	*Kalanchoe pinnata*	Fever	Leaf	Oral	Boiled

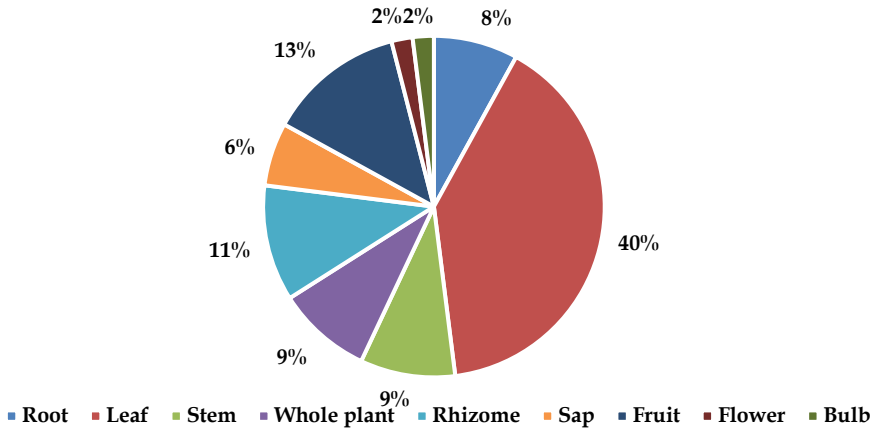


Figure 1. Parts of plants that have medicinal properties used in Pandansari Village.

Phytochemical screening was performed on two unidentified medicinal plants, *asam sembelekan* and *daun malaysia* (**Figure 2**), as reported by the Pandansari Village community. The results revealed notable differences in their secondary metabolite profiles, with the presence of saponins and terpenoids being the only shared constituents (**Table IV**). Notably, *asam sembelekan* exhibited the presence of phenolic compounds, which possess known antitussive (cough-relieving) properties[14]. This finding aligns somewhat with the reported use of this plant for ailments like coughs within the community[15]. Conversely, *daun malaysia* was found to contain alkaloids and flavonoids, secondary metabolites with established cholesterol-lowering activity[16,17]. This observation warrants further investigation into the potential anti-hyperlipidemic properties of *daun malaysia*, particularly considering its traditional use in the community for unspecified ailments.

a **b**

Figure 2. (**a**) *Asam sembelekan* and (**b**) *daun malaysia*.

Table IV. Phytochemical screening of *asam sembelekan* and *daun malaysia*.

Phytochemical compounds	Samples	
	Asam sembelekan	*Daun malaysia*
Alkaloids	-	+
Flavonoids	-	+
Saponins	+	+
Quinones	-	-
Phenols	+	-
Steroids	-	-
Terpenoids	+	+
Tannins	-	-

Note: +: Presence; -: Absence

A literature review identified previously reported secondary metabolite profiles for the 50 documented medicinal plants (**Table V**). Of the eight secondary metabolite classes tested on the two previously investigated plants, *I. cylindrica*, *M. peltata*, *D. zibethinus*, *J. gendarussa*, *Z. officinale*, *C. aurantifolia*, *H. rosa-sinensis*, and *C. xanthorrhiza* exhibited the greatest diversity, each containing six identified metabolite types. Conversely, *S. zalacca* lacked reports of any of the tested metabolites. Among the identified metabolites, flavonoids were the most prevalent, detected in 40 plants. This finding aligns with the established knowledge of flavonoids being one of the most widespread classes of secondary metabolites in various plant species[18]. Saponins and alkaloids followed in abundance, identified in 32 and 30 plants, respectively. Conversely, quinones were the least common, only reported in three plants. This scarcity is likely due to their derivation from the oxidation of hydroquinones, which may not be present in significant quantities within many medicinal plants[19].

Table V. Secondary metabolite from medicinal plants in Pandansari Village.

Plants	Alkaloids	Flavonoids	Saponins	Quinones	Phenols	Steroids	Terpenoids	Tannins	References
Areca catechu	+	+	-	-	-	-	+	+	20
Imperata cylindrica	+	-	+	-	+	+	+	+	21
Persea americana	+	+	+	-	-	-	-	-	22
Cordyline fruticosa	-	+	+	-	-	-	+	+	23
Merremia peltata	+	-	+	+	+	-	+	+	11
Anredera cordifolia	+	+	+	-	+	-	+	-	24
Muntingia calabura	-	+	+	-	+	-	+	+	25
Physalis angulata	+	-	-	-	-	-	-	+	26
Erythrina subumbrans	+	+	+	-	+	-	-	+	27
Durio zibethinus	+	+	+	-	-	+	+	+	28
Justicia gendarussa	+	+	+	-	+	+	+	-	29
Phyllanthus niruri	+	+	-	-	+	+	+	-	30
Zingiber officinale	+	+	+	-	-	+	+	+	31
Psidium guajava	-	-	-	-	-	-	+	+	32
Anacardium occidentale	-	+	-	-	+	-	+	-	33
Jatropha curcas	+	+	+	-	+	-	-	-	34
Citrus aurantifolia	+	+	+	-	+	+	-	+	35
Kyllinga brevifolia	+	+	-	-	-	-	-	+	36
Gardenia jasminoides	-	+	+	-	+	-	-	-	37
Plumeria acuminata	-	+	-	-	-	-	-	+	38
Melastoma malabathhricum	+	+	+	-	-	+	-	-	39
Carica papaya	-	+	+	-	-	+	-	+	40
Typhonium flagelliforme	-	+	-	-	-	-	-	+	41
Cocos nucifera	+	+	+	-	-	+	-	-	42
Moringa oleifera	-	-	-	-	-	-	-	-	43
Ocimum basilicum	+	+	+	-	-	-	-	+	44
Hibiscus sp.	-	+	-	-	-	-	-	-	45
Kampferia galanga	-	+	+	-	+	-	-	-	46
Chromolaena odorata	-	+	-	+	+	+	-	+	47
Orthosiphon aristatus	+	+	+	-	-	+	-	-	48
Curcuma zedoaria	-	+	+	-	-	-	-	-	49
Aloe vera	-	+	-	-	-	-	-	-	50
Hibiscus rosa-sinensis	+	+	+	-	-	+	-	+	11
Morinda citrifolia	+	-	+	+	+	-	+	+	51
Morus alba	-	+	+	-	-	+	-	+	52
Ananas comosus	-	+	+	-	-	+	+	+	53
Costus speciosus	-	+	-	-	-	-	-	-	54
Tinospora crispa	-	+	+	-	-	+	-	+	55
Passiflora foetida	+	-	-	-	-	+	-	-	56
Piper nigrum	+	+	-	-	+	-	-	-	57
Salacca zalacca	+	-	-	-	+	-	-	-	58
Cymbopogon citratus	-	-	-	-	-	-	-	-	59
Piper betle	+	+	+	-	-	-	+	+	60
Peperomia pellucida	-	+	+	-	-	-	-	+	61
Annona muricata	+	+	+	-	-	+	-	+	62
Elephantopus scaber	+	-	-	-	-	-	-	+	63
Saccharum officinarum	-	-	+	-	-	+	-	+	64
Curcuma xanthorrhiza	+	+	-	-	-	-	-	+	65
Kalanchoe pinnata	+	+	+	-	+	-	+	+	66

Note: +: Presence; -: Absence

CONCLUSION

This study documented 52 medicinal plants traditionally used by the Banjar and Javanese communities in Pandansari Village. These plants reportedly address various ailments, including male stamina enhancement, stiffness, hypertension, dysentery/diarrhea, flu/cough, wounds, fever, toothache, headache, allergies, cholesterol, diabetes, and vaginal discharge. Leaves were the most commonly utilized plant part, boiling the preferred processing method, and drinking the favored administration route. Further investigations are warranted to validate the reported efficacy of these plants and explore their potential bioactive compounds.

ACKNOWLEDGMENT

We are grateful to the residents of Pandansari Village, Kintap Sub-district, Tanah Laut Regency, South Kalimantan, for sharing their knowledge and information related to medicinal plants in their community. This research did not receive funding from either internal or external sources.

AUTHORS' CONTRIBUTION

Conceptualization: Sutomo, Alif Vera Aprilianes, Nani Kartinah, Arnida
Data curation: Sutomo, Alif Vera Aprilianes, Nani Kartinah, Arnida
Formal analysis: Sutomo, Alif Vera Aprilianes, Arnida, Khoirunnisa Muslimawati, Nabila Hadiah Akbar
Funding acquisition: -
Investigation: Alif Vera Aprilianes, Khoirunnisa Muslimawati, Nabila Hadiah Akbar
Methodology: Sutomo, Arnida, Khoirunnisa Muslimawati, Nabila Hadiah Akbar
Project administration: Sutomo, Nani Kartinah, Arnida
Resources: Sutomo, Arnida
Software: -
Supervision: Sutomo, Nani Kartinah, Arnida
Validation: Sutomo, Arnida
Visualization: Sutomo, Alif Vera Aprilianes
Writing - original draft: Alif Vera Aprilianes, Khoirunnisa Muslimawati, Nabila Hadiah Akbar
Writing - review & editing: Sutomo, Nani Kartinah, Arnida

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

REFERENCES

1. Handayani I, Saad H, Ratnakomala S, Lisdiyanti P, Kusharyoto W, Krause J, et al. Mining Indonesian Microbial Biodiversity for Novel Natural Compounds by a Combined Genome Mining and Molecular Networking Approach. Mar Drugs. 2021;19(6):316. DOI: 10.3390/md19060316; PMCID: PMC8227522; PMID: 34071728

2. Petrovska BB. Historical review of medicinal plants' usage. Pharmacogn Rev. 2012;6(11):1-5. DOI: 10.4103/0973-7847.95849; PMCID: PMC3358962; PMID: 22654398

3. Demie G, Negash M, Awas T. Ethnobotanical study of medicinal plants used by indigenous people in and around Dirre Sheikh Hussein heritage site of South-eastern Ethiopia. J Ethnopharmacol. 2018;220:87-93. DOI: 10.1016/j.jep.2018.03.033; PMID: 29601979

4. Az-Zahra FR, Sari NLW, Saputry R, Nugroho GD, Pribadi T, Sunarto, et al. Review: Traditional knowledge of the Dayak Tribes (Borneo) in the use of medicinal plants. Biodiversitas. 2021;22(1):4633-47. DOI: 10.13057/biodiv/d221057

5. Pradipta IS, Aprilio K, Febriyanti RM, Ningsih YF, Pratama MAA, Indradi RB, et al. Traditional medicine users in a treated chronic disease population: a cross-sectional study in Indonesia. BMC Complement Med Ther. 2023;23(1):120. DOI: 10.1186/s12906-023-03947-4; PMCID: PMC10102674; PMID: 37060056

6. Setiawan KEP, Wahyuningsih, Rizaldy DR, Kasimbar DC. Suwuk : Construction of the Javanese People's Mindset in Medicine. Javanologi Int J Javanese Stud. 2021;5(1):910-5. DOI: 10.20961/javanologi.v5i1.67938

7. Jamalie Z, Rif'at M. Dakwah Kultural Dialektika Islam dan Budaya dalam Tradisi Batatamba. Alhadharah J Ilmu Dakwah. 2012;11(21):61–7. DOI: 10.18592/alhadharah.v11i21.1786

8. Daud A. Islam dan Masyarakat Banjar: Deskripsi dan Analisis Kebudayaan Banjar. Jakarta: Rajagrafindo Persada; 1997.

9. Renjith V, Yesodharan R, Noronha JA, Ladd E, George A. Qualitative Methods in Health Care Research. Int J Prev Med. 2021;12:20. DOI: 10.4103/ijpvm.ijpvm_321_19; PMCID: PMC8106287; PMID: 34084317

10. Sutomo, Awaliyah VV, Arnida. Ethnobotanical Study and Phytochemical Screening of Medicinal Plants Used by Local People in Belangian Village, South Kalimantan. Borneo J Pharm. 2022;5(1):1-8. DOI: 10.33084/bjop.v5i1.2717

11. Sutomo S, Arnida A, Rizki MI, Triyasmono L, Nugroho A, Mintowati E, et al. Skrining Fitokimia dan Uji Kualitatif Aktivitas Antioksidan Tumbuhan Asal Daerah Rantau Kabupaten Tapin Kalimantan Selatan. J Pharmascience. 2016;3(1):66-74. DOI: 10.20527/jps.v3i1.5836

12. Liliwirianis N, Musa NLW, Zain WZWM, Kassim J, Karim SA. Premilinary Studies on Phytochemical Screening of Ulam and Fruit from Malaysia. J Chem. 2011;8:464595. DOI: 10.1155/2011/464595

13. Qasrin U, Setiawan A, Yulianti, Bintoro A. Studi tnobotani Tumbuhan Berkhasiat Obat Yang dimanfaatkan Masyarakat Suku Melayu Kabupaten Lingga Kepulauan Riau. J Belantara. 2020;3(2):139-52. DOI: 10.29303/jbl.v3i2.507

14. Dapaah G, Koffuor GA, Mante PK, Ben IO. The possible mode of antitussive and expectorant activity of the ethanol seed extracts of Picralima nitida ((Stapf) Th. & H. Durand). J Tradit Complement Med. 2016;7(1):133-40. DOI: 10.1016/j.jtcme.2016.05.003; PMCID: PMC5198831; PMID: 28053900

15. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. Medicines. 2018;5(3):93. DOI: 10.3390/medicines5030093; PMCID: PMC6165118; PMID: 30149600

16. Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, et al. Important Flavonoids and Their Role as a Therapeutic Agent. Molecules. 2020;25(22):5243. DOI: 10.3390/molecules25225243; PMCID: PMC7697716; PMID: 33187049

17. Kou S, Han B, Wang Y, Huang T, He K, Han Y, et al. Synergetic cholesterol-lowering effects of main alkaloids from Rhizoma Coptidis in HepG2 cells and hypercholesterolemia hamsters. Life Sci. 2016;151:50-60. DOI: 10.1016/j.lfs.2016.02.046; PMID: 26876917

18. Roy A, Khan A, Ahmad I, Alghamdi S, Rajab BS, Babalghith AO, et al. Flavonoids a Bioactive Compound from Medicinal Plants and Its Therapeutic Applications. Biomed Res Int. 2022;2022:5445291. DOI: 10.1155/2022/5445291; PMCID: PMC9192232; PMID: 35707379

19. Eyong KO, Kuete V, Efferth T. 10 - Quinones and Benzophenones from the Medicinal Plants of Africa. In: Kuete V, Ed. Medicinal Plant Research in Africa. Amsterdam: Elsevier; 2013. p. 351-91. DOI: 10.1016/B978-0-12-405927-6.00010-2

20. Fauzi, Widodo H, Haryanti S. Kajian Tumbuhan Obat yang Banyak Digunakan untuk Aprodisiaka oleh Beberapa Etnis Indonesia. Media Penelitian Pengembangan Kesehatan. 2019;29(1):51-64. DOI: 10.22435/mpk.v29i1.466

21. Maharani I, Ulmillah A, Kuswanto E. Pemberian Kombinasi Extract Alang-Alang (Imperata cylindrical) dan Kirinyuh (Chromolaena odorata) pada Tanaman Gulma (Ageratum conyzoides) di Lahan Tanaman Kopi Desa Ciptawaras kabupaten Lampung Barat. Organisms J Biosci. 2021;1(1):1- 11. DOI: 10.24042/organisms.v1i1.9377

22. Sulistiawati AANN, Prapti NKG, Lestari MPL. Pengaruh Pemberian Air Rebusan Leaf Alpukat (Persea Americana Mill) Terhadap Tekanan Darah Pasien Hipertensi di Wilayah Kerja Puskesmas II Denpasar Selatan. Coping Commun Pub Nurs. 2-15;3(3): 13939.

23. Fouedjou RT, Nguelefack-Mbuyo EP, Ponou BK, Ngeulefack TB, Barbono L, Tapondjou LA. Antioxidant Activities and Chemical Constituents of Extracts from Cordyline fruticosa (L.) A. Chev. (Agavaceae) and Eriobotrya japonica (Thunb) Lindl, (Rosaceae). Pharmacologia. 2016;7(2):103-13. DOI: 10.5567/pharmacologia.2016.103.113

24. Samirana PO, Swastini DA, Subratha IDGPY, Ariandi KA. Uji Aktivitas Penyembuhan Luka Extract Etabol Leaf Binahong (Anredera scandens (L.) Moq) pada Tikus Jantan Galur Wistar. J Farmasi Udayana. 2016;5:19-23.

25. Ginting GIN, Jayawarditha AAG, Dharmawan NS. Salep Extract Leaf Kersen Menurunkan Kadar Diabetic dan Migrasi Sel Polimorfonuklear pada Mencit Hiperglikemi. Indones Med Veterinus. 2021;10(2):211-22. DOI: 10.19087/imv.2021.10.2.211

26. Sutomo B, Kurnia D. 378 Resep Jus & Ramuan Herbal. Jakarta: Kawan Pustaka; 2016.

27. Pariata K, Mediastari AAPA, Suta IBP. Manfaat Dadap Serep (Eryhrina Sumbubrans) Untuk Mengatasi Fever Pada Anak. Widya Kesehatan. 2022;4(1):24-37. DOI: 10.32795/widyakesehatan.v4i1.2803

28. Rudiyansyah, Garson MJ. Secondary Metabolites from the Wood Bark of Durio zibethinus and Durio kutejensis. J Nat Prod. 2006;69(8):1218-21. DOI: 10.1021/np050553t

29. Widodo A, Khumaidi A, Lasongke PFA. Toksisitas Ekstrak Etanol dan Ekstrak Air dari Daun Jotang Kuda (Synedrella nodiflora (L.) Gaertn.), Daun Gandarusa (Justicia Gendarussa Burm.F.), dan Daun Pulutan (Urena lobata L.) dengan Brine Shrimp Lethality Test. J Farmasi Galenika Galenika J Pharm. 2019;5(2):198-205. DOI:10.22487/j24428744.2019.v5.i2.13935

30. Ervina MN, Mulyono Y. Etnobotani Meniran Hijau (Phyllanthus ninuri L) Sebagai Potensi Obat Kayap Ular (Herpes Zoster) dalam Tradisi Suku Dayak Ngaju. J Jejaring Matematika Sains. 2019;1(1):30-8. DOI: 10.36873/jjms.v1i1.134

31. Hadyprana S, Noer S, Supriyanti T. Uji Daya Hambat Ekstrak Jahe Putih Terhadap Pertumbuhan Pseudomonas aeruginosa dan Candida albicans Secara in Vitro. EduBiologia Biol Sci Edu J. 2021;1(2):142-8. DOI: 10.30998/edubiologia.v1i2.10142

32. Ricky D, Puspita, Mangklik G. 9. Studi Etnobotani Tumbuhan Obat di Desa Tumbang Jala – Kalimantan Tengahyang Masih dimanfaatkan Sebagai Obat Tradisional. In: Prosiding Seminar Nasional Kesehatan "Transformasi Bidang Kesehatan di Era Industri 4.0". Salatiga: Universitas Kristen Satya Wacana; 2019. p. 57-73.

33. Salehi B, Gültekin-Özgüven M, Kırkın C, Özçelik B, Morais-Braga MFB, Carneiro JNP, et al. Anacardium Plants: Chemical, Nutritional Composition and Biotechnological Applications. Biomolecules. 2019;9(9):465. DOI: 10.3390/biom9090465; PMCID: PMC6769990; PMID: 31505888

34. Rahu MI, Naqvi SHA, Memon NH, Idrees M, Kandhro F, Pathan NL, et al. Determination of antimicrobial and phytochemical compounds of Jatropha curcas plant. Saudi J Biol Sci. 2021;28(5):2867-76. DOI: 10.1016/j.sjbs.2021.02.019; PMCID: PMC8116963; PMID: 34012327

35. Nafisa S, Swandiny GF, Gangga E, Zaenudin YA. Antimicrobial Activity and Phytochemical Screening of Citrus aurantifolia Leaves Ethanolic Extract. J Ilmu Kefarmasian Indones. 2022;19(2):287-91. DOI: 10.35814/jifi.v19i2.1095

36. Isa N, Bakhari NA, Sarijo SH, Aziz A, Lockman Z. Kyllinga brevifolia mediated greener silver nanoparticles. AIP Conf Proceed. 2017;1901:020012. DOI: 10.1063/1.5010449

37. Kesavan K, Ghanasekaran J, Gurunagarajan S, Nayagam AAJ. Microscopic, Physicochemical and Phytochemical Analysis of Gardenia jasminoides (Ellis). Int J Pharm Pharm Sci. 2018;10(1):97-102. DOI: 10.22159/ijpps.2018v10i1.21665

38. Maya I, Sriwidodo. Review: Potensi Minyak Biji Sacha Inchi Sebagai Anti-aging dalam Formula Kosmetik. Majalah Farmasetika. 2022;7(5):407-23. DOI: 10.24198/mfarmasetika.v7i5.39510

39. Artawa MB, Sirat NM, Supariani NND. Efektivitas Pemanfaatan Getah Bunga Kamboja Untuk Menghilangkan Toothache Di Masa Pandemi COVID-19. J Kesehatan Gigi Dent Health J. 2021;8(2):27-33. DOI: 10.33992/jkg.v8i2.1491

40. Alfarabi M, Siagian FE, Cing JM, Suryowati T, Turhadi, Suyono MS, et al. Bioactivity and metabolite profile of papaya (Carica papaya) seed extract. Biodiversitas. 2022;23(9):4589-600. DOI: 10.13057/biodiv/d230926

41. Syahid SF, Kristina NN. Induksi dan Regenerasi Kulus Keladi Tikus (Typonium Flagelliforme Lodd) Secara In Vitro. J Penelitian Tanaman Industri Ind Crop Res J. 2007;13(4):142-6. DOI: 10.21082/jlittri.v13n4.2007.142-146

42. Sartika F. Daya Hambat Air Kelapa (Cocus nucifera) Terhadap Pertumbuhan Bakteri Salmonella typhi dan Escherichia coli. J Surya Medika JSM. 2019;4(2):12-6. DOI: 10.33084/jsm.v4i2.603

43. Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, Bertoli S. Cultivation, Genetic, Ethnopharmacology, Phytochemistry and Pharmacology of Moringa oleifera Leaves: An Overview. Int J Mol Sci. 2015;16(6):12791-835. DOI: 10.3390/ijms160612791; PMCID: PMC4490473; PMID: 26057747

44. Bilal A, Jahan N, Ahmed A, Bilal SN, Habib S, Hajra S. Phytochemical and pharmacological studies on Ocimum basilicum – A Review. Int J Cur Res Rev. 2012;4(23):73-83.

45. Tefu MOFI, Sabat DR. Tanaman Obat Tradisional Dokumentasi Pemanfaatan Tanaman Obat Masyarakat Suku Dawan (Amanuban). Yogyakarta: Deepublish; 2021.

46. Hayati EK, Ningsih R, Latifah L. Antioxidant Activity of Flavonoid from Rhizome Kaemferia galanga L. Extract. Alchemy J Chem. 2015;4(2):127-37. DOI: 10.18860/al.v4i2.3203

47. Munte N, Sartini, Lubis R. Skrining Fitokimia dan Antimikroba Ekstrak Daun Kirinyuh terhadap Bakteri Staphylococcus aureus dan Escherichia coli. BIOLINK J Biol Lingkungan Industri Kesehatan. 2017;2(2):132-40. DOI: 10.31289/biolink.v2i2.803

48. Noorcahyati. Tumbuhan Berkhasiat Obat Etnis Asli Kalimantan. Balikpapan: Balai Penelitian Teknologi Konservasi Sumber Daya Alam; 2012.

49. Himaja M, Ranjitha A, Raman MV, Anand M, Asif K. Phytochemical screening and antioxidant activity of rhizome part Curcuma zedoaria. Int J Res Ayurveda Pharm. 2010;1(2):414-7.

50. Mukherjee PK, Nema NK, Maity N, Mukherjee K, Harwansh RK. Phytochemical and Therapeutic Profile of Aloe vera. J Nat Remedies. 2014;14(1):1-26. DOI: 10.18311/jnr/2014/84

51. Putra WS. Kitab Herbal Nusantara: Kumpulan Resep & Ramuan Tanaman Obat Untuk Berbagai Gangguan Kesehatan. Yogyakarta: Katahati; 2015.

52. Lallo S, Hardianti B, Umar H, Trisurani W, Wahyuni A, Latifah M. Aktivitas Anti Inflamasi dan Penyembuhan Luka dari Ekstrak Kulit Batang Murbei (Morus alba L.). J Farmasi Galenika Galenika J Pharm. 2020;6(1):26-36. DOI: 10.22487/j24428744.2020.v6.i1.14661

53. Angelika L, Anissa N, Prasetya F. Pengaruh Jus Buah Nanas Kombinasi Madu sebagai Penurun Tekanan Darah pada Pasien Hipertensi. Proceed Mulawarman Pharm Conf. 2020;11(1):70-5. DOI: 10.25026/mpc.v11i1.396

54. Ajiningrum PS, Amilah S, Widyaningtyas PG. Efektivitas Ekstrak Rimpang Pacing (Costus speciosus), Daun Srikaya (Annona squamosa L.) dan Ekstrak Kombinasinya Terhadap Penurunan Jumlah Folikel Tersier dan Folikel De Graff Pada Mencit Betina (Mus musculus). J Pharm Sci. 2020;5(1):33-7. DOI: 10.53342/pharmasci.v5i1.164

55. Rahman M, Rahman MH, Chowdhury TA. Phytochemical and Biological Activity Studies of Tinospora crispa Stem. Dhaka Univ J Sci. 2020;68(2):167-70. DOI: 10.3329/dujs.v68i2.54616

56. Lim TK. Lansium domesticum 'Duku Group'. In: Edible Medicinal and Non Medicinal Plants. Dordrecht: Springer; 2012. p. 265-8. DOI: 10.1007/978-94-007-2534-8_33

57. Hikmawati NPE, Aulia C, Viransa VP. Kandungan Piperin dalam Ekstrak Buah Lada Hitam dan Buah Lada Putih (Piper nigrum L.) yang Diekstraksi dengan Variasi Konsentrasi Etanol Menggunakan Metode KLT-Densitometri. Media Farmasi J Ilmu Farmasi J Pharm Sci. 2016;13(2):173-85. DOI: 10.12928/mf.v13i2.7769

58. Setyawaty R, Aptuning RB, Dewanto. Preliminary Studies on the Content of Phytochemical Compounds on Skin of Salak Fruit (Salacca zalacca). Pharm J Indones. 2020;6(1):1-6. DOI: 10.21776/ub.pji.2020.006.01.1

59. Soraya C, Sunnati, Vivi M. Efek Antibakteri Extract Stem Serai (Cymbopogoncitarus) terhadap Pertumbuhan Enterococcus faecalis. Cakradonya Dent J. 2016;8(2):69-78.

60. Carolia N, Noventi W. Potensi Extract Leaf Sirih Hijau (Piper betle L.) Sebagai Alternatif Terapi Acne Vulgaris. Majority Med J Lampung Univ. 2016;5(1):140-5.

61. Hasanah HMM, Yahdi Y, Dewi YK. Studi Komparasi Kualitas Handsoap Esktrak Daun Sirih Hijau (Piper betle Linn), Daun Sirih Merah (Piper crocatum) dan Daun Sirih Cina (Peperomia pellucida). SPIN J Kimia Pendidikan Kimia. 2020;2(2):191-209. DOI: 10.20414/spin.v2i2.2392

62. Wullur AC, Schaduw J, Wardhani ANK. Identifikasi Alkaloid pada Leaf Sirsak (Annona muricata L.). J Ilmiah Farmasi. 2012;3(2):54-6.

63. Gunarti NS, Hidayah H. Flavonoid compounds of tapak liman plant (Elephantopus scaber) as antihyperuricemia. J Ilmiah Farmasi. 2022;Special:31-6. DOI: 10.20885/jif.specialissue2022.art4

64. Pathak V, Tiwari VK. Phytochemical Screening of Saccharum officinarum (Linn.) Stem. Int J Innov Sci Res Technol. 2017;2(8):291-305.

65. Rahmat E, Lee J, Kang Y. Javanese Turmeric (Curcuma xanthorrhiza Roxb.): Ethnobotany, Phytochemistry, Biotechnology, and Pharmacological Activities. Evid Based Complement Alternat Med. 2021;2021:9960813. DOI: 10.1155/2021/9960813; PMCID: PMC8214482; PMID: 34194529

66. Ramon P, Bergmann D, Abdulla H, Sparks J, Omoruyi F. Bioactive Ingredients in K. pinnata Extract and Synergistic Effects of Combined K. pinnata and Metformin Preparations on Antioxidant Activities in Diabetic and Non-Diabetic Skeletal Muscle Cells. Int J Mol Sci. 2023;24(7):6211. DOI: 10.3390/ijms24076211; PMCID: PMC10094480; PMID: 37047182

Borneo Journal of Pharmacy
Vol 7 Issue 2 May 2024
Pages 147 – 160

https://journal.umpr.ac.id/index.php/bjop/article/view/6599
DOI: https://doi.org/10.33084/bjop.v7i2.6599
e-ISSN: 2621-4814

Research Article

Utilization of Emulgel Watermelon (*Citrullus lanatus*) Flesh Extract as a Topical Antioxidant

Kori Yati *

Fitria Nugrahaeni

Rika Melinda

Lilis Rokimah Wati

Department of Pharmacy, Universitas Muhammadiyah Prof. DR. HAMKA, East Jakarta, Special Capital Region of Jakarta, Indonesia

*email: koriyati@uhamka.ac.id; phone: +6285212091201

Keywords:
Antioxidant stability
Characteristic physics test
Emulgel
Watermelon flesh extract

Abstract

The flesh of watermelon (*Citrullus lanatus*) contains carotenoid compounds that act as antioxidants. The purpose of this study was to determine the variation in the concentration of carbopol 940 on physical properties, irritation tests, and the stability of emulgel antioxidants against temperature and storage time. Evaluation of the physical properties of *C. lanatus* pulp extract emulgel includes organoleptic, homogeneity, dosage pH, dispersion, adhesion, emulsion type, viscosity as well as hedonic test and irritation test. Antioxidant stability testing of emulgel was carried out for 28 days at three temperature conditions: 4, 25, and 40°C and tested on days 0, 7, 14, 21, and 28 with the DPPH method. The results of the physical properties evaluation meet the requirements with pH values of 5.50–5.57, dispersion 5–6 cm, adhesion <4 seconds, viscosity 5624–15443 cPs, F2 and F3 hedonic tests are preferred by researchers, and irritation tests of all formulas show no irritation symptoms to all refiners. The results of antioxidant stability of emulgel after storage on the 28th day showed an average result of IC_{50} temperature of 4°C (112.4547 ± 0.1432 mg/L), 25°C (119.3170 ± 0.1966 mg/L), and 40°C (124.1554 ± 0.1317 mg/L). The results of stability analysis show that temperature and storage duration affect antioxidant stability. The higher the temperature and duration of storage, the antioxidant stability of emulgel decreases. Storage of *C. lanatus* flesh extract emulgel at 4°C was able to maintain antioxidant activity for 28 days of storage.

Received: January 17th, 2024
1st Revised: May 20th, 2024
Accepted: May 25th, 2024
Published: May 30th, 2024

INTRODUCTION

Watermelon (*Citrullus lanatus*) is a popular fruit rich in various health-promoting nutrients[1]. Beyond its well-known water content (approximately 91.45%), *C. lanatus* flesh also boasts a valuable nutritional profile, including vitamin C, thiamine, riboflavin, niacin, carbohydrates, fiber, and sugars (0.15% fat, 7.55% carbohydrates, 0.4% fiber, and 6.2% sugar)[2]. Importantly, *C. lanatus* possesses potent antioxidant properties due to the presence of carotenoid compounds like lycopene, phytoene, phytofluene, beta-carotene, and lutein[3]. These bioactive components contribute to its ability to combat free radicals and potentially mitigate cellular damage. Studies have demonstrated the significant antioxidant activity of *C. lanatus* flesh extract, with Mariani *et al.*[2] reporting a value of 16.619 mg/L. Given its established antioxidant properties, *C. lanatus* flesh presents a promising material for the development of skincare cosmetic products, particularly in the form of emulgels. *Citrullus lanatus* flesh extract has gained interest for its potential use in dermatological applications due to its unique properties. These include thixotropy, emolliency, a non-greasy feel, ease of spreading, lack of staining, long shelf life, water solubility, environmental friendliness, transparency, and a pleasant appearance[4]. Emulgels, a type of emulsion formulation (oil-in-water [O/W] or water-in-oil [W/O]), are increasingly utilized for drug delivery. When a gelling agent is incorporated

How to cite: Yati K, Nugrahaeni F, Melinda R, Wati LR. Utilization of Emulgel Watermelon (Citrullus lanatus) Flesh Extract as a Topical Antioxidant. Borneo J Pharm. 2024;7(2):147-60. doi:10.33084/bjop.v7i2.6599

into the emulsion, it transforms into a gel-like structure (emulgel). Notably, the concentration of the gelling agent significantly impacts the resulting viscosity of the emulgel[5]. This study investigates the effects of varying carbopol 940 concentrations on the physical properties, irritation potential, and stability of emulgel formulations containing *C. lanatus* flesh extract.

Carbopol 940, a commonly used gelling agent, significantly influences the consistency of emulgel formulations. Studies by Habiba *et al*.[6] demonstrated that varying carbopol 940 concentrations (0.5%, 0.75%, 1%, and 1.25%) in olive oil emulgel with moringa leaf extract resulted in distinct consistency variations, ranging from less viscous to quite dense and viscous. Notably, 1% carbopol 940 concentration has been shown to yield formulations with desirable physical properties and stability[7]. Beyond physical characteristics, ensuring the safety and user acceptance of topical formulations is crucial[8]. Irritation tests are essential to evaluate the potential for skin irritation caused by the emulgel extract[9]. Additionally, hedonic testing allows for the assessment of user preference regarding the formulation's sensory attributes[10].

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay was selected for its simplicity, speed, sensitivity, and minimal sample requirement[11]. This method measures the ability of the emulgel to scavenge free radicals, indirectly reflecting its antioxidant activity. DPPH is a stable free radical at room temperature that accepts an electron or hydrogen atom from an antioxidant compound, resulting in the formation of a stable molecule and a reduction in absorbance at 517 nm[12]. Similar to findings by Wulansari *et al*.[13], who reported a decrease in tamarind leaf extract's antioxidant capacity during storage at varying temperatures, we hypothesized that storage temperature and duration might influence the stability of the *C. lanatus* extract's antioxidant activity within the emulgel. Therefore, we evaluated the antioxidant stability of the emulgel formulations under different storage conditions. The emulgels were stored at 4°C, 25°C, and 40°C for 28 days. Antioxidant activity was assessed using the DPPH method at predetermined time points (days 0, 7, 14, 21, and 28) to investigate the combined effects of temperature and storage time. Based on the established knowledge that antioxidants are susceptible to oxidation[14], and the potential impact of temperature and storage on the activity of natural antioxidants like those found in *C. lanatus* extract[15], this study investigated the influence of carbopol 940 concentration on the physical properties, irritation potential, and, most importantly, the stability of the emulgel's antioxidant activity during storage at varying temperatures and durations.

MATERIALS AND METHODS

Materials

The following instruments were used in this study: analytical balance (Ohaus), pH meter (Hanna Instruments), Anton Paar viscometer (ViscoQC 300), oven (Memmert), UV-Vis spectrophotometer (Shimadzu), climatic chamber (Lneya), optical microscope (Novel), hotplate (Corning), micropipettes (Across Pro), Karl Fischer Titrator (Aquacounter AQV-300), UV Box Thin-layer chromatography (TLC) spotting display (Camag), homogenizer (Heidolph), adhesion tester, dispersion tester, TLC chamber, refrigerator (AQUA), and blender (Advan). Standard laboratory glassware was also used. *Citrullus lanatus* was obtained from the Kramat Jati main market, East Jakarta, Indonesia, and identified as *Citrullus lanatus* (Thunb.) Matsum. & Nakai by the National Research and Innovation Agency of the Republic of Indonesia, Cibinong (accession number B-579/II.6.2/IR.01.02/4/2023). The extract was prepared at the Indonesian Medicinal and Aromatic Crops Research Institute, Bogor. Other materials used include carbopol 940 (Dwilab Mandiri), triethanolamine (Dwilab Mandiri), paraffin liquid (Dwilab Mandiri), tween 80 (Dwilab Mandiri), propylene glycol (Dwilab Mandiri), methylparaben (Kimia Jaya Laboran), propylparaben (Kimia Jaya Laboran), span 80 (Labsains Chemical Center), distilled water (Fragrant Chemical), beta-carotene (Sigma Aldrich), DPPH (Sigma Aldrich), TLC plate GF_{254} (Merck), absolute methanol (Merck), vitamin C (Merck), methylene blue (Kimia Jaya Laboran), magnesium powder, and concentrated HCl (Universitas Muhammadiyah Prof. DR. HAMKA).

Methods

Preparation of C. lanatus flesh extract

Ripe *C. lanatus* fruits were obtained and thoroughly washed. The fruits were dissected to separate the red flesh, white flesh, and rind. The red flesh was cut into small pieces and manually pressed to remove excess moisture. Subsequently, the red

flesh was oven-dried at 50°C for 48 hours (2 x 24 hours). The dried material was then pulverized using a blender to obtain a fine powder for extraction[1]. A mass of 150.432 g of the red flesh powder was subjected to maceration extraction using 96% ethanol as the solvent. The mixture was macerated for 48 hours (2 x 24 hours) with constant agitation. The extract was then separated from the residue by filtration using filter paper. The residue was re-extracted with fresh 96% ethanol using the same maceration conditions. The combined filtrates from both maceration steps were concentrated using a rotary evaporator to remove the solvent and obtain a concentrated *C. lanatus* flesh extract.

Extract evaluation

The organoleptic properties (appearance, color, and odor) of the *C. lanatus* flesh extract were evaluated visually[16]. A qualitative screening for flavonoids was performed using a standard method[17]. Briefly, 0.5 g of extract was dissolved in 10 mL of hot methanol. Subsequently, 0.1 g of Mg powder and five drops of concentrated HCl were added. The formation of an orange, pink, or dark red color that persisted for at least three minutes was considered indicative of the presence of flavonoids. Thin-layer chromatography was employed to identify beta-carotene compounds. A mobile phase of chloroform : ethanol (1 : 1) and a stationary phase of silica gel 60 F_{254} were used[5]. Moisture content of the extract was determined using an automated Karl Fischer titrator. Approximately 0.05 g of the sample was weighed and introduced into the instrument for analysis[16].

Citrullus lanatus flesh extract antioxidant activity test

Citrullus lanatus flesh (10 mg) was weighed and dissolved in pro-analysis grade methanol in a 100 mL volumetric flask to prepare a 100 mg/L stock solution. The stock solution (600, 1000, 1400, 1800, or 2200 µL) was pipetted using a micropipette and diluted further with methanol to a final volume of 10 mL in separate volumetric flasks. This resulted in solutions with final concentrations of 6, 10, 14, 18, and 22 mg/L, respectively. Two milliliters of each extract concentration solution were pipetted into separate vials, followed by the addition of 2 mL of 0.1 mM DPPH solution. The vials were incubated in the dark for 30 minutes. The absorbance of each sample was then measured at 517 nm using a UV-Vis spectrophotometer. Three replicates were performed for each concentration. Vitamin C solutions (4, 5.5, 7, 8.5, and 10 mg/L) were prepared and used as a positive control following the same procedure[18]. The results of the extract solutions were compared to the vitamin C standard curve to determine the antioxidant activity.

Citrullus lanatus flesh extract emulgel formulation

The formulation for the *C. lanatus* flesh extract emulgel is detailed in **Table I**. Carbopol 940 was dispersed in twenty parts (w/w) purified water and allowed to hydrate for 24 hours. Triethanolamine was then gradually added under constant stirring until the gel base reached a pH of 6. Methylparaben and propylparaben were weighed, dissolved in propylene glycol, and incorporated into the gel base with continuous stirring until a homogenous mixture was obtained. An oil phase was prepared by combining liquid paraffin and span 80 in a separate container on a water bath set to 70°C. The water phase was prepared by mixing tween 80 with distilled water on a separate water bath set to 70°C. Both phases were stirred continuously throughout this process. The oil and water phases were then combined and homogenized using a suitable homogenizer. The homogenized emulsion was subsequently incorporated into the prepared gel base with continuous stirring until a uniform emulgel formed. Finally, the *C. lanatus* flesh extract was added to the emulgel and stirred until thoroughly homogeneous[19].

Table I. *Citrullus lanatus* flesh extract emulgel formula.

Materials	Formula (%)				Function
	F1	F2	F3	F4	
Citrullus lanatus flesh extract	0.25	0.25	0.25	0.25	Active substances
Carbopol 940	0.5	0.75	1	1.25	Gelling agent
TEA	0.18	0.55	0.8	1.02	Extermination
Propylene glycol	10	10	10	10	Humectants
Methylparaben	0.18	0.18	0.18	0.18	Preservatives
Propylparaben	0.02	0.02	0.02	0.02	Preservatives
Liquid paraffin	5	5	5	5	Oil phase
Span 80	1.4	1.4	1.4	1.4	Emulsifiers
Tween 80	3.6	3.6	3.6	3.6	Emulsifiers
Distilled water	ad 100	ad 100	ad 100	ad 100	Solvent

Evaluation of C. lanatus flesh extract emulgel formulation

Organoleptic test: The emulgel formulations were subjected to a visual evaluation of color, odor, and texture using the naked eye[20].

Homogeneity test: Approximately 0.1 g of each emulgel formulation was weighed and spread evenly in a thin layer onto a clean, transparent glass slide. The spread emulgel was visually inspected for homogeneity, ensuring the absence of coarse particles or uneven distribution[21].

pH test: The pH of the emulgel formulations was measured using a calibrated pH meter. Prior to measurement, the pH meter was calibrated with standard solutions of pH 4 and 7. About 10 g of each emulgel sample were weighed and transferred to a beaker. The pH electrode was immersed into the sample until a stable reading was obtained on the meter. The displayed pH value was then recorded[22].

Viscosity test: The viscosity of the emulgel formulations was determined using an Anton Paar viscometer. A 200 mL beaker was filled with a well-mixed sample of the emulgel. The appropriate spindle (specify size and model number) was carefully immersed into the sample, ensuring the fill line on the spindle was submerged. The viscometer was then turned on, and the spindle was rotated at a speed of 100 rpm. The viscosity readings were recorded in centipoise (cPs) after the readings stabilized.

Dispersion test: The spreadability of the emulgel formulations was evaluated using a simple method. A transparent glass plate was placed on a millimeter block paper base. About 0.5 g of the emulgel sample was spread uniformly onto the glass plate. A second transparent glass plate was carefully placed on top of the sample, and a weight of 50, 100, or 150 g was applied for one minute to ensure consistent contact. Following this, the upper glass plate was removed, and the diameter of the spread emulgel was measured using the underlying millimeter block paper. This procedure was repeated for each weight (50, 100, and 150 g) with fresh emulgel samples[23].

Adhesion test: The adhesive properties of the emulgel formulations were evaluated using a modified version of a previously described method[24]. Briefly, 0.25 g of each emulgel sample was applied to two pre-designated glass slides. A 1 kg weight was placed on top of the slides for 5 minutes to ensure uniform contact. The slides were then secured onto the testing apparatus, and an additional 80 g load was applied. The time taken for the emulgel to detach from the glass slides was recorded for each sample.

Phase separation (cycling test): The stability of the emulgel formulations against phase separation was evaluated using a thermal cycling test. Each formulation underwent six cycles over a 24-day period. Each cycle involved storing the emulgel for 48 hours at 4°C, followed by 48 hours at 45°C. After each cycle (every 96 hours), the emulgels were visually inspected for any signs of phase separation (creaming, sedimentation, or cracking)[25].

Determination of emulsion type: The type of the emulgel was determined using the methylene blue dye dilution method[20]. Briefly, 0.5 g of emulgel was spread onto a microscope slide. A drop of 1% methylene blue solution was then added to the emulgel sample on the slide. The slide was covered with a coverslip, and the distribution of the dye was observed under a microscope at a suitable magnification. If the dye diffused evenly throughout the emulgel, the emulsion was classified as O/W. Conversely, if the dye remained localized as discrete blue specks within the emulgel, the emulsion was classified as W/O.

Hedonic test: A hedonic test was conducted to evaluate the color, aroma, and texture of the prepared *C. lanatus* flesh extract emulgel[26]. Twenty student volunteers from the Faculty of Pharmacy and Science, Universitas Muhammadiyah Prof. DR. HAMKA, participated in the study.

Irritation test: Following approval from the Ethics Committee of Universitas Muhammadiyah Prof. DR. HAMKA number 03/23.10/02922 dated 2023, a closed patch test was conducted to assess the irritation potential of the emulgel formulations. Twenty healthy volunteers participated in this study. The emulgel was applied to a designated area (approximately 2 cm diameter) on the upper arm of each volunteer. The application site was then occluded with a waterproof plaster for 24 hours. During this period, the application sites were visually observed for signs of irritation at 4-hour intervals[27].

Antioxidant stability of C. lanatus flesh extract emulgel formulation

Emulgel formulations were stored at controlled temperatures of 4°C, 25°C, and 40°C for a period of 28 days. To assess the impact of storage on antioxidant stability, the DPPH method was employed on the emulgel samples at predetermined time points: days 0, 7, 14, 21, and 28[28].

Preparation of 0.1 mM DPPH solution: A 0.1 mM DPPH solution was prepared by dissolving 3.9432 mg of DPPH powder in analytical grade methanol. The solution was then brought to a final volume of 100 mL with additional methanol in a volumetric flask[18].

Determination of the maximum wavelength of DPPH: The maximum absorption wavelength (λ_{max}) of the DPPH solution was determined. Briefly, 2 mL of 0.1 mM DPPH solution in methanol was added to a cuvette. The absorbance was scanned across a wavelength range of 400-800 nm using a UV-Vis spectrophotometer to identify the λ_{max} of DPPH[18].

Measurement of antioxidant activity of C. lanatus flesh extract emulgel formulation: As much as 10 mg of emulgel were dissolved in methanol in a 10 mL volumetric flask to obtain a stock solution of 1000 mg/L. A series of five dilutions were prepared from the stock solution to achieve final concentrations of 45, 65, 85, 105, and 125 mg/L. Briefly, aliquots of 450, 650, 850, 1050, and 1250 µL of the stock solution were transferred to separate volumetric flasks and diluted to 10 mL with methanol. About 2 mL of each diluted solution were then combined with 2 mL of 0.1 mM DPPH solution and incubated in the dark for 30 minutes. The absorbance of each sample was measured at the λ_{max} of DPPH using a UV-Vis spectrophotometer. All measurements were performed in triplicate[18].

Data analysis

The antioxidant activity of the emulgel formulations was determined by calculating their % inhibition of DPPH radical oxidation according to **Equation 1**. This equation utilizes the absorbance values of the control DPPH solution ($Abs_{control}$) and the DPPH solution containing the sample (Abs_{sample}). The half-maximal inhibitory concentration (IC_{50}) value, representing the sample concentration required to inhibit 50% of DPPH activity, was determined using a linear regression equation derived from the formula ($y = a \pm bx$)[29]. The categorization of antioxidant activity followed the scale established by Blois[30]: very strong (<50 mg/L), strong (50-100 mg/L), medium (100-150 mg/L), low (150-200 mg/L), and very low (>200 mg/L).

$$\%inhibition = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \text{x} 100\% \qquad [1]$$

RESULTS AND DISCUSSION

The extraction process yielded 24.28% (w/w) of *C. lanatus* flesh extract, as detailed in **Table II**. The extract exhibited a characteristic watermelon odor, a thick consistency, and a brownish-red color, as shown in **Figure 1**. The moisture content of the extract, determined using a moisture test, was 18.1816%, which falls within the established range (5-30%) for viscous extracts[31]. Phytochemical screening revealed the presence of flavonoids *C. lanatus* flesh extract. The formation of a red-orange color upon addition of HCl and Mg powder serves as a characteristic test for flavonoid compounds (**Figure 2**). This color change indicates a reduction of the flavonoid components, further supporting their presence in the extract[32].

Table II. *Citrullus lanatus* flesh extract evaluation results.

Extract evaluation	Result
Yield (%)	24.28
Organoleptic	Form: viscous
	Color: brownish red
	Smell: typical of watermelon
Phytochemical screening (flavonoid)	Red-orange (positive flavonoids)
Water content (%)	18.18
TLC	Rf extract: 0.71
	Rf beta-carotene: 0.76

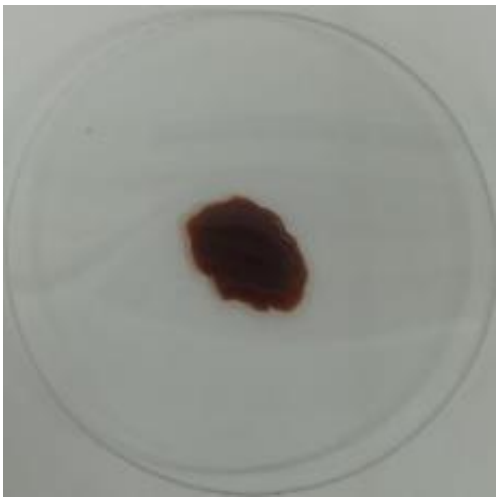


Figure 1. *Citrullus lanatus* flesh extract.

Figure 2. Flavonoid test results.

Thin-layer chromatography analysis was performed to identify the presence of beta-carotene in the *C. lanatus* flesh extract. Under visible and UV light at 254 and 366 nm, the extract exhibited an Rf value of 0.71, while the reference standard beta-carotene displayed an Rf value of 0.76 (**Figure 3**). Since the Rf values of the extract and standard fell within a close range (0 to 1)[33], the presence of beta-carotene in the *C. lanatus* flesh extract is confirmed.

Prior to emulgel preparation, the antioxidant activity of *C. lanatus* flesh extract was evaluated using the DPPH free radical scavenging assay and compared to vitamin C, a readily available and cost-effective standard with established strong antioxidant properties[34]. The carotenoid content of the extract was also determined using a UV-Vis spectrophotometer to identify the presence of these antioxidant pigments. Carotenoids typically exhibit absorption peaks within the 400-550 nm range[35]. Analysis of the *C. lanatus* flesh extract revealed three peaks at 502, 470, and 444 nm, suggesting the presence of carotenoid compounds.

The DPPH assay relies on the ability of antioxidants to scavenge the stable free radical DPPH, resulting in a color change from purple to yellow and a decrease in absorbance at the λ_{max} of DPPH (typically around 515-520 nm)[36,37]. In this study, the λ_{max} of the DPPH solution was determined to be 516 nm. To minimize light and oxygen exposure, known to degrade the DPPH solution[38], it was stored in the dark and protected with aluminum foil. Following the method described by Molyneux[37], samples containing the extract and DPPH solution were incubated for 30 minutes to allow for the characteristic slow-moving reaction between antioxidants and free radicals, evident by the color change.

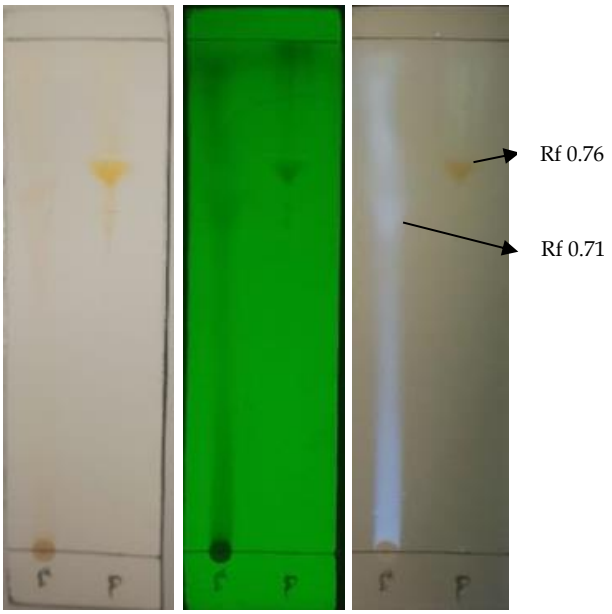


Figure 3. TLC results in visible light (**a**), UV 254 nm (**b**), and UV 366 nm (**c**). *Citrullus lanatus* flesh extract (S) and beta-carotene (P).

The DPPH assay results (**Table III**) revealed an IC_{50} value of 21.3876 mg/L for the *C. lanatus* flesh extract, compared to 8.6484 mg/L for vitamin C. Both the extract and vitamin C fall within the category of very strong antioxidants (IC_{50} <50 mg/L) based on established classifications[30]. However, the extract exhibited a higher IC_{50} value than vitamin C, which is likely due to the presence of various secondary metabolites with antioxidant properties within the extract, compared to the pure compound nature of vitamin C. Mariani *et al.*[2] reported a *C. lanatus* flesh extract IC_{50} value of 16.619 mg/L, highlighting potential variations in antioxidant activity arising from differences in plant growth location and sample treatment methods. The concentration of *C. lanatus* flesh extract incorporated into the emulgel formulations was 100-fold higher than the determined IC_{50} value of 2138.76 mg/L, translating to approximately 0.21% of the total emulgel weight[39]. This concentration was subsequently increased to 0.25% to potentially mitigate potential reductions in antioxidant activity arising from interactions with the emulgel base components[40].

Table III. *Citrullus lanatus* flesh extract and vitamin C antioxidant activity.

Sample	IC_{50} (mg/L)
Citrullus lanatus flesh extract	21.3876
Vitamin C	8.6484

The physical properties of the *C. lanatus* flesh extract emulgel formulations (F1-F4; **Figure 4**) are summarized in **Table IV**. All formulations exhibited a yellowish-white color and lacked stickiness. However, consistency varied across formulations: F1 had a slightly watery consistency, F2 possessed an emulgel-like consistency, F3 displayed a slightly viscous consistency, and F4 exhibited the most viscous consistency. This demonstrates that increasing carbopol 940 concentration significantly impacted consistency ($p < 0.05$) but did not affect odor or color. Homogeneity testing revealed that all formulations met the desired specifications, with no uneven color distribution or coarse particles observed. This ensures uniform distribution of active ingredients across each application[3].

pH testing is required to determine the degree of acidity of the preparation as it relates to consumer acceptance of a product[41]. The pH of the emulgels ranged from 5.50 to 5.57, exhibiting a slight decrease compared to the initial pH of 6 after the addition of the *C. lanatus* extract (weak acid, pH 5.31)[3]. However, these values remained within the normal human skin pH range (4.5 to 6.5)[42]. One-way ANOVA analysis showed no significant difference in pH between the formulations, indicating that carbopol 940 concentration did not influence pH.

Viscosity testing revealed a significant difference ($p < 0.05$) between the formulations. As expected, F4, containing the highest carbopol concentration (1.25%), displayed the highest viscosity, resulting in a thicker emulgel. All formulations met the standard viscosity range (6000-50000 cPs) for emulgel preparations according to SNI 16-4399-1996. The spreadability

(dispersion) of the emulgels decreased with increasing carbopol 940 concentration ($p < 0.05$). This aligns with the observed consistency variations, as higher carbopol concentrations lead to increased viscosity and reduced spreadability[43]. Nonetheless, all formulations met the dispersion criteria of 5 to 7 cm[44].

Adhesion time also increased with increasing carbopol concentration ($p < 0.05$). F4, with the highest carbopol content, exhibited the longest adhesion time. This is attributed to the more viscous nature of F4 compared to the other formulations. All formulations met the minimum adhesion requirement of 4 seconds for topical preparations[45]. The emulgels demonstrated good physical stability throughout six storage cycles at varying temperatures, with no observable phase separation between the oil and water phases. This suggests stability under both high and low temperatures[46]. Microscopic examination (4x/0.1 magnification) confirmed that all formulations (F1-F4) were O/W emulsions. As shown in **Figure 5**, the even distribution of methylene blue within each preparation confirms this classification.

Hedonic testing indicated that respondents preferred formulations F2 and F3 based on color, aroma, texture, and ease of application[26]. These findings suggest good user acceptance of the *C. lanatus* extract emulgel. Closed patch tests on volunteers' forearms revealed no irritation after 24-hour application of the emulgel preparations. This suggests good skin compatibility, likely due to the safe ingredients used and the pH remaining within the safe range for skin[47].

The results of this study demonstrate the successful development of stable *C. lanatus* flesh extract emulgels with varying carbopol 940 concentrations. While increasing carbopol concentration significantly impacted consistency and adhesion time, all formulations met the established criteria for physical properties. Notably, hedonic testing revealed user preference for formulations F2 and F3, suggesting good user acceptance. Importantly, the emulgels exhibited no irritation potential, indicating safety for topical application.

Figure 4. *Citrullus lanatus* flesh extract emulgel.

Table IV. *Citrullus lanatus* flesh extract emulgel evaluation results.

Physical evaluation	F1	F2	F3	F4
Organoleptic	Smell: distinctive Shape: rather watery Color: yellowish white	Smell: distinctive Shape: emulgel mass Color: yellowish white	Smell: distinctive Shape: slightly viscous Color: yellowish white	Smell: distinctive Shape: thick Color: yellowish white
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Ph	5.57 ± 0.0205	5.55 ± 0.0654	5.53 ± 0.0618	5.50 ± 0.0169
Viscosity (cPs)	5624.67 ± 83.2666	8423.67 ± 23.0940	15443.33 ± 582.8665	19070 ± 301.9934
Dispersion (cm)	6.18 ± 0.0750	5.81 ± 0.2346	5.55 ± 0.0704	5.25 ± 0.0625
Adhesion (seconds)	1.04 ± 0.0251	1.12 ± 0.0251	1.23 ± 0.0208	1.34 ± 0.0351
Cycling test	(-) No physical changes	(-) No physical changes	(-) No physical changes	(-) No physical changes
Emulsion type	O/W	O/W	O/W	O/W
Irritation test	No symptoms of irritation	No symptoms of irritation	No symptoms of irritation	No symptoms of irritation
Hedonic test (%)	93.75	100	100	80

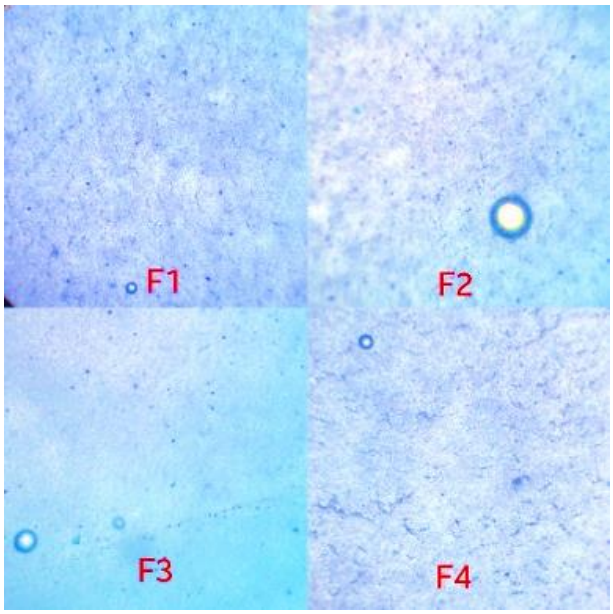


Figure 5. *Citrullus lanatus* flesh extract emulgel type results.

The F3 emulgel formulation with 1% carbopol 940 that exhibits appropriate physical properties was selected as the representative sample for antioxidant stability testing. As depicted in **Figure 6**, the IC_{50} value, a measure of antioxidant activity (lower IC_{50} indicates higher activity)[37], increased for emulgels stored at all three temperatures (4°C, 25°C, and 40°C) with increasing storage duration. This trend suggests a decrease in overall antioxidant activity during storage. The observed increase in IC_{50} values signifies a diminished capacity of the *C. lanatus* flesh extract within the emulgel to scavenge free radicals.

Two-way ANOVA analysis revealed a statistically significant interaction between storage temperature and storage time ($p < 0.05$). Specifically, the IC_{50} value increased with both longer storage duration and higher storage temperature. After 28 days, emulgels stored at 40°C exhibited the highest average IC_{50} values, while those stored at 4°C displayed the least pronounced decrease in activity. This phenomenon is likely attributed to the susceptibility of carotenoids, the primary antioxidant compounds in *C. lanatus* flesh, to degradation by heat, light, and oxygen. Carotenoid instability can lead to structural changes, such as the conversion from trans to cis isomers, rendering them more susceptible to oxidation[48].

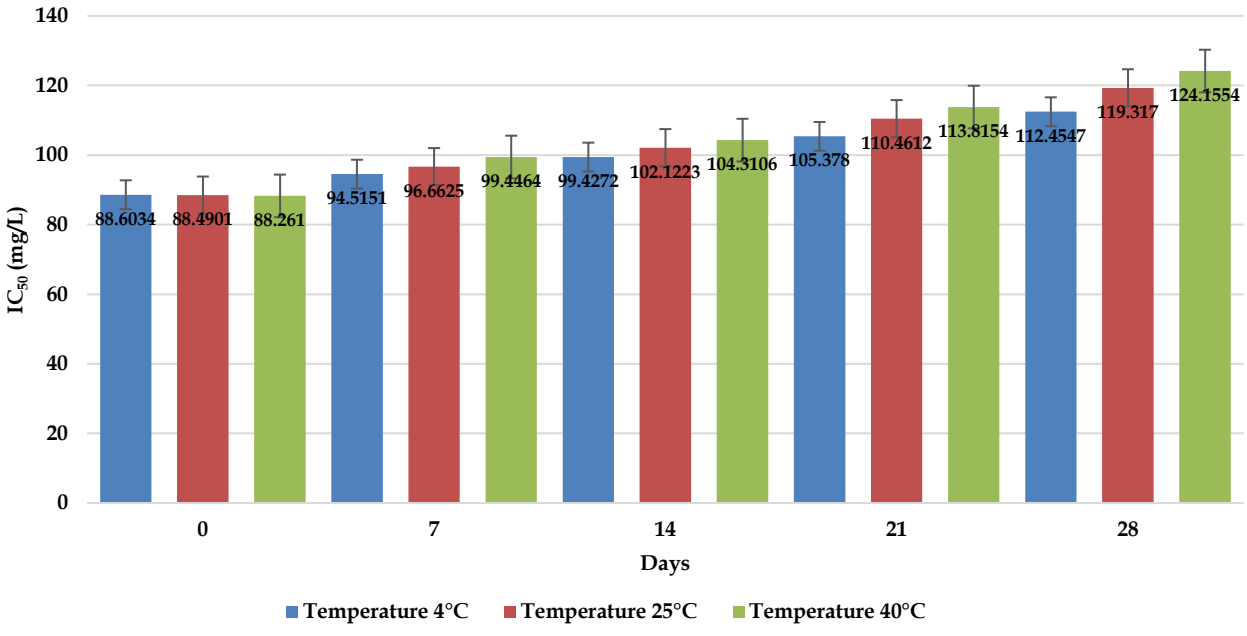


Figure 6. *Citrullus lanatus* flesh extract emulgel type results.

Carotenoids are susceptible to degradation through heat and oxidation, leading to the breakage of conjugated double bonds within their molecules[49]. This process reduces their biological activity and is often observed as a decrease in carotenoid content. Our findings align with this established knowledge, as evidenced by the observed stability of the emulgel's antioxidant activity when stored at 4°C compared to higher temperatures (25°C and 40°C). Similar results were reported by Aryayustama *et al*.[50], who demonstrated that pandan fruit carotenoid extract maintained its content during storage at 4°C for four weeks, while degradation occurred at higher temperatures (28°C and 45°C).

The observed temperature dependence of carotenoid stability can be attributed to their inherent chemical properties. Lower temperatures minimize the rate of thermal decomposition and oxidative reactions, thereby preserving the integrity and bioactivity of these antioxidant compounds[49]. Additionally, oxidation can lead to the formation of various degradation products with potentially altered or diminished biological activity compared to the parent carotenoid molecules[51].

CONCLUSION

This study investigated the influence of carbopol 940 concentration on the physical properties, irritation potential, and stability of *C. lanatus* flesh extract emulgel formulations. While variations in carbopol 940 concentration impacted physical properties like dispersion, adhesion, and viscosity, no significant differences were observed in hedonic preference or irritation. Notably, storage temperature and duration significantly affected the antioxidant stability of the emulgel formulations. Higher temperatures and longer storage periods led to decreased antioxidant activity. Importantly, emulgels stored at 4°C maintained significant antioxidant activity throughout the 28-day study period.

ACKNOWLEDGMENT

The authors gratefully acknowledge the financial support provided by the Research and Development Institute of Universitas Muhammadiyah Prof. DR. HAMKA (Grant number: SPK 300/F.03.07/2023).

AUTHORS' CONTRIBUTION

Conceptualization: Kori Yati, Fitria Nugrahaeni, Rika Melinda, Lilis Rokimah Wati
Data curation: Kori Yati, Fitria Nugrahaeni, Rika Melinda, Lilis Rokimah Wati
Formal analysis: Kori Yati, Fitria Nugrahaeni, Rika Melinda, Lilis Rokimah Wati
Funding acquisition: -
Investigation: Kori Yati, Fitria Nugrahaeni, Rika Melinda, Lilis Rokimah Wati
Methodology: Kori Yati, Fitria Nugrahaeni
Project administration: Kori Yati, Fitria Nugrahaeni, Rika Melinda, Lilis Rokimah Wati
Resources: Kori Yati, Fitria Nugrahaeni
Software: -
Supervision: Kori Yati, Fitria Nugrahaeni
Validation: Kori Yati, Fitria Nugrahaeni
Visualization: Kori Yati, Fitria Nugrahaeni
Writing - original draft: Kori Yati, Fitria Nugrahaeni, Rika Melinda, Lilis Rokimah Wati
Writing - review & editing: Kori Yati, Fitria Nugrahaeni

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

REFERENCES

1. Manivannan A, Lee ES, Ha K, Lee HE, Kim DS. Versatile Nutraceutical Potentials of Watermelon-A Modest Fruit Loaded with Pharmaceutically Valuable Phytochemicals. Molecules. 2020;25(22):5258. DOI: 10.3390/molecules25225258; PMCID: PMC7698065; PMID: 33187365

2. Mariani S, Rahman N, Supriadi. Uji Aktivitas Antioksidan Ekstrak Buah Semangka (Citrullus lanatus). J Akademika Kimia. 2018;7(3):107-14. DOI: 10.22487/j24775185.2018.v7.i3.11905

3. Ekayanti NPLS, Darsono FL, Wijaya S. Formulasi Sediaan Krim Pelembab Ekstrak Air Buah Semangka (Citrullus lanatus). J Pharm Sci Pract. 2019;6(1):36–43. DOI: 10.33508/jfst.v6i1.2011

4. Yadav SK, Mishra MK, Tiwari A, Shukla A. Emulgel: A New Approach for Enhanced Topical Drug Delivery. Int J Curr Pharm Res. 2016;9(1):15–9. DOI: 10.22159/ijcpr.2017v9i1.16628

5. Widayanti A, Fauziah DA, Radjab NS. Formulations Gel of Tuna Bone Collagen (Thunnus albacares) with HPMC as a Gelling Agent. Farmasains J Ilmiah Ilmu Kefarmasian. 2016;3(1):1-6.

6. Habiba SA, Tilarso DP, Putri AE. Pengaruh Konsentrasi Karbomer-940 pada Sediaan Emulgel Minyak Zaitun dan Ekstrak Daun Kelor. J Sains Kesehatan. 2022;4(2):138–46. DOI: 10.25026/jsk.v4i2.894

7. Handayani M, Mita N, Ibrahim A. Formulasi dan Optimasi Basis Emulgel Carbopol 940 dan Trietanolamin dengan Berbagai Variasi Konsentrasi. Proceed Mulawarman Pharm Conf. 2015;1(1):53–60. DOI: 10.25026/mpc.v1i1.8

8. Mursal ILP, Kusumawati AH, Puspasari DH. Pengaruh Variasi Konsentrasi Gelling Agent Carbopol 940 terhadap Sifat Fisik Sediaan Gel Hand Sanitizer Minyak Atsiri Daun Kemangi (Ocimum sanctum L.). Pharma Xplore J Sains Ilmu Farmasi. 2019;4(1):268-77. DOI: 10.36805/jpx.v4i1.617

9. Untari EK, Robiyanto. Uji Fisikokimia dan Uji Iritasi Sabun Antiseptik Kulit Daun Aloe vera (L.) Burm. F. J Jamu Indones. 2018;3(2):55–61. DOI: 10.29244/jji.v3i2.54

10. Nurdianti L, Rosiana D, Aji N. Evaluasi Sediaan Emulgel Anti Jerawat Tea Tree (Melaleuca alternifolia) Oil dengan Menggunakan HPMC sebagai Gelling Agent. J Pharmacopolium. 2018;1(1):23–31. DOI: 10.36465/jop.v1i1.392

11. Sadeer NB, Montesano D, Albrizio S, Zengin G, Mahomoodally MF. The Versatility of Antioxidant Assays in Food Science and Safety-Chemistry, Applications, Strengths, and Limitations. Antioxidants. 2020;9(8):709. DOI: 10.3390/antiox9080709; PMCID: PMC7464350; PMID: 32764410

12. Baliyan S, Mukherjee R, Priyadashini A, Vibhuti A, Gupta A, Pandey RP, et al. Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa. Molecules. 2022;27(4):1326. DOI: 10.3390/molecules27041326; PMCID: PMC8878429; PMID: 35209118

13. Wulansari ID, Admadi B, Mulyani S. Pengaruh Suhu Penyimpanan terhadap Kerusakan Antioksidan Ekstrak Daun Asam (Tamarindus indica L). J Rekayasa Manajemen Agroindustri. 2020;8(4):544-50. DOI: 10.24843/JRMA.2020.v08.i04.p07

14. Sharifi-Rad M, Kumar NVA, Zucca P, Varoni EM, Dini L, Panzarini E, et al. Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. Front Physiol. 2020;11:694. DOI: 10.3389/fphys.2020.00694; PMCID: PMC7347016; PMID: 32714204

15. Salin NSM, Saad WMM, Razak HRA, Salim F. Effect of Storage Temperatures on Physico-Chemicals, Phytochemicals and Antioxidant Properties of Watermelon Juice (Citrullus lanatus). Metabolites. 2022;12(1):75. DOI: 10.3390/metabo12010075; PMCID: PMC8780985; PMID: 35050198

16. Departemen Kesehatan Republik Indonesia. Parameter Standar Umum Ekstrak Tumbuhan Obat. Jakarta: Departemen Kesehatan Republik Indonesia; 2000.

17. Mangiwa S, Maryuni AE. Skrining Fitokimia dan Uji Antioksidan Ekstrak Biji Kopi Sangrai Jenis Arabika (Coffea arabica) Asal Wamena dan Moanemani, Papua. J Biol Papua. 2019;11(2):103-9. DOI: 10.31957/jbp.925

18. Pandawangi STW, Bachtiar A, Firmansyah D. Uji Aktivitas Antioksidan Krim Kombinasi Ekstrak Daun Jambu Biji (Psidium guajava L.) Dan Ekstrak Umbi Wortel (Daucus carota L.) Dengan Menggunakan Metode DPPH (2,2-diphenyl-1-picrylhydrazyl). Med Sains J Ilmiah Kefarmasian. 2018;3(1):31-42. DOI: 10.37874/ms.v3i1.62

19. Robby O, Gloria F, Saptawati T. Pengaruh Variasi Konsentrasi Ekstrak Daun Kelor (Moringa oleifera Lamk) Terhadap Efek Antiinflamasi Sediaan Emulgel. J Penelitian Kesehatan Suara Forikes. 2022;13(2):444-52. DOI: 10.33846/sf13231

20. Nugrahaeni F, Yati K, Sukmara. Pengaruh Variasi Gliseril Monostearat pada Krim Ekstrak Kulit Buah Naga Merah Super (Hylocereus costaricensis) dan Uji Antioksidannya. J Kefarmasian Indones. 2023;13(1):20-9. DOI: 10.22435/jki.v13i1.6099

21. Yenti R, Afrianti R, Qomariah S. Formulasi emulgel ekstrak etanol daun dewa (Gynura pseudochina (L.) DC) untuk pengobatan nyeri sendi terhadap tikus putih Jantan. In: Syam J, Syamsir Y, Editors. Prosiding Seminar Nasional dan Workshop "Perkembangan Terkini Sains Farmasi dan Klinik IV" Tahun 2014. Padang: Fakultas Farmasi Universitas Andalas; 2014. p. 56–63.

22. Kementerian Kesehatan Republik Indonesia. Farmakope Indonesia edisi IV. Jakarta: Direktorat Jenderal Pengawasan Obat dan Makanan; 1995.

23. Saryanti D, Setiawan I, Safitri RA. Optimasi Asam Stearat dan TEA pada Formula Sediaan Krim Ekstrak Kulit Pisang Kepok (Musa paradisiaca L.). J Riset Kefarmasian Indones. 2019;1(3):225-37. DOI: 10.33759/jrki.v1i3.44

24. Mutmainah, Kusmita L, Puspitaningrum I. Pengaruh Perbedaan Konsentrasi Ekstrak Etanol Kulit Buah Manggis (Garcinia mangostana L.) terhadap Karakteristik Fisik Sediaan Gel. J Ilmu Farmasi Farmasi Klinik J Pharm Sci Clin Pharm. 2014;11(Special Issue):98-104. DOI: 10.31942/jiffk.v0i0.1209

25. Najib A, Malik A, Ahmad AR, Handayani V, Syarif RA, Waris R. Standarisasi Ekstrak Air Daun Jati Belanda dan Teh Hijau. J Fitofarmaka Indones. 2017;4(2):241-5. DOI: 10.33096/jffi.v4i2.268

26. Qamariah N, Handayani R, Mahendra AI. Uji Hedonik dan Daya Simpan Sediaan Salep Ekstrak Etanol Umbi Hati Tanah. J Surya Medika JSM. 2022;7(2):124-31. DOI: 10.33084/jsm.v7i2.3213

27. Cahnia MS, Lestari U, Sani FK. Formulasi, Uji Efektivitas dan Uji Hedonik Masker Gel Peel Off Kombinasi Ekstrak Rimpang Kunyit (Curcuma longa L.) dan Madu (Mel depuratum) sebagai Peningkat Elastisitas Kulit. Med Sains J Ilmu Kefarmasian. 2022;7(2):177-90. DOI: 10.37874/ms.v7i2.328

28. Setianingsih D, Halim M. Uji Efektivitas dan Uji Stabilitas Formulasi Masker Gel Peel-Off Ekstrak Metanol Kulit Biji Pinang Yaki (Areca vestiaria Giseke). Indones Nat Res Pharm J. 2020;5(1):80-93. DOI: 10.52447/inspj.v5i1.1832

29. Souhoka FA, Hattu N, Huliselan M. Uji Aktivitas Antioksidan Ekstrak Metanol Biji Kesumba Keling (Bixa orellana L). Indones J Chem Res. 2019;7(1):25-31. DOI: 10.30598//ijcr.2019.7-fas

30. Blois MS. Antioxidant Determinations by the Use of a Stable Free Radical. Nature. 1958;181(4617):1119-200. DOI: 10.1038/1811199a0

31. Voigt R. Buku Pelajaran Teknologi Farmasi, Edisi Kelima. Yogyakarta: Gadjah Mada University Press; 1995.

32. Kho HE, Azlan A. Tang ST, Lim SM. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr Res. 2017;61(1):1361779. DOI: 10.1080/16546628.2017.1361779; PMCID: PMC5613902; PMID: 28970777

33. Stahl E. Analisis Kromatografi dan Mikroskopis Obat. Bandung: ITB Press; 1985.

34. Lung JKS, Destiani DP. Uji Aktivitas Antioksidan Vitamin A, C, E dengan metode DPPH. Farmaka. 2018;15(1):53-62. DOI: 10.24198/jf.v15i1.12805

35. Sirohi P, Verma H, Singh SK, Singh VK, Pandey J, Khusharia S, et al. Microalgal Carotenoids: Therapeutic Application and Latest Approaches to Enhance the Production. Curr Issues Mol Biol. 2022;44(12):6257-79. DOI: 10.3390/cimb44120427; PMCID: PMC9777246; PMID: 36547088

36. Hakim AR, Saputri R. Narrative Review: Optimasi Etanol sebagai Pelarut Senyawa Flavonoid dan Fenolik. J Surya Medika JSM. 2020;6(1):177-80. DOI: 10.33084/jsm.v6i1.1641

37. Molyneux P. The Use of the Stable Free Radical Diphenylpicryl-Hydrazyl (DPPH) for Estimating Antioxidant Activity. Songklanakarin J Sci Technol. 2004;26(2):211-9.

38. Gulcin I, Alwasel SH. DPPH Radical Scavenging Assay. Processes. 2023;11(8):2248. DOI: 10.3390/pr11082248

39. Sulistiana S, Darijanto ST. Formulasi Dan Evaluasi Mikroemulsi Gel Minyak Chamomile Serta Uji Aktivitas Antioksidan. Indones J Pharm Educ. 2022;2(1):52-66. DOI: 10.37311/ijpe.v2i1.11231

40. Suhery WN, Fernando A, Has N. Uji Aktivitas Antioksidan dari Ekstrak Bekatul Padi Ketan Merah dan Hitam (Oryza sativa L. Var. Glutinosa) dan Formulasinya dalam Sediaan Krim. Pharmacy J Farmasi Indones Pharm J Indones. 2016;13(1):102-15.

41. Elfiyani R, Nursal FK, Deviyolanda R, Shifa S. Pemanfaatan Ekstrak Kulit Putih Semangka Dalam Sediaan Masker Clay. JSFK J Sains Farmasi Klinik. 2023;10(2):218-25. DOI: 10.25077/jsfk.10.2.218-225.2023

42. Tranggono RI, Latifah F. Buku Pegangan Ilmu Pengetahuan Kosmetik. Jakarta: Media Pusindo; 2007.

43. Yuliandari M, Sa'adah H, Warnida H. Pengaruh Konsentrasi Carbopol 940 sebagai Gelling Agent terhadap Stabilitas Sifat Fisik Emulgel Hand Sanitizer Minyak Sereh Wangi (Cymbopogon nardus L.). Prosiding Seminar Nasional Kesehatan. 2021;1:117-24.

44. Garg A, Aggarwal D, Garg S, Singla AK. Spreading of Semisolid Formulations: An update. Pharm Technol. 2002;26(9):84-105.

45. Ulaen SPJ, Banne Y, Suatan RA. Pembuatan Salep Anti Jerawat dari Ekstrak Rimpang Temulawak (Curcuma xanthorrhiza Roxb.). J Ilmiah Farmasi. 2012;3(2):45-9.

46. Daud NS, Suryanti E. Formulasi Emulgel Antijerawat Minyak Nilam (Patchouli oil) Menggunakan Tween 80 dan Span 80 sebagai Pengemulsi dan HPMC sebagai Basis Gel. J Mandala Pharmacon Indones. 2017;3(2):90-5. DOI: 10.35311/jmpi.v3i02.3

47. Ardhany SD, Novaryatiin S, Pratama MRF, Utar Z. Irritation Test of Bawang Dayak (Eleutherine bulbosa (Mill.) Urb.) Extract Cream with Human Patch Test Method. J Farmasi Sains Praktis. 2021;7(1):74-80. DOI: 10.31603/pharmacy.v7i1.4854

48. González-Peña MA, Ortega-Regules AE, de Parrodi CA, Lozada-Ramírez JD. Chemistry, Occurrence, Properties, Applications, and Encapsulation of Carotenoids-A Review. Plants. 2023;12(2):313. DOI: 10.3390/plants12020313; PMCID: PMC9865331; PMID: 36679026

49. Šeregelj V, Estivi L, Brandolini A, Ćetković G, Šaponjac VT, Hidalgo A. Kinetics of Carotenoids Degradation during the Storage of Encapsulated Carrot Waste Extracts. Molecules. 2022;27(24):8759. DOI: 10.3390/molecules27248759; PMCID: PMC9782125; PMID: 36557892

50. Aryayustama MG, Wartini NM, Suwariani NP. Stabilitas Kadar Karotenoid Ekstrak Buah Pandan (Pandanus tectorius) pada Cahaya dan Suhu Penyimpanan. J Rekayasa Manajemen Agroindustri. 2018;6(3):218-24. DOI: 10.24843/JRMA.2018.v06.i03.p05

51. Pérez-Gálvez A, Viera I, Roca M. Carotenoids and Chlorophylls as Antioxidants. Antioxidants. 2020;9(6):505. DOI: 10.3390/antiox9060505; PMCID: PMC7346216; PMID: 32526968

Borneo Journal of Pharmacy
Vol 7 Issue 2 May 2024
Pages 161 – 171

https://journal.umpr.ac.id/index.php/bjop/article/view/6810
DOI: https://doi.org/10.33084/bjop.v7i2.6810
e-ISSN: 2621-4814

Research Article

Molecular Docking and Dynamics of *Xylocarpus granatum* as A Potential Parkinson's Drug Targeting Multiple Enzymes

Riyan Alifbi Putera Irsal [1,2]

Gusnia Meilin Gholam [1,3]

Dzikri Anfasa Firdaus [1,2]

Novian Liwanda [1]

Fernanda Chairunisa [4*]

[1] Department of Biochemistry, Institut Pertanian Bogor, Bogor, West Java, Indonesia
[2] Biomatics, Bogor, West Java, Indonesia
[3] Bioinformatics Research Center, Indonesian Institute of Bioinformatics, Malang, East Java, Indonesia
[4] Department of Biology, Universitas Nasional, South Jakarta, Special Capital Region of Jakarta, Indonesia

*email:
fernandachairunisa@civitas.unas.ac.id;
phone: +6281383388113

Keywords:
Adenosine A2A receptor
Catechol-O-methyltransferase
Monoamine oxidase-B
Neurology

Abstract

Parkinson's disease is a global health challenge affecting over 10 million individuals worldwide, leading to increased disability-adjusted life years (DALYs) and a rise in mortality rates. This study explores the potential anti-Parkinson's properties of *Xylocarpus granatum*, focusing on its interaction with key enzymes associated with the disease: catechol-O-methyltransferase (COMT), adenosine A2A receptor (A2AR), and monoamine oxidase-B (MAO-B). Using molecular docking and molecular dynamics approaches with YASARA Structure, the ethanol extract of *X. granatum* was investigated for its mechanism of action. Among 30 compounds, five demonstrated promising binding affinities. Structural flexibility analysis revealed minimal fluctuations in active-site residues, highlighting the stability of key complexes involving kaempferol, epicatechin, epigallocatechin, and native ligands. Molecular Mechanics Poisson–Boltzmann Surface Area (MM-PBSA) simulations provided insights into the binding energy of these complexes. Notably, kaempferol exhibited higher binding energy than the natural ligand, suggesting superior binding affinity. Analysis of the average radius of gyration (Rg) showcased control drug-MAO-B exhibited higher Rg values, indicating a more flexible protein conformation. Confirming mode stability with root mean square deviation (RMSD) analysis shows overall stability, except in the A2AR-bound complex. The study's collective findings underscore the structural stabilization of ligand-protein complexes, contributing valuable insights into the potential anti-Parkinson's properties of *X. granatum*. These discoveries hold promise for developing more effective therapies for Parkinson's disease and significantly contribute to the neurology field.

Received: March 8th, 2024
1st Revised: May 15th, 2024
Accepted: May 20th, 2024
Published: May 30th, 2024

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting millions worldwide. Over 10 million people currently live with PD, with a significant rise in disease burden observed since 2000[1]. This translates to a substantial increase in disability-adjusted life years (DALYs) and mortality rates[2]. Unfortunately, current therapies primarily manage PD symptoms, lacking the ability to slow or halt disease progression.

Parkinson's disease is characterized by both motor and non-motor features[3]. Motor symptoms like tremors, rigidity, bradykinesia (slowness of movement), and postural instability significantly impact patients' physical abilities. Additionally, PD can affect mental health by causing depression, anxiety, and cognitive impairment. These factors collectively contribute to a decline in quality of life, often leading to financial burdens associated with long-term care[4].

How to cite: Irsal RAP, Gholam GM, Firdaus DA, Liwanda N, Chairunisa F. Molecular Docking and Dynamics of Xylocarpus granatum as A Potential Parkinson's Drug Targeting Multiple Enzymes. Borneo J Pharm. 2024;7(2):161-71. doi:10.33084/bjop.v7i2.6810

The cardinal motor features of PD arise from the degeneration of dopaminergic neurons in the substantia nigra, a critical brain region responsible for dopamine production. Dopamine is a vital neurotransmitter responsible for movement control. Three key enzymes – catechol-O-methyltransferase (COMT), monoamine oxidase-B (MAO-B), and adenosine A2A receptor (A2AR) – all play a role in regulating dopamine levels. COMT breaks down dopamine, MAO-B contributes to its degradation, and A2AR modulates dopamine release[5-7].

The limitations of conventional therapies have spurred research into novel therapeutic approaches. Natural products, particularly those derived from plants, are a promising avenue for the development of new anti-Parkinson's agents. *Xylocarpus granatum* is a plant species that has attracted significant research interest due to its potential neuroprotective properties[8]. This activity may offer protection against the neuronal damage and degeneration characteristic of PD. Additionally, studies suggest that *X. granatum* possesses antidepressant-like effects in mice, potentially aiding in managing this common non-motor symptom in PD patients[9]. The reported pharmacological activities of *X. granatum* warrant further investigation into its potential as an anti-Parkinson's agent.

This study aims to elucidate the mechanism of action of an ethanol extract derived from *X. granatum* using computational approaches. We employ molecular docking and molecular dynamics simulations to investigate the interaction between this extract and three key enzymes implicated in PD pathogenesis: A2AR, COMT, and MAO-B. Molecular docking analysis provides insights into the binding interactions between the extract and these enzymes[10]. Subsequently, molecular dynamics simulations allow us to monitor the dynamic behavior and structural changes of the molecular complexes formed during these interactions[11].

MATERIALS AND METHODS

Materials

Three-dimensional structures of the target receptors, COMT, A2AR, and MAO-B enzymes, were retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/) using the following PDB codes: 3BWM, 3PWH, and 2V61, respectively. 3D structures of the test compounds and natural ligands (used as controls) were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Docking simulations were performed using YASARA Structure version 19.9.17 and BIOVIA Discovery Studio 2017 R2 Client 17.2. The computational hardware employed for these simulations consisted of an AMD Ryzen 5 3600 processor with 12 cores running at 3.6 GHz and 32 GB of RAM, operating under a Windows 11 Pro 64-bit operating system.

Methods

Preparation of receptors and ligands

Three-dimensional structures of all receptors were obtained from RCSB PDB in .pdb format. Water molecules and any nonessential residues were removed to prepare the protein structure for docking simulations. Hydrogen atoms were then added, and bond orders and hydrogenation states were adjusted using YASARA Structure to reflect a physiological pH of 7.4. This step ensures a more accurate representation of the *in vivo* environment where protein-ligand interactions occur. Thirty test compounds identified by Heryanto *et al*.[12] using GC/MS analysis were selected for docking simulations. The 3D structures of both the test compounds and a reference ligand (cite source of reference ligand) were retrieved from PubChem[13]. All ligand structures were prepared using YASARA Structure and underwent energy minimization to optimize atomic positions and obtain the lowest possible free energy state.

Molecular docking

Docking simulations were performed using the pre-configured script "dock_runscreening.mcr" within YASARA Structure. This script was executed 100 times to generate a statistically robust dataset of ligand-receptor interactions. The docking results were captured in .txt format for further analysis[14]. The docked ligand-receptor complexes were then analyzed using Discovery Studio to visualize and quantify the intermolecular interactions. This analysis focused on identifying and characterizing key interactions such as hydrogen bonds and hydrophobic interactions, which contribute to the binding

affinity between the ligand and receptor molecule. The visualization software allowed for the generation of two-dimensional interaction maps to depict these interactions in detail[15].

Density functional theory analysis

Density functional theory (DFT) calculations were performed using Gaussian 09W software to optimize the geometries of the selected molecular structures. Geometry optimization employed the B3LYP functional and the 3-21G* basis set. Subsequently, the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energies were calculated at the DFT level. These energy values served as the foundation for computing global chemical reactivity descriptors, including hardness (η), chemical potential (μ), softness (S), electronegativity (χ), and electrophilicity index (ω). All calculations adhered to the methodology established by Tamaciu *et al.*[16]. **Equations 1** and **2** were utilized to calculate the electron affinity (A) and ionization potential (I), respectively. The chemical potential (μ), electronegativity (χ), hardness (η), softness (S), and electrophilicity index (ω) were computed using **Equations 3** to **7**, respectively.

$$I = -E_{HOMO} \quad [1]$$

$$A = -E_{LUMO} \quad [2]$$

$$\mu = -\frac{(I+A)}{2} \quad [3]$$

$$\chi = \frac{(I+A)}{2} \quad [4]$$

$$\eta = \frac{(I-A)}{2} \quad [5]$$

$$S = \frac{1}{2\mu} \quad [6]$$

$$\omega = \frac{\mu 2}{2\mu} \quad [7]$$

Molecular dynamics simulation

The docked protein-ligand complex PDB file was loaded into YASARA Structure. The molecular dynamics (MD) simulation was performed using the "md_run.mcr" macro within YASARA Structure, with modifications to extend the simulation duration to 50 ns. The simulated system was maintained at physiological conditions: 300 K temperature, pH 7.4, and 0.9% NaCl. Following completion of the initial MD simulation, the simulation was continued using the protein-ligand complex obtained at the end of the first run. The YASARA Structure "md_analyze.mcr" macro was employed with default settings to analyze the MD simulation trajectories. This analysis yielded RMSD, radius of gyration (Rg), and root mean square fluctuation (RMSF) values for the protein and ligand[17,18].

Data analysis

Ligand binding energy analysis during the MD simulation was performed using the built-in "md_analyzebindenergy.mcr" macro within YASARA Structure, which employs the Molecular Mechanics Poisson–Boltzmann Surface Area (MM-PBSA) calculation method[17]. Quantitative analysis of the simulation results and data visualization were conducted using Microsoft Excel. Binding energy calculations were based on the previously reported **Equation 8** by Odhar *et al.*[19].

$$Binding\ Energy = E_{potRecept} + E_{solvRecept} + E_{potLigand} + E_{solvLigand} - E_{potComplex} - E_{solvComplex} \quad [8]$$

RESULTS AND DISCUSSION

Virtual screening identified 30 potential inhibitors of three PD-linked enzymes from *X. granatum* ethanol extract. As expected, all control drugs displayed more favorable (more negative) Gibbs free energy (ΔG) values compared to the identified *X. granatum* compounds (**Figure 1**). However, three *X. granatum* compounds exhibited ΔG values approaching those of the controls, warranting further investigation through *in silico* approaches like DFT and MD simulations. The ΔG reflects the binding affinity between a ligand and its target enzyme. A more negative ΔG value signifies stronger binding and potentially greater inhibitory activity[13,17]. While none of the identified *X. granatum* compounds surpassed the control

drugs in ΔG values, the three compounds identified for each enzyme show promise as potential alternative treatments. This highlights the importance of further exploration and development to exploit their therapeutic potential. Although not surpassing the controls, these *X. granatum* compounds with ΔG values approaching those of controls represent a promising starting point for discovering novel PD therapeutics.

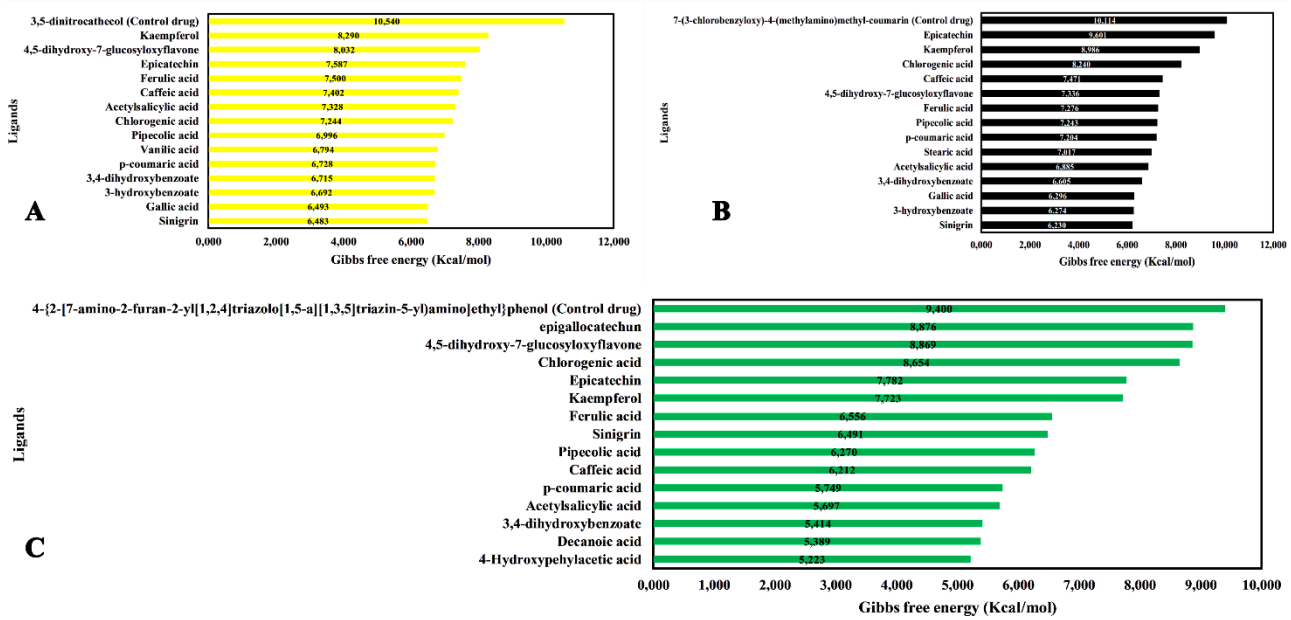


Figure 1. Changes in stability of PD enzymes in 50 ns. (**a**) MM-PBSA, (**b**) Rg, and (**c**) RMSD.

Docking simulations were performed to investigate the binding interactions between the top three identified compounds (epigallocatechin, kaempferol, and epicatechin) and three target enzymes: COMT, A2AR, and MAO-B (**Figure 2**). Each enzyme possesses distinct active site residues: COMT (ASP141, HIS142, TRP143, LYS144), A2AR (ALA63, GLU169, ASN253, ALA277, HIS278), and MAO-B (TYR60, PRO102, LEU164, PHE168, LEU171, CYS172, ILE198, ILE199, GLN206, TYR326, PHE343). Epigallocatechin interacted with the active site of A2AR (GLU169, ASN253, HIS278) via a hydrogen bond with ASN253 and van der Waals interactions elsewhere. The control drug for A2AR bound to four residues (ALA63, GLU169, ASN253, HIS278) but lacked a hydrogen bond with ASN253, relying solely on van der Waals interactions, which may be less favorable for binding affinity.

Kaempferol formed hydrophobic interactions with two COMT active site residues (HIS142 and TRP143). The control drug for COMT interacted with all COMT active sites, exhibiting various interactions (salt bridge, hydrogen bond, hydrophobic, and van der Waals). However, the interaction with ASP141 might be unfavorable. Epicatechin bound to seven COMT active sites (TYR60, LEU171, CYS172, ILE198, ILE199, TYR326, and PHE343), forming a combination of three hydrogen bonds, two hydrophobic interactions, and two van der Waals interactions. The control drug for COMT interacted with all active sites, exhibiting one hydrogen bond, one pi-sulfur bond, one attractive charge interaction, five hydrophobic interactions, and van der Waals interactions with the remaining residues.

In this study, hydrogen bonds (conventional and carbon-hydrogen) and hydrophobic interactions were observed, contributing to the overall binding strength of the ligand-enzyme complexes. Generally, a higher number of hydrogen bonds and hydrophobic interactions correlate with increased binding energy[20]. However, our findings also revealed the presence of an unfavorable negative-negative interaction between the control drug for A2AR (4-{2-[(7-amino-2-furan-2-yl[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-yl)amino]ethyl}phenol) and epigallocatechin, potentially affecting drug activity due to repulsive forces between atoms[21]. Overall, the three test ligands derived from *X. granatum* displayed distinct binding characteristics, warranting further investigation using molecular dynamics simulations to assess the stability of these complexes.

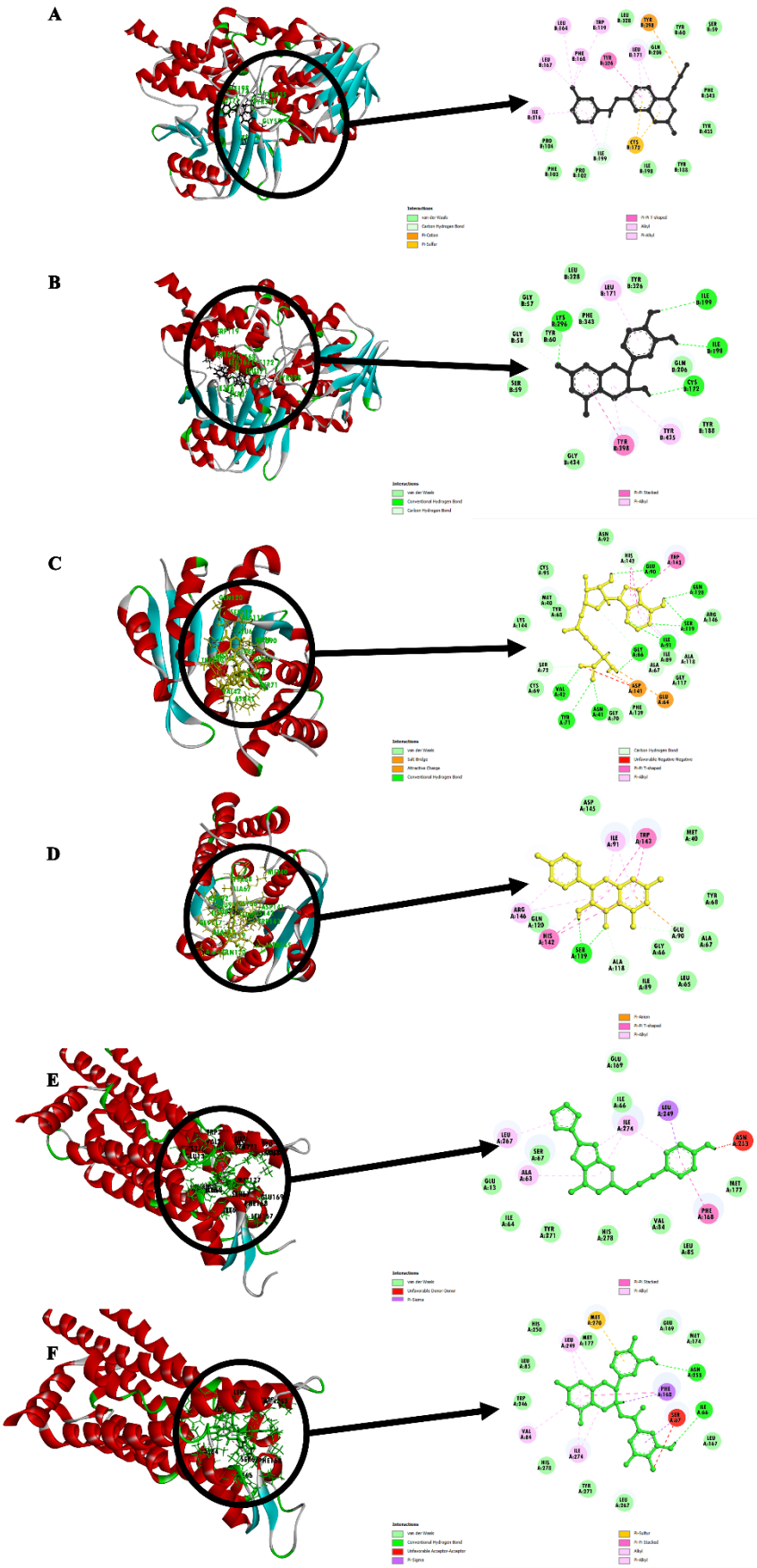


Figure 2. Docked ligand-receptor complexes of (**A**) 7-(3-chlorobenzyloxy)-4-(methylamino)methyl-coumarin-MAOB, (**B**) epicatechin-MAOB, (**C**) 3,5-dinitrocathecol-COMT, (**D**) kaempferol-COMT, (**E**) 4-{2-[7-amino-2-furan-2-yl[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-yl)amino]ethyl}phenol-A2AR, (**F**) epigallocatechin-A2AR.

Density functional theory calculations were performed to investigate the electronic properties of the three identified compounds (kaempferol, epicatechin, and epigallocatechin) (**Figure 3**). The HOMO-LUMO energy gap, an indicator of molecular softness, was calculated. Kaempferol exhibited the most negative energy gap (-0.27408 eV), followed by epicatechin (-0.20037 eV) and epigallocatechin (-0.20673 eV). A lower energy gap corresponds to a softer molecule. The complete summary of DFT calculations is presented in **Table I**. Notably, all three ligands displayed a favorable characteristic: low hardness combined with high softness, suggesting their potential as promising phytochemicals.

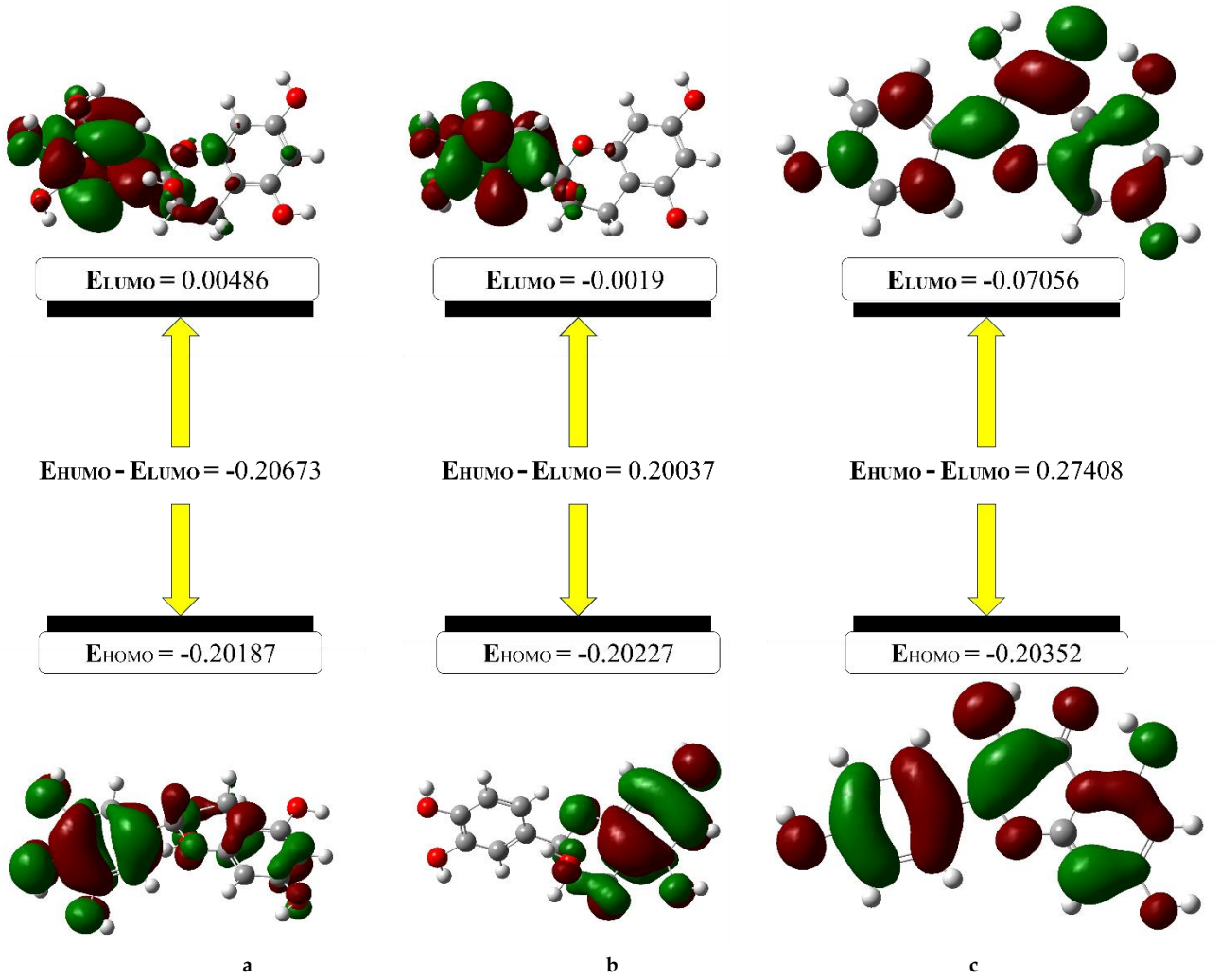


Figure 3. Visualization of HOMO, LUMO, and energy gap of selected compounds which were further analyzed with DFT. (**a**) epigallocatechin, (**b**) epicatechin, and (**c**) kaempferol.

Table I. Calculation analysis of DFT studies on selected compounds.

Compounds	EHOMO (eV)	ELUMO (eV)	Energy gap (eV)	Ionization potential (eV)	Affinity (eV)	Chemical potential (eV)	Electronegativity (eV)	Hardness (eV)	Softness (eV)	Electrophilicity index (eV)
Epicatechin	-0.20227	-0.0019	-0,20037	0.20227	0.0019	-0.102085	0.102085	0.100185	4.99077	0.05326
Kaempferol	-0.20352	-0.07056	-0,27408	0.20352	0.07056	-0.13704	0.13704	0.06648	7.52106	0.14124
Epigallocatechin	-0.20187	0.00486	-0,20673	0.20187	-0.00486	-0.098505	0.098505	0.103365	4.82393	0.04694

Frontier molecular orbitals (HOMO and LUMO) were investigated to gain insights into the optical, electrical properties, and potential interactions of the studied compounds (kaempferol, epicatechin, and others). Additionally, the HOMO-LUMO ΔG, η, μ, S, χ, and ω were calculated to further explore their electronic properties and chemical reactivity. The ΔG reflects compound stability, with a larger gap indicating greater stability. Based on the calculated ΔG values, kaempferol was predicted to be the most stable compound. The μ reflects the tendency of a molecule to lose or gain electrons. Both η and S are interrelated and describe the ease of electron donation or acceptance, respectively, influencing reactivity. Ionization

potential and electron affinity are crucial parameters for calculating χ and absolute η. Finally, ω indicates the electron-accepting tendency of a molecule. Collectively, these frontier molecular orbital parameters provide valuable insights into various aspects of the studied compounds, aiding in the identification of potential drug candidates or enzyme inhibitors[16,22-24].

Ligand binding to a receptor can induce subtle but significant changes in the receptor's structure. In this study, we investigated these alterations by analyzing RMSF of the active-site residues in the complexes formed between kaempferol, epicatechin, and their native ligands with COMT, A2AR, and MAO-B enzymes over a 50 ns molecular dynamics simulation. The RMSF serves as a measure of protein structural flexibility, with higher values indicating greater mobility. The RMSF values for the active-site residues (**Table II**) are particularly noteworthy. Notably, the complexes formed by kaempferol, epicatechin, and their native ligands with COMT and MAO-B enzymes exhibit minimal RMSF values, suggesting robust stability within the protein-ligand interactions. As reported by Dash *et al*.[25], RMSF calculations capture the dynamic behavior of protein backbones, with elevated values indicating increased flexibility. Furthermore, Biswas *et al*.[26] proposed that ligand-protein complexes with RMSF values below 1.4 Å for each residue suggest a stable interaction. Gratifyingly, all our tested ligand complexes satisfy this criterion, indicating favorable protein-ligand binding.

Table II. RMSF of PD-linked enzymes active sites.

RMSF (Å)								
A2AR			COMT			MAO-B		
Residues	Epigallocatechin	4-[2-[(7-amino-2-furan-2-yl[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-yl)amino]ethyl]phenol	Residues	Kaempferol	3,5-dinitrocathecol	Residues	3,5-dinitrocathecol	7-(3- chlorobenzyloxy)-4-(methylamino)methyl-coumarin
ALA163	1.803	1.060	ASP141	0.604	0.599	TYR60	0.601	0.655
GLU169	1.573	1.301	HIS142	0.525	0.643	PRO102	0.823	0.859
ASN253	1.64	1.459	TRP143	0.920	1.035	LEU164	0.965	1.056
ALA277	1.078	0.903	LYS144	0.832	0.891	PHE168	0.646	0.605
HIS278	1.977	1.389	-	-	-	LEU171	0.712	0.648
-	-	-	-	-	-	CYS172	1.332	1.644
-	-	-	-	-	-	ILE198	1.025	1.234
-	-	-	-	-	-	ILE199	0.459	0.524
-	-	-	-	-	-	GLN206	0.563	0.467
-	-	-	-	-	-	TYR326	0.532	0.674
-	-	-	-	-	-	PHE343	0.448	0.454

Molecular dynamics simulations revealed ligand-induced alterations in protein backbone flexibility. The RMSF values indicated minimal fluctuations in the active site residues of complexes with kaempferol, epicatechin, and native ligands, suggesting stable protein-ligand interactions (**Figure 4A**). This aligns with the proposed stability criteria[26] and findings by Dash *et al*.[25]. These minimal RMSF values suggest favorable interactions between the studied compounds and their target enzymes, COMT and MAO-B, potentially influencing their effectiveness as inhibitors or modulators.

Free energy calculations from MM-PBSA simulations provided insights into ligand binding affinities (**Figure 4A**). Positive ΔG values suggest favorable binding, consistent with YASARA's methodology[27]. Notably, kaempferol displayed a higher ΔG than the natural ligand for COMT, indicating a potentially stronger interaction. This finding highlights kaempferol's promise for further investigation in drug development. Overall, the positive ΔG values suggest that the studied compounds form stable complexes with their respective enzymes, supporting their potential as therapeutic candidates.

The Rg values provided insights into protein complex structures (**Figure 4B**). Control drug-MAO-B exhibited a higher Rg value, indicating a looser protein structure compared to other complexes. This aligns with Dash *et al*.[25] and suggests increased flexibility. The Rg serves as a measure of protein compactness, offering a valuable indicator for biological contexts. In this study, Rg facilitated a comparative analysis of protein structures relative to their hydrodynamic radius. As highlighted by Justino *et al*.[28], Rg measurements contribute to our understanding of protein-environment interactions.

Stability of ligand binding modes is crucial for reliable MD simulations. The RMSD of the protein backbone in the final 5 ns of the simulation was used to assess stability (defined as RMSD <2 Å) based on criteria from Chairunisa *et al*.[18] (**Figure 4C**). All complexes, except two binding A2AR, exhibited stability with consistently low RMSD values throughout the 50 ns simulation. This collectively suggests structural stabilization of the ligand-protein complex, supported by the lower RMSD values observed in ligand-docked proteins compared to their unbound counterparts[29]. The observed stability underscores the robustness of the MD simulations and the validity of the ligand binding modes.

This study employed a computational approach to investigate the interactions between the ethanol extract of *X. granatum* and potential anti-Parkinson's targets. The findings provide insights into ligand-induced protein flexibility changes, binding affinities, and structural stability. Notably, kaempferol displayed promising potential as a COMT inhibitor. We believe this work contributes valuable information for the development of more effective PD treatments.

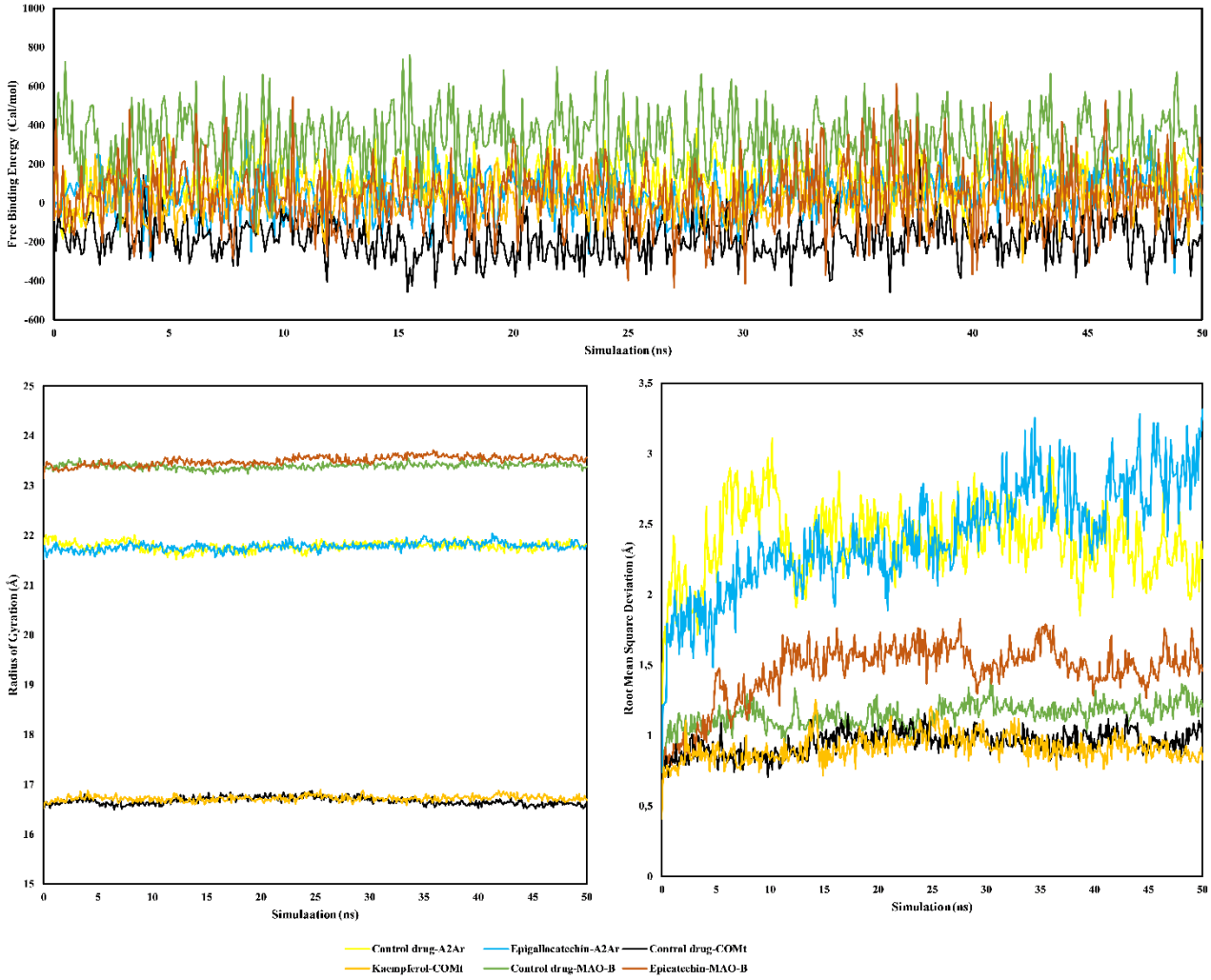


Figure 4. Changes in stability of PD enzymes in 50 ns. (**a**) MM-PBSA, (**b**) Rg, and (**c**) RMSD.

CONCLUSION

In silico docking simulations identified several *X. granatum* compounds with promising binding affinities to A2AR, COMT, and MAO-B, key enzymes implicated in PD. Notably, five compounds exhibited high binding energies to each target protein: A2AR (epigallocatechin, 4,5-dihydroxy-7-glucoloxyflavonone, chlorogenic acid, epicatechin, and kaempferol), COMT (kaempferol, 4,5-dihydroxy-7-glucoloxyflavonone, epigallocatechin, epicatechin, and ferulic acid), and MAO-B (epicatechin, kaempferol, chlorogenic acid, caffeic acid, and stearic acid). Analysis of the MAO-B-ligand complex revealed a more flexible protein conformation, potentially enhancing ligand binding. Overall stability of the ligand-protein interactions was confirmed by RMSD analysis, except for the A2AR complex, which may warrant further investigation. These findings suggest that *X. granatum* may possess structural stabilization effects on key PD-related proteins, highlighting its potential as a therapeutic candidate. Future studies exploring *in vitro* and *in vivo* models are warranted to validate these *in silico* observations and elucidate the precise mechanisms of action. This research offers valuable insights that could pave the way for the development of novel and more effective therapies for PD.

ACKNOWLEDGMENT

This research received no external funding.

AUTHORS' CONTRIBUTION

Conceptualization: Riyan Alifbi Putera Irsal, Gusnia Meilin Gholam
Data curation: Riyan Alifbi Putera Irsal, Gusnia Meilin Gholam, Dzikri Anfasa Firdaus, Novian Liwanda, Fernanda Chairunisa
Formal analysis: Riyan Alifbi Putera Irsal, Gusnia Meilin Gholam, Dzikri Anfasa Firdaus, Novian Liwanda, Fernanda Chairunisa
Funding acquisition: -
Investigation: Riyan Alifbi Putera Irsal, Gusnia Meilin Gholam
Methodology: Riyan Alifbi Putera Irsal, Gusnia Meilin Gholam
Project administration: Riyan Alifbi Putera Irsal, Gusnia Meilin Gholam
Resources: Riyan Alifbi Putera Irsal, Gusnia Meilin Gholam, Fernanda Chairunisa
Software: Riyan Alifbi Putera Irsal, Gusnia Meilin Gholam
Supervision: Riyan Alifbi Putera Irsal, Gusnia Meilin Gholam, Dzikri Anfasa Firdaus, Novian Liwanda, Fernanda Chairunisa
Validation: Riyan Alifbi Putera Irsal, Gusnia Meilin Gholam, Fernanda Chairunisa
Visualization: Riyan Alifbi Putera Irsal, Gusnia Meilin Gholam
Writing - original draft: Riyan Alifbi Putera Irsal, Gusnia Meilin Gholam
Writing - review & editing: Riyan Alifbi Putera Irsal, Gusnia Meilin Gholam, Dzikri Anfasa Firdaus, Novian Liwanda, Fernanda Chairunisa

DATA AVAILABILITY

All of the data is contained within this manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Ou Z, Oan J, Tang S, Duan D, Yu D, Nong H, et al. Global Trends in the Incidence, Prevalence, and Years Lived with Disability of Parkinson's Disease in 204 Countries/Territories From 1990 to 2019. Front Public Health. 2021;9:776847. DOI: 10.3389/fpubh.2021.776847; PMCID: PMC8688697; PMID: 34950630

2. Kouli A, Torsney KM, Kuan WL. Parkinson's Disease: Etiology, Neuropathology, and Pathogenesis. In: Stoker TB, Greenland JC, editors. Parkinson's Disease: Pathogenesis and Clinical Aspects. Brisbane (AU): Codon Publications; 2018. DOI: 10.15586/codonpublications.parkinsonsdisease.2018.ch1

3. DeMaagd G, Philip A. Parkinson's Disease and Its Management: Part 1: Disease Entity, Risk Factors, Pathophysiology, Clinical Presentation, and Diagnosis. P T. 2015;40(8):504-32. PMCID: PMC4517533; PMID: 26236139

4. Magrinelli F, Picelli A, Tocco P, Federico A, Roncari L, Smania N, et al. Pathophysiology of Motor Dysfunction in Parkinson's Disease as the Rationale for Drug Treatment and Rehabilitation. Parkinsons Dis. 2016;2016:9832839. DOI: 10.1155/2016/9832839; PMCID: PMC4913065; PMID: 27366343

5. Jankovic J, Tan EK. Parkinson's disease: etiopathogenesis and treatment. J Neurol Neurosurg Psychiatry. 2020;91(8):795-808. DOI: 10.1136/jnnp-2019-322338; PMID: 32576618

6. Regensburger M, Ip CW, Kohl Z, Schrader C, Urban PP, Kassubek J, et al. Clinical benefit of MAO-B and COMT inhibition in Parkinson's disease: practical considerations. J Neural Transm. 2023;130(6):847-61. DOI: 10.1007/s00702-023-02623-8; PMCID: PMC10199833; PMID: 36964457

7. Boulaamane Y, Ibrahim MAA, Britel MR, Maurady A. In silico studies of natural product-like caffeine derivatives as potential MAO-B inhibitors/AA 2A R antagonists for the treatment of Parkinson's disease. J Integr Bioinform. 2022;19(4):20210027. DOI: 10.1515/jib-2021-0027; PMCID: PMC9800045; PMID: 36112816

8. Dey D, Quispe C, Hossain R, Jain D, Ahmed Khan R, Janmeda P, et al. Ethnomedicinal Use, Phytochemistry, and Pharmacology of Xylocarpus granatum J. Koenig. Emran T Bin, editor. Evid Based Complement Altern Med. 2021;2021:8922196. DOI: 10.1155/2021/8922196; PMCID: PMC8423563; PMID: 34504541

9. Yin X, Li X, Hao Y, Zhao Y, Zhou J, Shi H. Xylocarpin H, a Limonoid of Xylocarpus granatum, Produces Antidepressant-Like Activities in Mice. J Behav Brain Sci. 2015;5(11):524-32. DOI: 10.4236/jbbs.2015.511050

10. Agu PC, Afiukwa CA, Orji OU, Ezeh EM, Ofoke IH, Ogbu CO, et al. Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. Sci Rep. 2023;13(1):13398. DOI: 10.1038/s41598-023-40160-2; PMCID: PMC10435576; PMID: 37592012

11. Hollingsworth SA, Dror RO. Molecular Dynamics Simulation for All. Neuron. 2018;99(6):1129-43. DOI: 10.1016/j.neuron.2018.08.011; PMCID: PMC6209097; PMID: 30236283

12. Heryanto R, Putra CA, Khalil M, Rafi M, Putri SP, Karomah AH, et al. Antioxidant Activity and Metabolite Profiling of Xylocarpus granatum Extracts Using Gas Chromatography-Mass Spectrometry. Metabolites. 2023;13(2):156. DOI: 10.3390/metabo13020156; PMCID: PMC9958973; PMID: 36837775

13. Irsal RAP, Seno DSH, Safithri M, Kurniasih R. Penapisan Virtual Senyawa Aktif Sirih Merah (Piper Crocatum) sebagai Inhibitor Angiotensin Converting Enzyme. J Farmamedika Pharmamedika J. 2022;7(2):104-13. DOI: 10.47219/ath.v7i2.157

14. Durai P, Shin H, Achek A, Kwon H, Govindaraj RG, Panneerselvam S, et al. Toll-like receptor 2 antagonists identified through virtual screening and experimental validation. FEBS J. 2017;284(14):2264-83. DOI: 10.1111/febs.14124; PMID: 28570013

15. Gholam GM, Darmawan NI, Siregar JE, Artika IM. Selected Polyphenols from Date (Phoenix dactylifera) as Anti-Virulence of Candida albicans Through Multiple Enzyme Targets. Biointerface Res Appl Chem. 2022;13(4):386. DOI: 10.33263/BRIAC134.386

16. Talmaciu MM, Bodoki E, Oprean R. Global chemical reactivity parameters for several chiral beta-blockers from the Density Functional Theory viewpoint. Clujul Med. 2016;89(4):513-8. DOI: 10.15386/cjmed-610; PMCID: PMC5111492; PMID: 27857521

17. Land H, Humble MS. YASARA: A Tool to Obtain Structural Guidance in Biocatalytic Investigations. In: Bornscheuer UT, Höhne M, editors. Protein Engineering. Methods in Molecular Biology. New York (US): Humana Press: 2018. p. 43–67. DOI: 10.1007/978-1-4939-7366-8_4

18. Chairunisa F, Safithri M, Andrianto D, Kurniasih R, Irsal RAP. Molecular Docking of Red Betel Leaf Bioactive Compounds (Piper crocatum) as Lipoxygenase Inhibitor. Indones J Pharm Sci Technol. 2023;10(2):90-103. DOI: 10.24198/ijpst.v10i2.38934

19. Odhar HA, Hashim AF, Humad SS. Molecular docking analysis and dynamics simulation of salbutamol with the monoamine oxidase B (MAO-B) enzyme. Bioinformation. 2022;18(3):304-9. DOI: 10.6026/97320630018304; PMCID: PMC9722423; PMID: 36518132

20. Ferenczy GG, Kellermayer M. Contribution of hydrophobic interactions to protein mechanical stability. Comput Struct Biotechnol J. 2022;20:1946-56. DOI: 10.1016/j.csbj.2022.04.025; PMCID: PMC9062142; PMID: 35521554

21. Dhorajiwala TM, Halder ST, Samant L. Computer-Aided Docking Studies of Phytochemicals from Plants Salix Subserrata and Onion as Inhibitors of Glycoprotein G of Rabies Virus. Biomed Biotechnol Res J. 2019;3(4):269-76. DOI: 10.4103/bbrj.bbrj_124_19

22. Roohi H, Mohtamadifar N. The role of the donor group and electron-accepting substitutions inserted in π-linkers in tuning the optoelectronic properties of D-π-A dye-sensitized solar cells: a DFT/TDDFT study. RSC Adv. 2022;12(18):11557-73. DOI: 10.1039/d2ra00906d; PMCID: PMC9006569; PMID: 35425060

23. Chattaraj PK, Duley S. Electron Affinity, Electronegativity, and Electrophilicity of Atoms and Ions†. J Chem Eng Data. 2010;55(5):1882-6. DOI: 10.1021/je900892p

24. Zhan CG, Nichols JA, Dixon DA. Ionization Potential, Electron Affinity, Electronegativity, Hardness, and Electron Excitation Energy: Molecular Properties from Density Functional Theory Orbital Energies. J Phys Chem A. 2003;107(20):4184-95. DOI: 10.1021/jp0225774

25. Dash R, Ali MC, Dash N, Azad MAK, Hosen SMZ, Hannan MA, et al. Structural and Dynamic Characterizations Highlight the Deleterious Role of SULT1A1 R213H Polymorphism in Substrate Binding. Int J Mol Sci. 2019;20(24):6256. DOI: 10.3390/ijms20246256; PMCID: PMC6969939; PMID: 31835852

26. Biswas S, Mahmud S, Mita MA, Afrose S, Hasan MR, Shimu MSS, et al. Molecular Docking and Dynamics Studies to Explore Effective Inhibitory Peptides Against the Spike Receptor Binding Domain of SARS-CoV-2. Front Mol Biosci. 2022;8(10):1305-15. DOI: 10.3389/fmolb.2021.791642; PMCID: PMC8851422; PMID: 35187069

27. Genheden S, Ryde U. The MM/PBSA and MM/GBSA methods to estimate ligand-binding affinities. Expert Opin Drug Discov. 2015;10(5):449-61. DOI: 10.1517/17460441.2015.1032936; PMCID: PMC4487606; PMID: 25835573

28. Justino GC, Nascimento CP, Justino MC. Molecular dynamics simulations and analysis for bioinformatics undergraduate students. Biochem Mol Biol Educ. 2021;49(4):570-82. DOI: 10.1002/bmb.21512; PMID: 33844418

29. Ng HW, Laughton CA, Doughty SW. Molecular dynamics simulations of the adenosine A2a receptor: structural stability, sampling, and convergence. J Chem Inf Model. 2013;53(5):1168-78. DOI: 10.1021/ci300610w; PMID: 23514445

Borneo Journal of Pharmacy
Vol 7 Issue 2 May 2024
Pages 172 – 186

https://journal.umpr.ac.id/index.php/bjop/article/view/6820
DOI: https://doi.org/10.33084/bjop.v7i2.6820
e-ISSN: 2621-4814

Research Article

Chemoinformatic-aided Antidiabetic Analysis of the Therapeutic Potential of Phytoconstituents in *Eremomastax speciosa* Extracts

Sulyman Olalekan Ibrahim [1*]
Halimat Yusuf Lukman [2]
Israel Ehizuelen Ebhohimen [3]
Halimah Funmilayo Babamale [1]
Fatimah Ronke Abdulkadir [4]
Abdulmumeen Amao Hamid [4]
Marili Funmilayo Zubair [1]
Olubunmi Atolani [4]

[1] Department of Industrial Chemistry, University of Ilorin, Ilorin, Kwara State, Nigeria
[2] Department of Chemical Sciences, Summit University, Offa, Kwara State, Nigeria
[3] Department of Biochemistry, Ambrose Alli University, Ekpoma, Edo State, Nigeria
[4] Department of Chemistry, University of Ilorin, Ilorin, Kwara State, Nigeria

*email: ibrahim.sio@unilorin.edu.ng; phone: +2348030661412

Keywords:
α-amylase
Antihyperglycemic
Metformin
Molecular docking
Phytochemicals

Abstract

This research attempts to establish the antihyperglycemic potential of *Eremomastax speciosa*, a medicinal plant utilized in traditional West African diabetes therapy, through virtual simulation. While numerous reports have validated its biological potency, studies on the drug-likeness and antidiabetic properties of its compounds are limited. The *in silico* pharmacological, and toxicological profile of aqueous, methanolic/methylene phytochemicals from previously reported work was analyzed using Swiss ADME and Protox II online server. The docking process was performed using PyRx-0.8, coupled with AutoDock Vina. Phytochemicals that aligned with Lipinski's rules for drugs were then subjected to a virtual docking simulation. This simulation replicated the inhibitory effects of *E. speciosa* phytochemicals on sodium-glucose co-transporters (SGLT2) and α-amylase, similar to metformin, an FDA-approved antidiabetic medicine utilized as a control. Phytochemicals such as 8, 9,10-dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol (-6.6 kcal/mol), 11-isopropylidenetricyclo[4.3.1.1(2,5)]undec-3-en-10-one (-7.9 kcal/mol), 4-(1,5-dihydroxy-2,6,6-trimethylcyclohex-2-enyl)but-3-en-2-one (-7.3 kcal/mol), and N-methyl-N-4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl]-acetamide (-7.5 kcal/mol) exhibits superior binding affinities to the specific proteins targeted, compared to metformin, implying that *E. speciosa* is a source of druggable antidiabetic molecules that can be enhanced to achieve better efficacy.

Received: March 10th, 2024
1st Revised: May 13th, 2024
Accepted: May 20th, 2024
Published: May 30th, 2024

INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder characterized by hyperglycemia, remains a global health crisis[1,2]. The vast majority (>95%) of diabetes cases are diagnosed as type 2 diabetes mellitus (T2DM), resulting from insulin resistance and impaired pancreatic β-cell function[3,4]. This condition significantly contributes to morbidity and mortality worldwide[5]. While various treatment options exist, including insulin preparations and synthetic oral hypoglycemics, the discovery and development of novel antidiabetic medications remains a research priority[6].

Plants have long been recognized for their potential to treat and manage various ailments[7]. Notably, medicinal plants have been documented to possess insulin-mimicking properties, regulate insulin secretion, and inhibit carbohydrate-digesting enzymes, making them promising candidates for exploring and developing new diabetes therapies[8]. *Eremomastax speciosa*, also known as "Edem Iduodut" or "Ndadad Edem" among the Ibibio people of Nigeria and as "African blood tonic" in

How to cite: Ibrahim SO, Lukman HY, Ebhohimen IE, Babamale HF, Abdulkadir FR, Hamid AA, et al. Chemoinformatic-aided Antidiabetic Analysis of the Therapeutic Potential of Phytoconstituents in Eremomastax speciosa Extracts. Borneo J Pharm. 2024;7(2):172-86. doi:10.33084/bjop.v7i2.6820

Cameroon, is an indigenous plant native to tropical regions, particularly Africa[9]. Traditionally, its leaves and roots are used in decoctions, infusions, or poultices to treat various illnesses, including diabetes, anemia, constipation, diarrhea, and malaria[10,11]. The lack of scientific data regarding the antidiabetic potential and the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of its bioactive compounds necessitates further investigation.

Advancements in computer technology have facilitated the use of *in silico* methods, such as virtual screening and network analysis, to investigate the pharmacological and therapeutic potential of phytochemicals found in medicinal plants[12,13]. These computational tools enable the rapid prediction of phytochemicals with promising pharmacological profiles, potentially accelerating the discovery of novel and more effective plant-derived antidiabetic drugs[14]. Establishing a streamlined approach for the initial *in silico* prediction of bioactive compounds, followed by subsequent *in vitro* and *in vivo* validation, has the potential to significantly improve the efficiency of evaluating the medicinal properties of plants[15]. This study aims to utilize online servers and molecular docking simulations to evaluate the ADMET, pharmacokinetics, and drug-likeness properties of bioactive compounds identified in *E. speciosa*. Additionally, we investigate the plant's potential antidiabetic activity using *in silico* methods.

MATERIALS AND METHODS

Materials

The computational analyses were performed on a Lenovo T460 personal computer equipped with an Intel® Core™ i5-6300U CPU (2.4-2.5 GHz, 6th generation), 16 GB RAM, and a 64-bit operating system with an x64-based processor running Microsoft Windows 10. The following software programs were utilized, including ChemDraw Ultra v.12.0, PyRx-0.8 software coupled with AutoDock Vina 1.1.2, and BIOVIA Discovery Studio Visualizer v.16.1.0.1535. Online web servers were employed for property prediction and toxicity analysis, including SwissADME (http://www.swissadme.ch/index.php), ProTox II (https://tox-new.charite.de/protox_II/index.php?site=compound_input), ChEMBL (https://www.ebi.ac.uk/chembl/), and PubChem (https://pubchem.ncbi.nlm.nih.gov/).

Methods

Data mining of E. speciosa phytochemicals

Information regarding the bioactive phytochemicals present in *E. speciosa* was obtained from a literature review conducted by Siwe *et al*.[16]. This review identified ten phytochemicals reported in the aqueous extract and 14 phytochemicals reported in the methanolic/methylene extract. This dataset of twenty-four putatively bioactive compounds formed the basis for the current study.

Prediction of ADME and toxicity

The SwissADME was employed to predict the physicochemical properties, ADME parameters, and drug-likeness of the identified small phytochemicals[17]. This online platform facilitates the exploration of these properties, aiding in the drug discovery process. Canonical SMILES strings generated using ChemDraw were uploaded into SwissADME to obtain predictions for physicochemical properties (e.g., lipophilicity, water solubility), pharmacokinetics, drug-likeness, and medicinal chemistry friendliness. To assess the potential *in silico* toxicity profile of the identified compounds, the canonical SMILES formula obtained from ChemDraw was used for analysis with Protox II[18].

Retrieval, preparation, and identification of protein active sites

To predict the potential antidiabetic properties of the identified druggable phytochemicals from *E. speciosa*, we employed *in silico* molecular docking simulations. Metformin and acarbose, established antidiabetic drugs, were used as references to understand their mechanisms of action. Information regarding their mechanisms was retrieved from the ChEMBL database. Metformin is known to inhibit sodium-glucose co-transporter 2 (SGLT2)[19], while acarbose acts as an α-amylase inhibitor[20]. The crystal structures of human SGLT2 (PDB ID 8HDH) and α-amylase (PDB ID 4GQR) were obtained from the Protein Data Bank (https://www.rcsb.org/). These protein structures were then optimized for docking simulations. The binding site coordinates for each enzyme were identified by analyzing the co-crystallized inhibitors within the respective

protein structures, utilizing the Discovery Studio Visualizer. Docking simulations were performed for each phytochemical against SGLT2 and α-amylase. The docking scores and the most favorable binding poses for each complex were documented[21].

Ligand preparation and molecular docking

To gain insights into the potential mechanisms of action of the identified phytochemicals, *in silico* docking simulations were performed. The known mechanisms of standard drugs metformin (SGLT2 inhibitor) and acarbose (α-amylase inhibitor) were retrieved from the ChEMBL database. Crystal structures of these standard drugs, along with all phytochemicals identified through GC-MS analysis, were downloaded in SDF format from PubChem. Open Babel was employed to prepare and optimize the downloaded structures for docking simulations. The PyRx platform coupled with AutoDock Vina was then utilized for docking simulations. Briefly, the phytoconstituents isolated from the methanolic and aqueous extracts of *E. speciosa* were docked into the active sites of human α-amylase and SGLT2. The protein structures used for docking were retrieved from the Protein Data Bank, focusing on co-crystallized forms to incorporate the bound ligand information. The specific amino acid residues constituting the active sites were selected based on the downloaded PDB structures[22].

Data analysis

Following the docking simulations, the ligand-protein complex with the most favorable binding affinity (ΔG; kcal/mol) and pose was selected. This complex was then saved in the PDB file format for further analysis. The Discovery Studio Visualizer was employed to visualize and analyze the intermolecular interactions formed between the ligands and the target protein[23].

RESULTS AND DISCUSSION

Tables I to **VI** detail the physicochemical properties of the aqueous extract (AES) and methanolic extract (MES) of *E. speciosa*. While some compounds were identified in both extracts, independent analysis was conducted to establish a reference library of compounds present in each. Molecular weight (MW) is a crucial physicochemical property affecting processes like absorption and interaction with targets[24,25]. Lipinski's rule of five suggests favorable drug-like properties for compounds with MW below 500 Da, logP less than 5, and a limited number of hydrogen bond donors and acceptors[13,26,27]. All identified *E. speciosa* phytochemicals complied with these rules, indicating good oral bioavailability potential. The majority of identified compounds in the AES, with the exception of trilinolein (MW 879.83 g/mol, rotatable bonds 50, relative formula mass (MR) >250, and heavy atom 63), exhibited a molecular weight below 500 g/mol and an atomic weight <40. Additionally, all AES compounds possessed a topological polar surface area (TPSA) below 100 and contained a limited number of hydrogen bond acceptors and donors (less than 10 and 5, respectively). Notably, the physicochemical properties of the MES compounds followed a similar trend, with the exception of 4,22-cholestadien-3-one, which displayed a molecular weight exceeding 500 g/mol (**Tables I** and **II**).

Table I. Physicochemical properties of phytochemicals from AES.

Phytochemicals	MW	#Heavy atoms	#Aromatic heavy atoms	Fraction Csp3	#Rotatable bonds	#H-bond acceptors	#H-bond donors	MR	TPSA
9-oxabicyclo[3.3.1]nonane-2,6-diol	158.19	11	0	1	0	3	2	39.75	49.69
2,7-dioxaisotwistane	140.18	10	0	1	0	2	0	36.4	18.46
5-hydroxy-9-oxabicyclo[3.3.1] nonan-2-one	156.18	11	0	0.88	0	3	1	38.83	46.53
11-isopropylidenetricyclo [4.3.1.1(2,5)]undec-3-en-10-one	202.29	15	0	0.64	0	1	0	62.32	17.07
4-(1,5-dihydroxy-2,6,6-trimethylcyclohex-2-enyl)but-3-en-2-one	224.3	16	0	0.62	2	3	2	63.84	57.53
7-methyl-Z-tetradecen-1-ol acetate	268.43	19	0	0.82	13	2	0	84.64	26.3
ethyl iso-allocholate	436.62	31	0	0.96	6	5	3	122.89	86.99
Trilinolein	879.38	63	0	0.74	50	6	0	277.12	78.9
Olean-12-en-3-one	424.7	31	0	0.9	0	1	0	133.92	17.07
α-amyrin	426.72	31	0	0.93	0	1	1	135.14	20.23

Table II. Physicochemical properties of phytochemicals from MES.

Phytochemicals	MW	#Heavy atoms	#Aromatic heavy atoms	Fraction Csp3	#Rotatable bonds	#H-bond acceptors	#H-bond donors	M R	TPSA
9-oxabicyclo[3.3.1]nonane-2,6-diol	158.19	11	0	1	0	3	2	39.75	49.69
2,5-methano-2H-furo[3,2-b]pyran, hexahydro (2,7-dioxaisotwistane)	140.18	10	0	1	0	2	0	36.4	18.46
6,6-dimethyl-10-methylene-1-oxa-spiro[4.5]decane	180.29	13	0	0.83	0	1	0	55.96	9.23
N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl]-acetamide	266.34	19	0	0.71	6	4	0	76.65	49.85
9,10-dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol	196.29	14	0	1	0	2	2	55.86	40.46
2-pentadecanone, 6,10,14-trimethyl	268.48	19	0	0.94	12	1	0	88.84	17.07
Phytol	296.53	21	0	0.9	13	1	1	98.94	20.23
Isophytol	296.53	21	0	0.9	13	1	1	98.98	20.23
Z-(13,14-epoxy)tetradec-11-en-1-ol acetate	268.39	19	0	0.81	13	3	0	78.81	38.83
E,Z-1,3,12-nonadecatriene	294.47	21	0	0.68	14	2	2	94.35	40.46
7-methyl-Z-tetradecen-1-ol acetate	268.43	19	0	0.82	13	2	0	84.64	26.3
Ethyl iso-allocholate	424.7	31	0	0.9	1	1	0	134.18	17.07
Lupenone	382.62	28	0	0.81	4	1	0	122.18	17.07
4,22-cholestadien-3-one	502.77	36	0	0.97	7	4	1	147.9	59.06

Tables III and **IV** present the MLOGP values for the identified phytochemicals in AES and MES. These values indicate the relative preference of a compound for partitioning between lipophilic (fatty) and hydrophilic (watery) environments. Generally, the phytochemicals in AES exhibited significant lipophilicity, with most MLOGP values falling below 4.5 (**Table III**). This suggests that these compounds preferentially partition into the lipophilic phase, potentially favoring absorption across cell membranes. Exceptions include trilinolein, olean-12-en-3-one, and α-amyrin. Conversely, MES displayed a trend towards higher lipophilicity, with a few compounds, such as ethyl iso-allocholate and lupenone, having MLOGP values exceeding 4.5 (**Table IV**).

Table III. Lipophilicity properties of phytochemicals from AES.

Phytochemicals	iLOGP	XLOGP3	WLOGP	MLOGP	Silicos-IT LogP	Consensus LogP
9-oxabicyclo[3.3.1]nonane-2,6-diol	1.53	-0.05	0.05	0.02	0.38	0.38
2,7-dioxaisotwistane	2.01	0.95	1.1	0.9	1.53	1.3
5-hydroxy-9-oxabicyclo[3.3.1] nonan-2-one	0.9	0.16	0.61	0.28	1.36	0.66
11-isopropylidenetricyclo [4.3.1.1(2,5)]undec-3-en-10-one	2.48	2.77	3.12	3.21	2.83	2.88
4-(1,5-dihydroxy-2,6,6-trimethylcyclohex-2-enyl)but-3-en-2-one	2.29	0.72	1.6	1.14	2.04	1.56
7-methyl-Z-tetradecen-1-ol acetate	3.74	6.18	5.27	4.33	5.49	5
ethyl iso-allocholate	3.99	2.71	3.93	3.46	3.49	3.52
Trilinolein	12.82	20.34	17.43	9.25	20.55	16.08
Olean-12-en-3-one	4.53	8.84	8.38	6.82	7.51	7.21
α-amyrin	4.77	9.01	8.02	6.92	6.52	7.05

Table IV. Lipophilicity properties of phytochemicals from MES.

Phytochemicals	iLOGP	XLOGP3	WLOGP	MLOGP	Silicos-IT LogP	Consensus LogP
9-oxabicyclo[3.3.1]nonane-2,6-diol	1.53	-0.05	0.05	0.02	0.38	0.38
2,5-methano-2H-furo[3,2-b]pyran, hexahydro (2,7-dioxaisotwistane)	2.01	0.95	1.1	0.9	1.53	1.3
6,6-dimethyl-10-methylene-1-oxa-spiro[4.5]decane	2.75	2.81	3.3	2.88	3.65	3.08
N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl]-acetamide	3.01	0.27	0.19	0.94	1.18	1.12
9,10-dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol	2.33	1.14	1.55	2.09	1.68	1.76
2-pentadecanone, 6,10,14-trimethyl	4.39	6.95	6.01	4.79	6.18	5.66
Phytol	4.71	8.19	6.36	5.25	6.57	6.22
Isophytol	4.88	7.83	6.36	5.25	6.57	6.18
Z-(13,14-epoxy)tetradec-11-en-1-ol acetate	268.39	19	0	0.81	13	3
E,Z-1,3,12-nonadecatriene	294.47	21	0	0.68	14	2
7-methyl-Z-tetradecen-1-ol acetate	268.43	19	0	0.82	13	2
Ethyl iso-allocholate	424.7	31	0	0.9	1	1
Lupenone	382.62	28	0	0.81	4	1
4,22-cholestadien-3-one	502.77	36	0	0.97	7	4

Water solubility is another critical factor for absorption and drug formulation[28]. While specific solubility values were not determined, the presence of these compounds in hydrophilic extracts suggests favorable interaction with the aqueous environment, potentially enhancing biological activity. SwissADME predictions indicated solubility ranging from low to moderate for various compounds, suggesting potential for bioabsorption and diverse therapeutic applications depending on solubility characteristics. **Tables V** and **VI** present the hydrophilicity properties of AES and MES, respectively. As expected, all extracts exhibited good solubility in water due to their hydrophilic nature. Investigating the potential for drug interactions, we evaluated the inhibitory effects of these extracts on cytochrome P450 (CYP) isoenzymes. Interestingly, none of the compounds identified in AES showed any inhibitory activity against CYP isoenzymes (**Table VII**).

Gastrointestinal absorption (GIA), blood-brain barrier (BBB) permeability, and P-glycoprotein (P-gp) substrate potential were evaluated using *in silico* tools. Additionally, all AES components displayed favorable absorption characteristics, with high GIA and the ability to permeate BBB, except for trilinolein, olean-12-en-3-one, and α-amyrin. In contrast, the analysis of MES components revealed that most exhibited significant inhibition of the CYP2C9 isoenzyme, potentially leading to drug interactions (**Table VIII**). Despite this concern, MES components generally demonstrated favorable ADME profiles, with high GIA and BBB permeability. Notably, ethyl iso-allocholate, lupenone, and 4,22-cholestadien-3-one deviated from this trend, displaying non-permeability to glycoprotein substrates. Generally, the compounds exhibited high predicted GIA, indicating good oral bioavailability[29]. Exceptions included trilinolein, olean-12-en-3-one, α-amyrin, and several others, suggesting these compounds may require alternative administration routes or formulation strategies for optimal bioavailability. Most compounds were predicted to be non-BBB permeant, potentially limiting their utility for central nervous system (CNS)-related conditions[30]. However, some compounds with lower molecular weight and fewer hydrogen bonds might have the potential to cross the BBB for targeted brain therapies[31]. P-glycoprotein is an efflux pump that limits intestinal absorption of certain drugs. While some *E. speciosa* compounds were predicted P-gp substrates, further investigation is needed to determine the practical impact on their bioavailability.

Table V. Water solubility properties of phytochemicals from AES.

Phytochemicals	ESOL LogS	ESOL Solubility (mg/mL)	ESOL Solubility (mol/L)	ESOL Class	Ali LogS	Ali Solubility (mg/mL)	Ali Solubility (mol/L)	Ali Class	Silicos-IT LogSw	Silicos-IT Solubility [illegible]	Silicos-IT Solubility (mol/L)	Silicos-IT class
9-oxabicyclo[3.3.1]nonane-2,6-diol	-0.79	2.57E+01	1.62E-01	Very soluble	-0.54	4.53E+01	2.87E-01	Very soluble	0.25	2.79E+02	1.76E+00	Soluble
2,7-dioxaisotwistane	-1.31	6.90E+00	4.92E-02	Very soluble	-0.92	1.67E+01	1.19E-01	Very soluble	-0.62	3.35E+01	2.39E-01	Soluble
5-hydroxy-9-oxabicyclo[3.3.1] nonan-2-one	-0.91	1.93E+01	1.23E-01	Very soluble	-0.69	3.16E+01	2.02E-01	Very soluble	-0.9	1.98E+01	1.27E-01	Soluble
11-isopropylidenetricyclo[4.3.1.1(2,5)]undec-3-en-10-one	-2.84	2.93E-01	1.45E-03	Soluble	-2.78	3.33E-01	1.64E-03	Soluble	-2.24	1.17E+00	5.81E-03	Soluble
4-(1,5-dihydroxy-2,6,6-trimethylcyclohex-2-enyl)but-3-en-2-one	-1.55	6.29E+00	2.80E-02	Very soluble	-1.51	6.99E+00	3.12E-02	Very soluble	-1.49	7.26E+00	3.24E-02	Soluble
7-methyl-Z-tetradecen-1-ol acetate	-4.54	7.75E-03	2.89E-05	Moderately soluble	-6.52	8.17E-05	3.04E-07	Poorly soluble	-4.92	3.24E-03	1.21E-05	Moderately soluble
ethyl iso-allocholate	-3.86	6.05E-02	1.39E-04	Soluble	-4.19	2.82E-02	6.45E-05	Moderately soluble	-3.39	1.77E-01	4.05E-04	Soluble
Trilinolein	-14.81	1.37E-12	1.56E-15	Insoluble	-22.31	4.26E-20	4.84E-23	Insoluble	-15.35	3.96E-13	4.50E-16	Insoluble
Olean-12-en-3-one	-8.04	3.85E-06	9.07E-09	Poorly soluble	-9.08	3.51E-07	8.26E-10	Poorly soluble	-7.86	5.86E-06	1.38E-08	Poorly soluble
α-amyrin	-8.16	2.94E-06	6.89E-09	Poorly soluble	-9.33	2.02E-07	4.72E-10	Poorly soluble	-6.71	8.23E-05	1.93E-07	Poorly soluble

Table VI. Water solubility properties of phytochemicals from MES.

Phytochemicals	ESOL LogS	ESOL Solubility (mg/mL)	ESOL Solubility (mol/L)	ESOL Class	Ali LogS	Ali Solubility (mg/mL)	Ali Solubility (mol/L)	Ali Class	Silicos-IT LogSw	Silicos-IT Solubility [illegible]	Silicos-IT Solubility (mol/L)	Silicos-IT class
9-oxabicyclo[3.3.1]nonane-2,6-diol	-0.79	2.57E+01	1.62E-01	Very soluble	-0.54	4.53E+01	2.87E-01	Very soluble	0.25	2.79E+02	1.76E+00	Soluble
2,5-methano-2H-furo[3,2-b]pyran, hexahydro (2,7-dioxaisotwistane)	-1.31	6.90E+00	4.92E-02	Very soluble	-0.92	1.67E+01	1.19E-01	Very soluble	-0.62	3.35E+01	2.39E-01	Soluble
6,6-dimethyl-10-methylene-1-oxa-spiro[4.5]decane	-2.73	3.37E-01	1.87E-03	Soluble	-2.66	3.94E-01	2.18E-03	Soluble	-3.25	1.00E-01	5.57E-04	Soluble
N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl]-acetamide	-1.27	1.45E+01	5.43E-02	Very soluble	-0.88	3.53E+01	1.32E-01	Very soluble	-1.53	7.86E+00	2.95E-02	Soluble
9,10-dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol	-1.78	3.29E+00	1.68E-02	Very soluble	-1.58	5.12E+00	2.61E-02	Very soluble	-1.33	9.12E+00	4.65E-02	Soluble
2-pentadecanone, 6,10,14-trimethyl	-5.09	2.18E-03	8.11E-06	Moderately soluble	-7.12	2.03E-05	7.56E-08	Poorly soluble	-5.55	7.50E-04	2.79E-06	Moderately soluble
Phytol	-5.98	3.10E-04	1.05E-06	Moderately soluble	-8.47	9.94E-07	3.35E-09	Poorly soluble	-5.51	9.06E-04	3.05E-06	Moderately soluble
Isophytol	-5.75	5.23E-04	1.76E-06	Moderately soluble	-8.1	2.35E-06	7.92E-09	Poorly soluble	-5.51	9.06E-04	3.05E-06	Moderately soluble
Z-(13,14-epoxy)tetradec-11-en-1-ol acetate	-3.49	8.74E-02	3.26E-04	Soluble	-5.05	2.41E-03	8.98E-06	Moderately soluble	-4.16	1.87E-02	6.98E-05	Moderately soluble
E,Z-1,3,12-nonadecatriene	-4.31	1.45E-02	4.93E-05	Moderately soluble	-6.27	1.57E-04	5.32E-07	Poorly soluble	-3.88	3.88E-02	1.32E-04	Soluble
7-methyl-Z-tetradecen-1-ol acetate	-4.54	7.75E-03	2.89E-05	Moderately soluble	-6.52	8.17E-05	3.04E-07	Poorly soluble	-4.92	3.24E-03	1.21E-05	Moderately soluble
Ethyl iso-allocholate	-8.43	1.58E-06	3.72E-09	Poorly soluble	-9.83	6.28E-08	1.48E-10	Poorly soluble	-7.44	1.54E-05	3.63E-08	Poorly soluble
Lupenone	-6.69	7.89E-05	2.06E-07	Poorly soluble	-7.71	7.41E-06	1.94E-08	Poorly soluble	-5.75	6.74E-04	1.76E-06	Moderately soluble
4,22-cholestadien-3-one	-7.68	1.05E-05	2.09E-08	Poorly soluble	-9.33	2.34E-07	4.66E-10	Poorly soluble	-6.62	1.21E-04	2.41E-07	Poorly soluble

Table VII. Pharmacokinetics properties of phytochemicals from AES.

Phytochemicals	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	log Kp (cm/s)
9-oxabicyclo[3.3.1]nonane-2,6-diol	High	No	No	No	No	No	No	No	-7.3
2,7-dioxaisotwistane	High	Yes	No	No	No	No	No	No	-6.48
5-hydroxy-9-oxabicyclo[3.3.1] nonan-2-one	High	Yes	No	No	No	No	No	No	-7.14
11-isopropylidenetricyclo[4.3.1.1(2,5)]undec-3-en-10-one	High	Yes	No	No	Yes	No	No	No	-5.57
4-(1,5-dihydroxy-2,6,6-trimethylcyclohex-2-enyl)but-3-en-2-one	High	Yes	No	No	No	No	No	No	-7.16
7-methyl-Z-tetradecen-1-ol acetate	High	Yes	No	No	No	Yes	No	No	-3.55
ethyl iso-allocholate	High	No	Yes	No	No	No	No	No	-7.04
Trilinolein	Low	No	Yes	No	No	No	No	No	2.78
Olean-12-en-3-one	Low	No	No	No	No	No	No	No	-2.61
α-amyrin	Low	No	No	No	No	No	No	No	-2.51

Table VIII. Pharmacokinetics properties of phytochemicals from MES.

Phytochemicals	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	log Kp (cm/s)
9-oxabicyclo[3.3.1]nonane-2,6-diol	High	No	No	No	No	No	No	No	-7.3
2,5-methano-2H-furo[3,2-b]pyran, hexahydro (2,7-dioxaisotwistane)	High	Yes	No	No	No	No	No	No	-6.48
6,6-dimethyl-10-methylene-1-oxa-spiro[4.5]decane	High	Yes	No	No	No	Yes	No	No	-5.4
N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl]-acetamide	High	No	No	No	No	No	No	No	-7.73
9,10-dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol	High	Yes	No	No	No	No	No	No	-6.69
2-pentadecanone, 6,10,14-trimethyl	High	No	Yes	No	No	Yes	No	No	-3
Phytol	Low	No	Yes	No	No	Yes	No	No	-2.29
Isophytol	Low	No	Yes	No	No	Yes	No	No	-2.55
Z-(13,14-epoxy)tetradec-11-en-1-ol acetate	High	Yes	No	Yes	No	Yes	Yes	No	-4.74
E,Z-1,3,12-nonadecatriene	High	Yes	Yes	Yes	No	Yes	Yes	Yes	-4.08

7-methyl-Z-tetradecen-1-ol acetate	High	Yes	No	No	No	Yes	No	No	-3.55
Ethyl iso-allocholate	Low	No	No	No	No	No	No	No	-2.1
Lupenone	Low	No	No	No	No	Yes	No	No	-3.29
4,22-cholestadien-3-one	Low	No	No	No	No	No	No	No	-3.52

Analysis based on Lipinski's Rule of Five indicated that all identified compounds within the extracts adhered to this rule, violating no more than two of its criteria (**Tables IX** and **X**). This suggests favorable drug-likeness properties, implying potential for oral bioavailability. Additionally, the extracts exhibited significant bioavailability scores, further supporting their potential for drug development. With the exception of trilinolein and 4,22-cholestadien-3-one, which displayed synthetic accessibility scores of 8.46 and 6.78, respectively, all other identified compounds possessed values <6.5. Lower synthetic accessibility scores generally indicate greater ease of synthesis. Furthermore, none of the identified compounds triggered a pain alert, suggesting a favorable safety profile (**Tables XI** and **XII**). This absence of pain alerts is a promising finding for the potential development of these compounds into safe and efficacious drugs. The combined analysis using Lipinski's rule, bioavailability scores, and ProTox-II predictions suggests that the identified *E. speciosa* phytochemicals possess promising drug-like properties and low predicted toxicity[18]. These findings indicate their potential as safe and viable candidates for further drug development.

Table IX. Drug-likeness and bioavailability score of phytochemicals from AES.

Phytochemicals	Lipinski #violations	Ghose #violations	Veber #violations	Egan #violations	Muegge #violations	Bioavailability Score
9-oxabicyclo[3.3.1]nonane-2,6-diol	0	2	0	0	1	0.55
2,7-dioxaisotwistane	0	2	0	0	1	0.55
5-hydroxy-9-oxabicyclo[3.3.1] nonan-2-one	0	2	0	0	1	0.55
11-isopropylidenetricyclo[4.3.1.1(2,5)] undec-3-en-10-one	0	0	0	0	1	0.55
4-(1,5-dihydroxy-2,6,6-trimethylcyclohex-2-enyl)but-3-en-2-one	0	0	0	0	0	0.55
7-methyl-Z-tetradecen-1-ol acetate	1	0	1	0	1	0.55
ethyl iso-allocholate	0	1	0	0	0	0.55
Trilinolein	2	4	1	1	3	0.17
Olean-12-en-3-one	1	3	0	1	2	0.55
α-amyrin	1	3	0	1	2	0.55

Table X. Drug-likeness and bioavailability score of phytochemicals from MES.

Phytochemicals	Lipinski #violations	Ghose #violations	Veber #violations	Egan #violations	Muegge #violations	Bioavailability Score
9-oxabicyclo[3.3.1]nonane-2,6-diol	0	2	0	0	1	0.55
2,5-methano-2H-furo[3,2-b]pyran, hexahydro (2,7-dioxaisotwistane)	0	2	0	0	1	0.55
6,6-dimethyl-10-methylene-1-oxa-spiro[4.5]decane	0	0	0	0	2	0.55
N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl]-acetamide	0	0	0	0	0	0.55
9,10-dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol	0	0	0	0	1	0.55
2-pentadecanone, 6,10,14-trimethyl	1	1	1	1	2	0.55
Phytol	1	1	1	1	2	0.55
Isophytol	1	1	1	1	2	0.55
Z-(13,14-epoxy)tetradec-11-en-1-ol acetate	0	0	1	0	0	0.55
E,Z-1,3,12-nonadecatriene	0	0	1	0	1	0.55
7-methyl-Z-tetradecen-1-ol acetate	1	0	1	0	1	0.55
Ethyl iso-allocholate	1	3	0	1	2	0.55
Lupenone	1	1	0	1	2	0.55
4,22-cholestadien-3-one	2	4	0	1	1	0.17

Table XI. Medicinal chemistry properties of phytochemicals from AES.

Phytochemicals	PAINS #alerts	Brenk #alerts	Leadlikeness #violations	Synthetic Accessibility
9-oxabicyclo[3.3.1]nonane-2,6-diol	0	2	0	0
2,7-dioxaisotwistane	0	2	0	0
5-hydroxy-9-oxabicyclo[3.3.1] nonan-2-one	0	2	0	0
11-isopropylidenetricyclo[4.3.1.1(2,5)]undec-3-en-10-one	0	0	0	0
4-(1,5-dihydroxy-2,6,6-trimethylcyclohex-2-enyl)but-3-en-2-one	0	0	0	0
7-methyl-Z-tetradecen-1-ol acetate	1	0	1	0
ethyl iso-allocholate	0	1	0	0
Trilinolein	2	4	1	1
Olean-12-en-3-one	1	3	0	1
α-amyrin	1	3	0	1

Table XII. Medicinal chemistry properties of phytochemicals from MES.

Phytochemicals	PAINS #alerts	Brenk #alerts	Leadlikeness #violations	Synthetic Accessibility
9-oxabicyclo[3.3.1]nonane-2,6-diol	0	2	0	0
2,5-methano-2H-furo[3,2-b]pyran, hexahydro (2,7-dioxaisotwistane)	0	2	0	0
6,6-dimethyl-10-methylene-1-oxa-spiro[4.5]decane	0	0	0	0
N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl]-acetamide	0	0	0	0
9,10-dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol	0	0	0	0
2-pentadecanone, 6,10,14-trimethyl	1	1	1	1
Phytol	1	1	1	1
Isophytol	1	1	1	1
Z-(13,14-epoxy)tetradec-11-en-1-ol acetate	0	0	1	0
E,Z-1,3,12-nonadecatriene	0	0	1	0
7-methyl-Z-tetradecen-1-ol acetate	1	0	1	0
Ethyl iso-allocholate	1	3	0	1
Lupenone	1	1	0	1
4,22-cholestadien-3-one	2	4	0	1

An evaluation of the predicted toxicity of identified compounds in both the aqueous and methanolic/methylene chloride extracts was conducted. All compounds, except N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl]-acetamide, exhibited relatively high LD_{50} values, indicating low acute toxicity. N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl]-acetamide displayed a predicted LD_{50} of 41 mg/kg, placing it within Toxicity Class 2 (moderately toxic). *In silico* analysis also predicted potential carcinogenicity for 7-methyl-Z-tetradecen-1-ol acetate (prediction probability: 0.50) within the aqueous extract with LD_{50} of 3460 mg/kg. Four compounds in the aqueous extract (ethyl iso-allocholate [0.57], trilinolein [0.57], olean-12-en-3-one [0.64], and α-amyrin [0.98]) and three within the methanolic/methylene chloride extract (ethyl iso-allocholate [0.57], 4,22-cholestadien-3-one [0.99], and 3-acetoxy-7,8-epoxylanostan-11-ol [0.99]) showed predicted tendencies to induce immunotoxicity (prediction probability ≥0.50) (**Tables XIII** and **XIV**). While the majority of identified compounds pose minimal acute toxicity concerns, the predicted carcinogenicity and immunotoxicity of certain compounds warrant further investigation. In particular, 7-methyl-Z-tetradecen-1-ol acetate and the compounds identified with potential immunotoxicity should be subjected to more in-depth analysis to confirm or refute the *in silico* predictions.

Table XIII. Toxicological profile of phytochemicals from AES.

Phytochemicals	LD_{50} (mg/kg) / Tox Class	Prediction accuracy (%)	Immunotoxicity	Carcinogenicity	Mutagenicity	Cytotoxicity
9-oxabicyclo[3.3.1]nonane-2,6-diol	3100 / 5	69.26	Inactive	Inactive	Inactive	Inactive
2,7-dioxaisotwistane	7800 / 6	69.26	Inactive	Inactive	Inactive	Inactive
5-hydroxy-9-oxabicyclo[3.3.1] nonan-2-one	20000 / 6	68.07	Inactive	Inactive	Inactive	Inactive
11-isopropylidenetricyclo[4.3.1.1(2,5)]undec-3-en-10-one	5000 / 5	69.26	Inactive	Inactive	Inactive	Inactive
4-(1,5-dihydroxy-2,6,6-trimethylcyclohex-2-enyl)but-3-en-2-one	1000 / 4	68.07	Inactive	Inactive	Inactive	Inactive
7-methyl-Z-tetradecen-1-ol acetate	3460 / 5	100	Inactive	Active (0.50)	Inactive	Inactive
ethyl iso-allocholate	5000 / 5	72.9	Active (0.57)	Inactive	Inactive	Inactive
Trilinolein	3700 / 6	70.97	Active (0.57)	Inactive	Inactive	Inactive
Olean-12-en-3-one	5000 / 5	72.9	Active (0.64)	Inactive	Inactive	Inactive
α-amyrin	7000 / 6	100	Active (0.98)	Inactive	Inactive	Inactive

Table XIV. Toxicological profile of phytochemicals from MES.

Phytochemicals	LD_{50} (mg/kg) / Tox Class	Prediction accuracy (%)	Immunotoxicity	Carcinogenicity	Mutagenicity	Cytotoxicity
9-oxabicyclo[3.3.1]nonane-2,6-diol	3100 / 5	69.26	Inactive	Inactive	Inactive	Inactive
2,5-methano-2H-furo[3,2-b]pyran, hexahydro (2,7-dioxaisotwistane)	7800 / 6	69.26	Inactive	Inactive	Inactive	Inactive
6,6-dimethyl-10-methylene-1-oxa-spiro[4.5]decane	5000 / 5	69.26	Inactive	Inactive	Inactive	Inactive
N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl]-acetamide	41 / 2	68.07	Inactive	Inactive	Inactive	Inactive
9,10-dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol	2000 / 4	70.97	Inactive	Inactive	Inactive	Inactive
2-pentadecanone, 6,10,14-trimethyl	5000 / 5	100.00	Inactive	Inactive	Inactive	Inactive
Phytol	5000 / 5	100.00	Inactive	Inactive	Inactive	Inactive
Isophytol	340 / 4	100.00	Inactive	Inactive	Inactive	Inactive
Z-(13,14-epoxy)tetradec-11-en-1-ol acetate	3460 / 5	70.97	Inactive	Active (0.63)	Active (0.58)	Inactive
E,Z-1,3,12-nonadecatriene	5000 / 5	70.97	Inactive	Inactive	Inactive	Inactive
7-methyl-Z-tetradecen-1-ol acetate	3460 / 5	100.00	Inactive	Active (0.50)	Inactive	Inactive
Ethyl iso-allocholate	5000 / 5	72.90	Active (0.57)	Inactive	Inactive	Inactive
Lupenone	2300 / 5	70.97	Inactive	Inactive	Inactive	Inactive
4,22-cholestadien-3-one	5000 / 5	72.90	Active (0.99)	Inactive	Inactive	Inactive

Table XV present the results of the *in silico* binding affinity (docking scores) of *E. speciosa* compounds against the profiled targets. The tested compounds generally displayed favorable binding affinities, with scores exceeding that of the standard control (-5.1 kcal/mol). Notably, 11-isopropylidenetricyclo[4.3.1.1(2,5)]undec-3-en-10-one exhibited the strongest binding affinity (-7.9 kcal/mol), while 2,7-dioxaisotwistane displayed the weakest affinity (-5.8 kcal/mol) within this set of compounds. However, it is important to note that acarbose, a well-established antidiabetic drug, demonstrated a slightly higher binding affinity (-7.7 kcal/mol) compared to the most potent *E. speciosa* compound. Interestingly, three compounds, 9,10-dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol, 11-isopropylidenetricyclo[4.3.1.1(2,5)]undec-3-en-10-one, and 6,6-dimethyl-10-methylene-1-oxa-spiro[4.5]decane, shared the highest binding affinity among the tested compounds within a different target profile, with identical scores of -6.2 kcal/mol.

Table XV. ΔG of top *E. speciosa* compounds with SGLT2 and α-amylase.

Compounds	ΔG (kcal/mol)	
	SGLT2	α-amylase
2,7-dioxaisotwistane	-5.8	-4.6
9,10-dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol	-6.6	-6.2
9-oxabicyclo[3.3.1]nonane-2,6-diol	-6.2	-5.4
11-isopropylidenetricyclo [4.3.1.1(2,5)]undec-3-en-10-one	-7.9	-7.1
4-(1,5-dihydroxy-2,6,6-trimethylcyclohex-2-enyl)but-3-en-2-one	-7.3	-6.2
5-hydroxy-9-oxabicyclo [3.3.1] nonan-2-one	-6.6	-5.4
6,6-dimethyl-10-methylene-1-oxa-spiro[4.5]decane	-6.5	-6.2
E,Z-1,3,12-nonadecatriene	-6.9	-5.2
N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl]-acetamide	-7.5	-6.0
Z-(13,14-epoxy)tetradec-11-en-1-ol acetate	-6.6	-5.6
Metformin	-5.1	-
Acarbose	-	-7.7

Docking simulations were validated to ensure the selected compounds bind to the SGLT2 protein at the same site as the co-crystallized (native) ligand (**Figure 1**). The 2D interaction diagrams for the top three compounds with SGLT2 are presented in **Figure 2**. 11-isopropylidenetricyclo[4.3.1.1(2,5)]undec-3-en-10-one formed nine interactions with SGLT2, primarily through van der Waals and alkyl bonds. Interacting amino acid residues included SER508, ALA446, ALA447, LEU452, PHE504, GLN448, GLN451, TYR455, and GLY507. 4-(1,5-dihydroxy-2,6,6-trimethylcyclohex-2-enyl)but-3-en-2-one exhibited 13 interactions with SGLT2, including two hydrogen bonds with PHE152 and SER156. Other interacting residues included ILE456, VAL443, VAL444, VAL459, TYR455, PHE504, GLN451, ALA447, LEU452, TRP440, and THR153. N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl]-acetamide displayed the most extensive network of interactions with SGLT2, forming 18 bonds, including three hydrogen bonds with TRP291, GLN457, and THR87. Additional interacting residues included SER287, ALA102, LYS321, ASN75, PHE98, PHE453, GLU99, VAL95, VAL157, VAL286, GLY79, GLY83, HIS80, LEU84, and TYR290.

Docking simulations were performed to investigate the potential interaction between α-amylase and bioactive compounds identified in *E. speciosa*. The results for 4-(1,5-dihydroxy-2,6,6-trimethylcyclohex-2-enyl)but-3-en-2-one (compound name) are depicted in **Figure 3**. This compound exhibited thirteen interactions with α-amylase, including two hydrogen bonds with ASP197 and THR163. Additional interacting residues involved in van der Waals forces included GLU233, ALA198, HIS101, TYR62, LEU165, LEU162, TRP59, TRP58, ASP300, HIS299, and ARG195. For comparison, the docking profile of acarbose, a known α-amylase inhibitor, is presented in **Figure 4**. Acarbose displayed a higher number and wider variety of interactions with α-amylase, including van der Waals forces, hydrogen bonds, and unfavorable donor-donor interactions.
Molecular docking simulations were performed to assess the potential antidiabetic activity of *E. speciosa* compounds by evaluating their binding affinity to key diabetes-related targets: SGLT2 and α-amylase[32,33]. Lower binding energy signifies stronger ligand-target interactions. All *E. speciosa* compounds displayed higher binding affinities for SGLT2 compared to metformin, a common antidiabetic drug. This suggests their potential as potent SGLT2 inhibitors, potentially reducing glucose reabsorption and mitigating diabetic kidney complications[34,35]. While the compounds exhibited lower binding affinities for α-amylase compared to acarbose, another antidiabetic drug, a significant interaction was observed. This suggests their potential to inhibit α-amylase activity and regulate postprandial glucose levels, making them promising candidates for diabetes management.

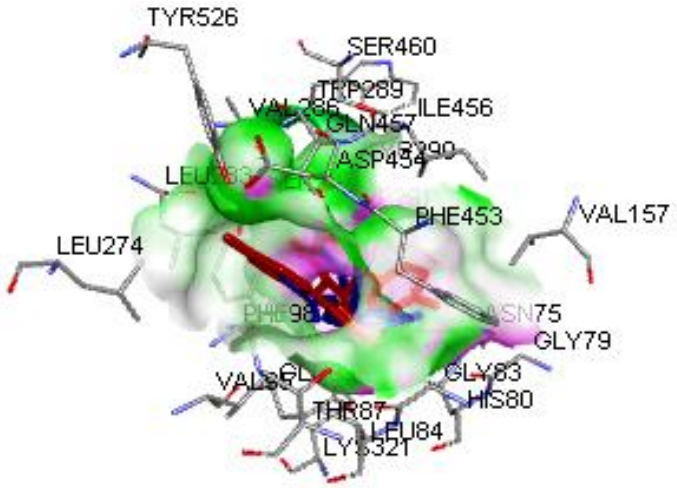
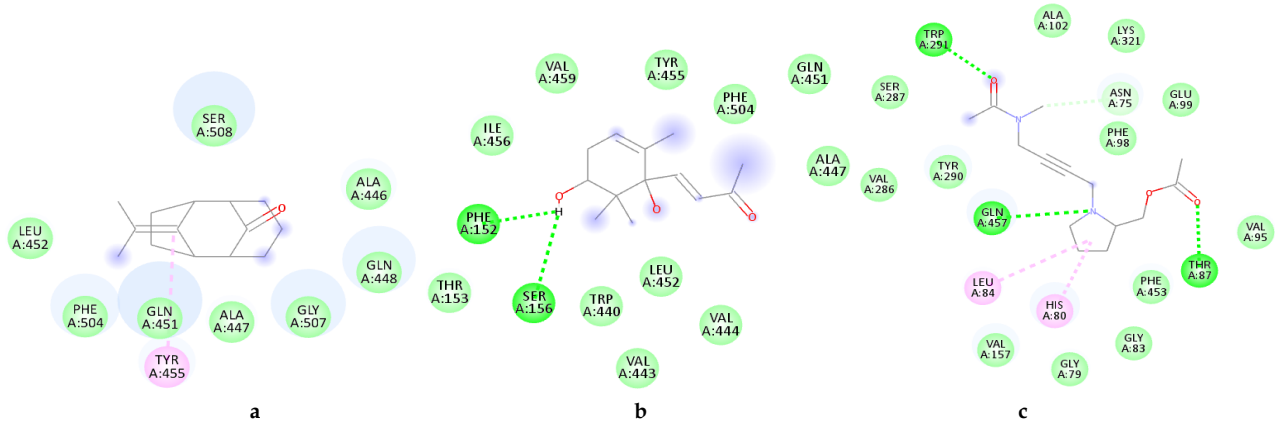


Figure 1. 3D structure of the super-imposition of N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl]-acetamide (red) and the native ligand (blue) in the binding pockets of SGLT2.



a **b** **c**

Figure 2. 2D binding interaction of 11-isopropylidenetricyclo[4.3.1.1(2,5)]undec-3-en-10-one (**a**), 4-(1,5-dihydroxy-2,6,6-trimethylcyclohex-2-enyl)but-3-en-2-one (**b**), and N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl]-acetamide (**c**) with SGLT2. Van der Waals (light green), hydrogen (bright green), and Pi-alkyl (pink) interactions.

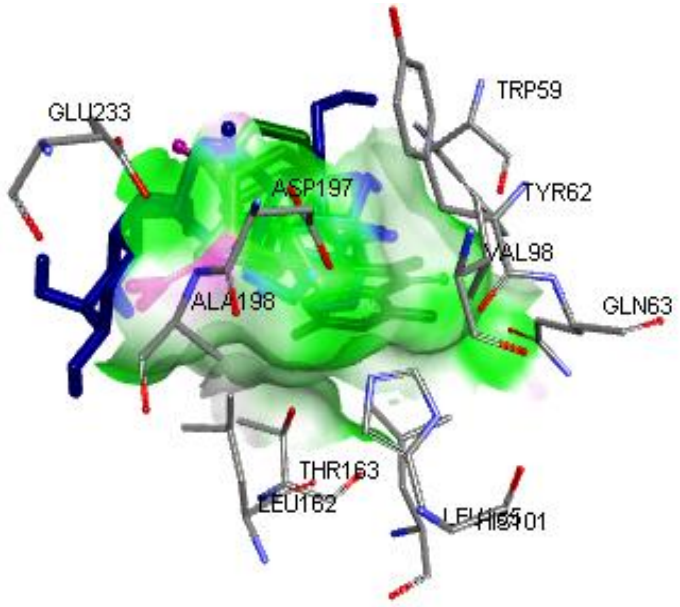
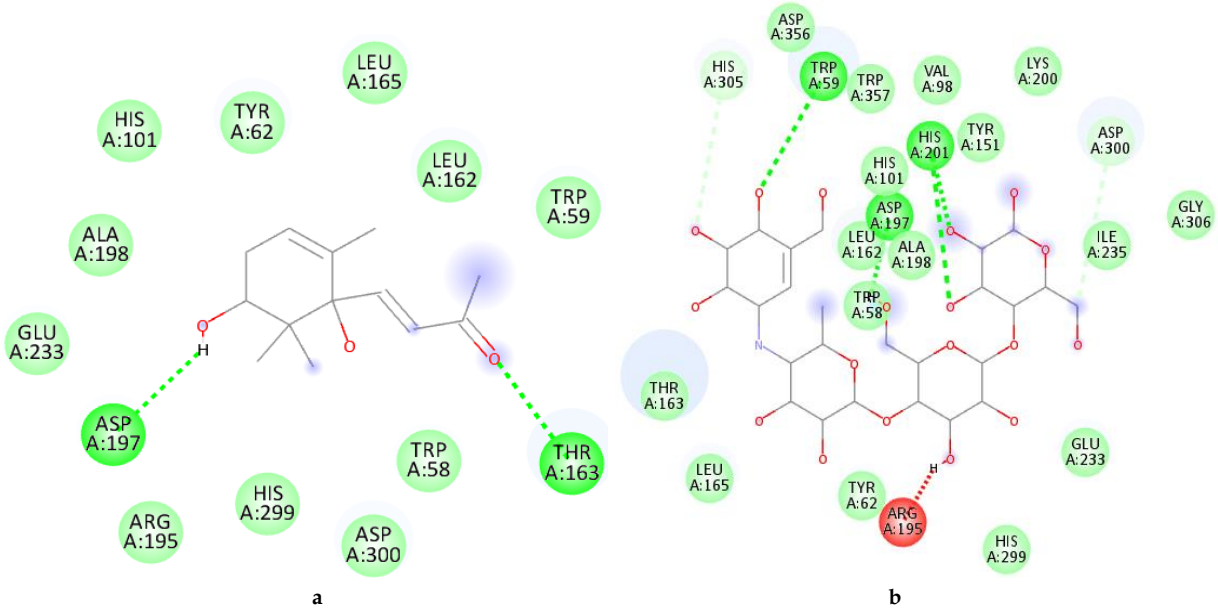


Figure 3. 3D structure of the super-imposition of 9,10-dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol (red), 11-isopropylidenetricyclo[4.3.1.1(2,5)]undec-3-en-10-one (purple), 6,6-dimethyl-10-methylene-1-oxa-spiro[4.5]decane (orange) and the native ligand (blue) in the binding pockets of α-amylase.



a **b**

Figure 4. 2D binding interaction of 4-(1,5-dihydroxy-2,6,6-trimethylcyclohex-2-enyl)but-3-en-2-one (**a**) and acarbose (**b**) with α-amylase. Van der Waals (light green), hydrogen (bright green), and unfavorable donor-donor (red) interactions.

Plant-based drug discovery is gaining significant interest due to the potential for lower toxicity and fewer side effects compared to synthetic medications[36,37]. However, the success of a compound as a therapeutic candidate is highly dependent on its physicochemical properties[35]. Here, we investigated the drug-likeness and antidiabetic potential of *E. speciosa* phytochemicals. This study identified *E. speciosa* phytochemicals with promising drug-like properties, low predicted toxicity, and potential for antidiabetic activity through SGLT2 and α-amylase inhibition. Further *in vitro* and *in vivo* studies are warranted to validate these findings and explore their therapeutic potential for diabetes management.

CONCLUSION

In silico assessment of *E. speciosa* compounds revealed promising drug-like properties and broad applicability in pharmaceutical development. The identified compounds exhibited a range of hydrophobicities, suggesting their potential

for various administration routes beyond oral delivery. This is further supported by the favorable bioavailability scores, placing them within the acceptable range for druggable candidates. Additionally, the *in silico* inhibition profiles against diabetic targets suggest a potential role for these compounds in diabetes management. However, further investigations are warranted to validate these findings. *In vitro* and *in vivo* studies are recommended to confirm the antidiabetic activity and establish the binding stability of the compounds with the identified diabetic targets.

ACKNOWLEDGMENT

The authors gratefully acknowledge Siwe *et al*. for their significant contributions to this research.

AUTHORS' CONTRIBUTION

Conceptualization: Sulyman Olalekan Ibrahim, Halimat Yusuf Lukman, Israel Ehizuelen Ebhohimen, Halimah Funmilayo Babamale, Abdulmumeen Amao Hamid, Marili Funmilayo Zubair, Olubunmi Atolani
Data curation: Sulyman Olalekan Ibrahim, Halimat Yusuf Lukman, Israel Ehizuelen Ebhohimen, Halimah Funmilayo Babamale, Fatimah Ronke Abdulkadir, Abdulmumeen Amao Hamid, Marili Funmilayo Zubair, Olubunmi Atolani
Formal analysis: Sulyman Olalekan Ibrahim, Halimat Yusuf Lukman, Israel Ehizuelen Ebhohimen, Halimah Funmilayo Babamale, Fatimah Ronke Abdulkadir, Abdulmumeen Amao Hamid, Marili Funmilayo Zubair, Olubunmi Atolani
Funding acquisition: -
Investigation: Sulyman Olalekan Ibrahim, Halimat Yusuf Lukman, Israel Ehizuelen Ebhohimen, Halimah Funmilayo Babamale, Fatimah Ronke Abdulkadir, Abdulmumeen Amao Hamid, Marili Funmilayo Zubair, Olubunmi Atolani
Methodology: Sulyman Olalekan Ibrahim, Halimat Yusuf Lukman, Israel Ehizuelen Ebhohimen, Halimah Funmilayo Babamale, Fatimah Ronke Abdulkadir, Abdulmumeen Amao Hamid, Marili Funmilayo Zubair, Olubunmi Atolani
Project administration: Sulyman Olalekan Ibrahim
Resources: Sulyman Olalekan Ibrahim, Olubunmi Atolani
Software: Sulyman Olalekan Ibrahim, Halimat Yusuf Lukman, Israel Ehizuelen Ebhohimen, Halimah Funmilayo Babamale, Abdulmumeen Amao Hamid, Marili Funmilayo Zubair, Olubunmi Atolani
Supervision: Sulyman Olalekan Ibrahim, Olubunmi Atolani
Validation: Sulyman Olalekan Ibrahim, Halimat Yusuf Lukman, Israel Ehizuelen Ebhohimen, Halimah Funmilayo Babamale, Abdulmumeen Amao Hamid, Marili Funmilayo Zubair, Olubunmi Atolani
Visualization: Sulyman Olalekan Ibrahim, Olubunmi Atolani
Writing - original draft: Sulyman Olalekan Ibrahim, Halimat Yusuf Lukman, Israel Ehizuelen Ebhohimen, Halimah Funmilayo Babamale, Fatimah Ronke Abdulkadir, Abdulmumeen Amao Hamid, Marili Funmilayo Zubair, Olubunmi Atolani
Writing - review & editing: Sulyman Olalekan Ibrahim, Halimat Yusuf Lukman, Israel Ehizuelen Ebhohimen, Halimah Funmilayo Babamale, Abdulmumeen Amao Hamid, Marili Funmilayo Zubair, Olubunmi Atolani

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Andrade C, Gomes NG, Duangsrisai S, Andrade PB, Pereira DM, Valentao P. Medicinal plants utilized in Thai Traditional Medicine for diabetes treatment: ethnobotanical surveys, scientific evidence and phytochemicals. J Ethnopharmacol. 2020;263:113177. DOI: 10.1016/j.jep.2020.113177; PMID: 32768637

2. Hussain A, Bhowmik B, do Vale Moreira NC. COVID-19 and diabetes: Knowledge in progress. Diabetes Res Clin Pract. 2020;162:108142. DOI: 10.1016/j.diabres.2020.108142; PMCID: PMC7144611; PMID: 32278764

3. Rachdaoui N. Insulin: the friend and the foe in the development of type 2 diabetes mellitus. Int J Mol Sci. 2020;21(5):1770. DOI: 10.3390/ijms21051770; PMCID: PMC7084909; PMID: 32150819

4. Hameed I, Masoodi SR, Mir SA, Nabi M, Ghazanfar K, Ganai BA. Type 2 diabetes mellitus: from a metabolic disorder to an inflammatory condition. World J Diabetes. 2015;6(4):598-612. DOI: 10.4239/wjd.v6.i4.598; PMCID: PMC4434080; PMID: 25987957

5. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, et al. Pathophysiology of Type 2 Diabetes Mellitus. Int J Mol Sci. 2020;21(17):6275. DOI: 10.3390/ijms21176275; PMCID: PMC7503727; PMID: 32872570

6. Sacks KN, Friger M, Shoham-Vardi I, Abokaf H, Spiegel E, Sergienko R, et al. Prenatal exposure to gestational diabetes mellitus as an independent risk factor for long-term neuropsychiatric morbidity of the offspring. Am J Obstet Gynecol. 2016;215(3):380.e1-7. DOI: 10.1016/j.ajog.2016.03.030; PMID: 27018463

7. Astutik S, Pretzsch J, Kimengsi JN. Asian medicinal plants' production and utilization potentials: A review. Sustainability. 2019;11(19):5483. DOI: 10.3390/su11195483

8. Pundarikakshudu K, Shah PA, Patel MG. Chapter 1 - A comprehensive review of Indian medicinal plants effective in diabetes management: Current status and future prospects. In: Naeem M, Aftab T, editors. Antidiabetic Medicinal Plants: Applications and Opportunities. Cambridge; Academic Press: 2024. p. 3-73. DOI: 10.1016/B978-0-323-95719-9.00013-6

9. Nchegang B, Mezui C, Longo F, Nkwengoua Z, Amang A, Tan P. Effects of the aqueous extract of Eremomastax speciosa (Acanthaceae) on sexual behavior in normal male rats. Biomed Res Int. 2016;2016:9706429. DOI: 10.1155/2016/9706429; PMCID: PMC4971301; PMID: 27525283

10. Effiong GS, Ebe NU, Akpanyung EO, Emeka RA, Nwuzor EO. Effects of Ethanol Leaf Extract of Eremomastax speciosa (African Blood Tonic) on Female Reproductive Hormones of Albino Wistar Rats. Int J Biochem Bioinform Biotechnol Stud. 2020;5(1):1-12.

11. Onoja SO, Eke C, Ejiofor E, Madubuike KG, Ezeja MI, Omeh YN, et al. antioxidant, anti-inflammatory and anti-nociceptive properties of hydro-methanol extract of Eremomastax speciosa (Hochst.) Cufod leaf. Afr J Tradit Complement Altern Med. 2017;14(6):56-63. DOI: 10.21010/ajtcam.v14i6.6

12. Barlow D, Buriani A, Ehrman T, Bosisio E, Eberini I, Hylands P. In-silico studies in Chinese herbal medicines' research: evaluation of in-silico methodologies and phytochemical data sources, and a review of research to date. J Ethnopharmacol. 2012;140(3):526-34. DOI: 10.1016/j.jep.2012.01.041; PMCID: PMC7126886; PMID: 22326356

13. Ibrahim SO, Lukman HY, Zubair MF, Amusan OT, Abdulkadri FR, Lawal B, et al. An Insight into the Physicochemical, Drug-likeness, Pharmacokinetics and Toxicity Profile of Kigelia africana (Lam) Bioactive Compounds. Al-Bahir J Eng Pure Sci. 2024;4(1):4. DOI: 10.55810/2313-0083.1050

14. Rehman A, Bukhari SA, Akhter N, Hussain MAI, Chaudhary Z. In Silico identification of novel phytochemicals that target SFRP4: An early biomarker of diabesity. PLoS One. 2023;18(11):e0292155. DOI: 10.1371/journal.pone.0292155; PMCID: PMC10635506; PMID: 37943820

15. Jamtsho T, Yeshi K, Perry MJ, Loukas A, Wangchuk P. Approaches, Strategies and Procedures for Identifying Anti-Inflammatory Drug Lead Molecules from Natural Products. Pharmaceuticals. 2024;17(3):283. DOI: 10.3390/ph17030283; PMCID: PMC10974486; PMID: 38543070

16. Siwe GT, Ernestine NZ, Amang A, Mezui C, Choudhary I, Tan P. Comparative GC-MS analysis of two crude extracts from Eremomastax speciosa (Acanthaceae) leaves. J Med Plant Stud. 2019;7(2):25-9.

17. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017;7(1):42717. DOI: 10.1038/srep42717; PMCID: PMC5335600; PMID: 28256516

18. Banerjee P, Eckert AO, Schrey AK, Preissner R. ProTox-II: a webserver for the prediction of toxicity of chemicals. Nucleic Acids Res. 2018;46(W1):W257-63. DOI: 10.1093/nar/gky318; PMCID: PMC6031011; PMID: 29718510

19. Kalra S, Jacob J, Baruah MP. Metformin + Sodium-glucose Co-transporter-2 Inhibitor: Salutogenic Lifestyle Mimetics in a Tablet? Indian J Endocrinol Metab. 2018;22(1):164-6. DOI: 10.4103/ijem.ijem_266_17; PMCID: PMC5838898; PMID: 29535955

20. Oboh G, Ogunsuyi OB, Ogunbadejo MD, Adefegha SA. Influence of gallic acid on α-amylase and α-glucosidase inhibitory properties of acarbose. J Food Drug Anal. 2016;24(3):627-34. DOI: 10.1016/j.jfda.2016.03.003; PMCID: PMC9336674; PMID: 28911570

21. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010;31(2):455-61. DOI: 10.1002/jcc.21334; PMCID: PMC3041641; PMID: 19499576

22. Antoni C, Vera L, Devel L, Catalani MP, Czarny B, Cassar-Lajeunesse E, et al. Crystallization of bi-functional ligand protein complexes. J Strcut Biol. 2013;182(3):246-54. DOI: 10.1016/j.jsb.2013.03.015; PMID: 23567804

23. Aribisala JO, Sabiu S. Cheminformatics identification of phenolics as modulators of penicillin-binding protein 2a of Staphylococcus aureus: A structure–activity-relationship-based study. Pharmaceutics. 2022;14(9):1818. DOI: 10.3390/pharmaceutics14091818; PMCID: PMC9503099; PMID: 36145565

24. Manaia EB, Abuçafy MP, Chiari-Andréo BG, Silva BL, Junior JAO, Chiavacci LA. Physicochemical characterization of drug nanocarriers. Int J Nanomedicine. 2017;12:4991-5011. DOI: 10.2147/ijn.s133832; PMCID: PMC5516877; PMID: 28761340

25. Saravanakumar A, Sadighi A, Ryu R, Akhlaghi F. Physicochemical properties, biotransformation, and transport pathways of established and newly approved medications: a systematic review of the top 200 most prescribed drugs vs. the FDA-approved drugs between 2005 and 2016. Clin Pharmacokinet. 2019;58(10):1281-94. DOI: 10.1007/s40262-019-00750-8; PMCID: PMC6773482; PMID: 30972694

26. Kadri A, Aouadi K. In vitro antimicrobial and α-glucosidase inhibitory potential of enantiopure cycloalkylglycine derivatives: Insights into their in silico pharmacokinetic, druglikeness, and medicinal chemistry properties. J Appl Pharm Sci. 2020;10(6):107-15. DOI: 10.7324/JAPS.2020.10614

27. Lipinski CA. Lead-and drug-like compounds: the rule-of-five revolution. Drug Discov Today Technol. 2004;1(4):337-41. DOI: 10.1016/j.ddtec.2004.11.007; PMID: 24981612

28. Riaz M, Zia-Ul-Haq M, Saad B. Anthocyanins and human health: biomolecular and therapeutic aspects. Cham: Springer; 2016. DOI: 10.1007/978-3-319-26456-1

29. Ahmed AH, Alkali YI. In silico pharmacokinetics and molecular docking studies of lead compounds derived from Diospyros mespiliformis. PharmaTutor. 2019;7(3):31-7. DOI: 10.29161/PT.v7.i3.2019.31

30. Welcome MO. Blood brain barrier inflammation and potential therapeutic role of phytochemicals. PharmaNutrition. 2020;11:100177. DOI: 10.1016/j.phanu.2020.100177

31. Pardridge WM. Drug transport across the blood–brain barrier. J Cereb Blood Flow Metab. 2012;32(11):1959-72. DOI: 10.1038/jcbfm.2012.126; PMCID: PMC3494002; PMID: 22929442

32. Pinzi L, Rastelli G. Molecular docking: shifting paradigms in drug discovery. Int J Mol Sci. 2019;20(18):4331. DOI: 10.3390/ijms20184331; PMCID: PMC6769923; PMID: 31487867

33. Jiao X, Jin X, Ma Y, Yang Y, Li J, Liang L, et al. A comprehensive application: Molecular docking and network pharmacology for the prediction of bioactive constituents and elucidation of mechanisms of action in component-based Chinese medicine. Comput Biol Chem. 2021;90:107402. DOI: 10.1016/j.compbiolchem.2020.107402; PMID: 33338839

34. Gong L, Feng D, Wang T, Ren Y, Liu Y, Wang J. Inhibitors of α-amylase and α-glucosidase: Potential linkage for whole cereal foods on prevention of hyperglycemia. Food Sci Nutr. 2020;8(12):6320-37. DOI: 10.1002/fsn3.1987; PMCID: PMC7723208; PMID: 33312519

35. Swain SS, Hussain T. Combined Bioinformatics and Combinatorial Chemistry Tools to Locate Drug-Able Anti-TB Phytochemicals: A Cost-Effective Platform for Natural Product-Based Drug Discovery. Chem Biodivers. 2022;19(11):e202200267. DOI: 10.1002/cbdv.202200267

36. Nasim N, Sandeep IS, Mohanty S. Plant-derived natural products for drug discovery: current approaches and prospects. Nucleus. 2022;65(3):399-411. DOI: 10.1007/s13237-022-00405-3; PMCID: PMC9579558; PMID: 36276225

37. Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou E. Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges and Future Perspectives. Microorganisms. 2021;9(10):2041. DOI: 10.3390/microorganisms9102041; PMCID: PMC8541629; PMID: 34683362

Borneo Journal of Pharmacy
Vol 7 Issue 2 May 2024
Pages 187 – 197

https://journal.umpr.ac.id/index.php/bjop/article/view/6507
DOI: https://doi.org/10.33084/bjop.v7i2.6507
e-ISSN: 2621-4814

Review Article

A Review of Antifungal Resistance in West Africa

Abraham Bwalhuma Muhindo [1,2,3*]
Adamu Almustapha Aliero [4]
Festo Mwebaze Syalhasha [5]
Ibrahim Ntulume [2]
Emmanuel Eilu [2]
Martin Odoki [2]
Joe Mutebi [6]

[1] Department of Medical Laboratory Sciences, Mbarara University of Science and Technology, Mbarara, Mbarara District, Uganda
[2] Department of Microbiology and Immunology, Kampala International University, Ishaka, Bushenyi District, Uganda
[3] Department of Microbiology, Premium Medical Services, Freetown, Western Area Urban District, Sierra Leone
[4] Department of Microbiology, Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria
[5] Institute of Public Health and Management, Clarke International University, Kampala, Kampala District, Uganda
[6] Department of Computing, Kampala International University, Ishaka, Bushenyi District, Uganda

*email: abrahambwalhuma@gmail.com; phone: +256485421373

Keywords:
Antifungal resistance
Candida
Dermatophytes
Fungal infection
West Africa

Abstract

Knowledge of local and regional antimicrobial resistance (AMR) is important for clinical decision-making. However, surveillance capacity for fungal infections let alone antifungal resistance is lacking throughout West Africa, and current antifungal resistance data are sparse. We sought to address this gap by summarizing all available high-quality data on antifungal resistance in West Africa. We searched the PubMed database, African Journals Online archives, and free web searches in October and December 2023 using the terms "antifungal resistance" and "West Africa" to find articles published from 2010 onwards. Only 11 articles were included in our analysis most of which were cross-sectional and descriptive in design; relatively high levels of antifungal resistance (AFR) to commonly used antifungals were reported including (24-75%) resistance to fluconazole and ketoconazole, two of the most frequently-prescribed antifungals in this region. There is a high level of resistance to griseofulvin, ketoconazole, cotrimoxazole, and fluconazole among dermatophyte infections (80-100%) with 100% resistance to amphotericin B, ketoconazole, and fluconazole reported by the invasive fungal disease-causing pathogen *Cryptococcus neoformans*. Resistance to commonly used anti-fungal drugs is prevalent; raising concern that these drugs may no longer be useful for treating moderate or severe fungal infections in West Africa hence calling for countries to promote acceptance of antimicrobial stewardship as a programmatic strategy not just focused on bacterial resistance but also fungal resistance including pharmacy management, laboratory complete mycological investigations and dissemination of standard fungal susceptibility profiles.

Received: January 2nd, 2024
1st Revised: May 6th, 2024
Accepted: May 13th, 2024
Published: May 30th, 2024

INTRODUCTION

"*Emerging from the shadows of the bacterial antimicrobial resistance pandemic, fungal infections are growing, and are ever more resistant to treatments, becoming a public health concern worldwide*," said Dr Hanan Balkhy, WHO Assistant Director-General, Antimicrobial Resistance (AMR)[1]. Approximately 1 million individuals have invasive fungal infections (Candida, Cryptococcus, Aspergillus, and Pneumocystis) worldwide, resulting in the death of 1.7 million people annually[2,3]. While the benefits of appropriate antifungal usage to treat fungal infections are well established, all antifungal use carries a risk of

How to cite: Muhindo AB, Aliero AA, Syalhasha FM, Ntulume I, Eilu E, Odoki M, et al. Chemoinformatic-aided Antidiabetic Analysis of the Therapeutic Potential of Phytoconstituents in Eremomastax speciosa Extracts. Borneo J Pharm. 2024;7(2):187-97. doi:10.33084/bjop.v7i2.6507

inducing antifungal resistance (AFR). The emergence of resistant or even multi-resistant strains is of concern, because of the limited antifungal arsenal[4]. The emerging global health threat of fungal diseases is compounded by the rapid emergence of antifungal resistance and, in many settings, limited access to quality diagnostics and treatment[5-7].

There are four major classes of antifungal medicines (azoles, echinocandins, pyrimidines, and polyenes) mostly used in clinical practice, and only a few others are under development[8-11]. With the growing prevalence of human fungal infections, especially in immunocompromised patients, these diseases have become a worldwide public health issue[12]. The immune system status of the host determines the outcome of the disease and therefore infections may progress or range from limited cutaneous or subcutaneous to invasive, disseminated, and life-threatening infections[13]. Invasive Fungal infections (IFI) have been observed to increase in the last decades due to an increased number of immunosuppressed patients or persons resulting in increased use of systemic antifungal drugs to treat these infections eg echinocandins, polyenes, triazoles, and flucytosine[14]. Overuse and misuse especially prophylactically have resulted in stronger selective pressure on fungi[15].

The WHO Fungal Priority Pathogens List[1] represents the first global response to identify and prioritize fungal pathogens and their impact on global public health and to consider the unmet research and development needs grouped the priority fungal pathogens into those of critical, high, and medium priority. Global warming, increased international travel, lack of globally affordable diagnostic platforms, and increasing resistance have increased the incidence of mycoses yet the prevention and control of fungal diseases remains a major challenge, especially in resource-poor countries, despite their likely large burden[16]. Although such reports are concerning, the burden of Antifungal Resistance (AFR) in the West Africa region is not well published. Additionally, knowledge of the situation in many parts of the world further complicates the problem[17].

Better knowledge of the burden and proportion of infections caused by drug-resistant fungi in low-resource settings would raise awareness of the need to prevent the rise and spread of drug resistance[18]. Understanding current levels of AFR throughout West Africa could improve clinical practice by guiding empirical antifungal choice. Toward this end, we reviewed the available evidence on the burden of AFR among fungal pathogens in West Africa to ascertain the common antifungal pathogens isolated in West Africa, summarize the antifungal sensitivity profiles of common fungal pathogens to available antifungal agents, and to critically analyze current clinical and diagnostic practices and future research interventions to address antifungal resistance.

SEARCH STRATEGY AND SELECTION CRITERIA

A comprehensive literature search was conducted in October and December 2023 to identify relevant studies on antifungal resistance (AFR) in West Africa (**Figure 1**). We searched the PubMed database (www.ncbi.nlm.nih.gov/pubmed/), African Journals Online archives (https://www.ajol.info/index.php/ajol), Google Scholar (https://scholar.google.com/), and ScienceDirect (https://www.sciencedirect.com/). The search terms "antifungal resistance" and "West Africa" were used to identify articles published from 2010 onwards. Only English language articles were included.

Inclusion criteria for full-text articles were reported the proportion of AFR among clinical fungal isolates obtained from human subjects or environmental samples in any West African country (including Cameroon, Ghana, Nigeria, Ivory Coast, Sierra Leone, Guinea, Central African Republic, Mali, Liberia, Niger, and others), as well as described the studied patient population, isolated organisms, specific laboratory methods used for antifungal susceptibility testing, and interpretation methods for minimum inhibitory concentration breakpoints or zone diameters of inhibition as outlined by the Clinical and Laboratory Standards Institute (CLSI)[19,20]. To ensure the inclusion of contemporary and relevant AFR data, only studies published from 2010 onwards were considered. Data extraction focused on fungal species isolated, the number of isolates tested for AFR, specific antifungals tested, and the percentage of organisms resistant to each antifungal agent.

Our initial search strategy identified 413 articles: 257 from PubMed, 133 from AJOL, and 23 from other free web sources. After screening, 100 articles were deemed relevant for further evaluation. Of these, 33 full-text articles were accessible. We excluded 22 articles for the following reasons: three lacked data on fungal resistance, six reported antifungal resistance by a specific antifungal group, and 13 lacked information on isolate identity or specific laboratory methods employed. The remaining 11 articles were included in this review (**Table I**). These studies investigated patterns of antifungal resistance (AFR) in various African countries: five focused on Cameroon[21-23,28,31], four on Nigeria[24,26,27,29], one on Ivory Coast[30], and one

on Ghana[25]. A total of 2,721 subjects were included across the selected studies. Notably, Cameroon contributed the highest number of studies (45%).

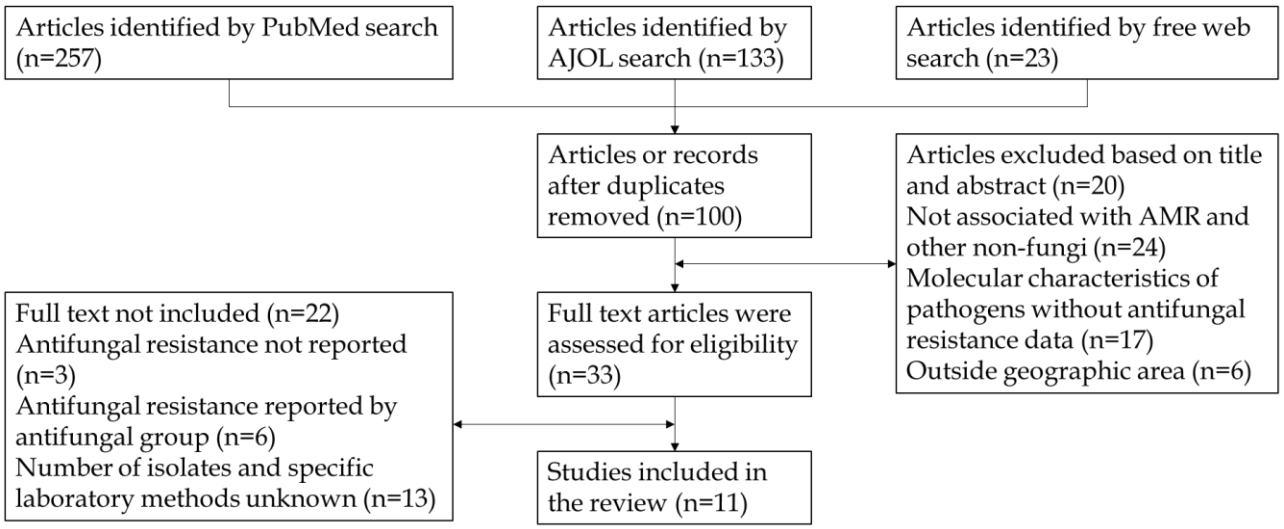


Figure 1. Selection of publications for inclusion.

Table I. Summary of studies included in the analysis.

Country	Year	Fungal group	Sample source/patient populations	Number of study participants/samples
Nigeria	2011	*Candida* spps	HIV/AIDS patients	200
Cameroon	2012	*Candida* spps	HIV/AIDS patients	304
Ghana	2012	*Candida* spps	General outpatients	528
Ivory Coast	2012	*Candida* spps	Women with leukorrhoea	150
Cameroon	2013	*Candida* spps	HIV/AIDS patients	207
Cameroon	2016	*Trichophyton* spps	Diabetic patients	152
Cameroon	2016	*Cryptococcus* spps	Environmental isolates	350
Cameroon	2020	*Candida* spps	HIV/AIDS patients	378
Nigeria	2020	*Tinea* spps	Children attending elementary school	301
Nigeria	2020	Dermatophytes	University students	119
Nigeria	2021	Dermatophytes	Children attending elementary school	32

ANTIFUNGAL RESISTANCE PATTERNS

A review of the laboratory methodologies employed within the studies included in this review revealed concerning levels of resistance among fungal pathogens to commonly used antifungals in West Africa (**Table II**). Notably, *Candida* spp., a prevalent fungal genus, exhibited resistance rates ranging from 24% to 75% for fluconazole and ketoconazole, two of the most frequently prescribed antifungal medications in the region[21-25]. Similarly, high resistance rates (80-100%) were observed for griseofulvin, ketoconazole, cotrimoxazole, and fluconazole among school children diagnosed with dermatophyte infections[26,27]. Additionally, a study investigating environmental samples reported 100% resistance to amphotericin B, ketoconazole, and fluconazole among *Cryptococcus* spp., including both *Cryptococcus gattii* and *Cryptococcus neoformans*, fungal pathogens responsible for invasive fungal diseases[28].

FUNGAL ORGANISMS

Several studies have identified *Candida* spps. (particularly *C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis*, *C. parapsilosis*, and *C. pseudotropicalis*) as the predominant fungal isolates from immunocompromised patients[21-25,29,30]. Conversely, dermatophytes (*Trichophyton enotrephon, T. bullosum, T. simii, T. tonsurans, Microsporum audouinii, M. canis, T. concentricum, T. mentagrophytes, T. rubrum,* and *T. violaceum*) were the most prevalent isolates among school children and university students[26,27,31]. An alarming trend highlighted in the reviewed literature is the emergence of single fungal isolates exhibiting resistance to multiple antifungal drugs. This phenomenon appears to be most common among *Candida* spp. isolated from

immunocompromised patients, particularly those with HIV/AIDS[21-25]. Notably, susceptibility to nystatin remained generally good across *Candida* spp., suggesting its potential as a first-line empirical treatment for Candida infections[32]. The identification of multi-drug resistant fungi underscores the critical need for promoting appropriate antifungal usage and routine microbiological sampling of infected patients, especially in resource-limited settings[33]. The collective evidence presented here strongly suggests the existence of AMR, particularly against widely used antifungal drugs (**Table II**).

Table II. Antifungal resistance patterns from selected studies in West Africa.

Authors	Year	Country	Method	Organism	AMP	NYS	FCY	GRS	MIC	FLU	ITR	VOR	KET	ECO	CTM
Njunda *et al.*[21]	2012	Cameroon	Disc diffusion	*C. albicans* (n=175)	92	23	-	-	-	124	-	-	23	24	-
Njunda *et al.*[22]	2013	Cameroon	Broth microdilution	*C. albicans* (n=103)	5	76	11	-	9	16	10	4	2	8	-
				C. dubliniensis	0	3	0	-	0	2	0	0	0	0	-
				(n=3)	1	13	1	-	3	2	1	1	0	5	-
				C. tropicalis (n=23)	0	2	1	-	0	0	0	0	0	0	-
				C. famata (n=4)											
Abrantes *et al.*[23]	2016	Cameroon	Broth microdilution	*C. albicans* (n=92)	4	-	6	-	0	46	47	46	-	-	-
				C. dubliniensis	1	-	-	-	-	0	0	0	-	-	-
				(n=10)											
				C. glabrata (n=24)	1	-	-	-	16	1	4	1	-	-	-
				C. krusei (n=3)	1	-	-	-	0	2	0	1	-	-	-
				C. tropicalis (n=4)	2	-	-	-	0	0	0	0	-	-	-
				C. kefyr/para/lusi	1	-	-	-	-	0	0	0	-	-	-
				(n=2)											
Dongmo *et al.*[28]	2014	Cameroon	Broth microdilution	*C. neoformans*	4	4	-	-	-	64	-	-	1	-	-
Eba *et al.*[31]	2016	Cameroon	Broth microdilution	*Trichopyton* spps.	67	-	-	96	19	-	98	-	79	-	-
Nweze *et al.*[24]	2011	Nigeria	Broth microdilution	*C. albicans* (n=54)	0	-	5	-	-	9	6	1	-	-	-
				C. dubliniensis	0	-	1	-	-	3	1	0	-	-	-
				(n=9)	0	-	2	-	-	0	0	1	-	-	-
				C. tropicalis (n=22)	0	-	2	-	-	2	1	0	-	-	-
				C. parapsilopsis											
				(n=18)	0	-	0	-	-	0	1	0	-	-	-
				C. guilliermondii											
				(n=11)											
Ayodele *et al.*[29]	2020	Nigeria	Disc diffusion	*T. rubrum* (n=156)	156	-	-	-	33	156	156	-	156	-	33
				M. ferrugineum	51	-	-	-	0	51	51	-	51	-	0
				(n=51)											
				T. mentagrophytes	17	-	-	-	0	0	17	-	17	-	0
				(n=17)											
				T. verrucosum	4	-	-	-	4	4	4	-	4	-	4
				(n=4)											
David *et al.*[26]	2020	Nigeria	Broth microdilution	*M. canis* (n=6)	-	1	-	-	-	5	-	-	2	-	6
				T. concentricum	-	3	-	-	-	5	-	-	4	-	5
				(n=6)											
				T. mentagrophytes	-	4	-	-	-	12	-	-	6	-	12
				(n=12)											
				T. rubrum (n=7)	-	4	-	-	-	7	-	-	4	-	7
				T. violaceum (n=7)	-	5	-	-	-	7	-	-	5	-	7
Ungokore *et al.*[27]	2021	Nigeria	Disc diffusion	*T. eriotrephon*	1	-	-	0	-	2	-	-	4	-	-
				(n=10)	1	-	-	0	-	0	-	-	1	-	-
				T. bullosum (n=2)	1	-	-	0	-	0	-	-	5	-	-
				T. simii (n=12)	1	-	-	1	-	2	-	-	1	-	-
				T. tonsurans (n=3)	0	-	-	0	-	0	-	-	1	-	-
				M. audouinii (n=2)											
Djohan *et al.*[30]	2012	Ivory Coast	Semi-solid medium microdilution	*C. albicans* (n=45)	0	-	-	-	-	2	22	11	-	-	-
Feglo *et al.*[25]	2012	Ghana	Semi-solid medium microdilution	*C. albicans* (n=33)	9	-	3	-	-	0	2	0	-	-	-
				C. dubliniensis	0	-	4	-	-	0	0	0	-	-	-
				(n=4)	1	-	0	-	-	0	3	0	-	-	-
				C. glabrata (n=12)	2	-	1	-	-	0	0	0	-	-	-
				C. krusei (n=3)	3	-	0	-	-	0	0	0	-	-	-
				C. tropicalis (n=8)											

Notes: AMP-amphotericin B, NYS-nystatin, FCY-flucytosine, GRS-griseofulvin, MIC-micafungin, FLU-fluconazole, ITR-itraconazole, VOR-voriconazole, KET-ketoconazole, ECO- econazole, CTM-clotrimazole

This review summarizes the findings of 11 studies highlighting significant resistance across West Africa to antifungals crucial for everyday use. Notably, most Candida species exhibited limited susceptibility to fluconazole, a commonly-used first-line antifungal in the region and recommended by the WHO for managing superficial and systemic mycoses[34]. Furthermore, Cryptococcus isolates (both *C. neoformans* and *C. gatti* variants) obtained from the environment displayed concerning resistance to amphotericin B, ketoconazole, and fluconazole[35]. Cryptococcus, a globally distributed invasive

fungus, presents a substantial therapeutic challenge due to the lack of documented human-to-human transmission yet possesses established virulence mechanisms that enable infection, dissemination, and ultimately, host mortality[36].

Antifungal resistance to commonly used drugs like fluconazole, ketoconazole, cotrimoxazole, and amphotericin B appears prevalent in West Africa and could be a growing concern, particularly among immunocompromised HIV/AIDS patients. However, as highlighted in the 2022 WHO report on fungal priority pathogens[1], this resistance is likely under-reported due to limited access to diagnostic testing, inadequate microbiology support, and inconsistencies in laboratory standards. The scarcity of readily available diagnostic tools, such as rapid antigen assays, and the high costs or lack of access to polymerase chain reaction (PCR) testing significantly hinder diagnosis, leading to underreported cases[37]. These limitations in antifungal susceptibility testing often contribute to antifungal misuse and overuse, further exacerbating resistance development[38]. The limited data on antifungal resistance in West Africa could stem from several factors, including low clinician awareness of fungal infections and a shortage of healthcare personnel equipped to diagnose mycoses[39]. Even when some antifungal resistance information exists, inadequate national laboratory strategic plans, particularly regarding mycoses, might hinder proper communication to healthcare providers across the region[40].

PREVENTION OF THE DEVELOPMENT AND SPREAD OF ANTIFUNGAL RESISTANCE

Antifungal resistance is likely to become an even greater challenge in West Africa and may be compounded by overuse of antifungals, the lack of antifungal prescription oversight, and the paucity of relevant local information and data on antifungal resistance[41]. To address these issues, existing antimicrobial stewardship programs should be strengthened or, where they are not yet in place, they should be developed and implemented in all regional referral hospitals in response to these challenges[42]. Based on our findings, there are variations in the incidence and prevalence of fungal conditions dependent on local clinical practice with varying antifungal resistance. Therefore, each country in the West African region needs to contextualize the fight during the design or formation of local priorities for better-targeted public health outcomes[43]. The area of particular focus should be immunocompromised patients presenting with Candida-like symptoms. Existing but limited resources should be directed equally at discovering the causes and offering appropriate treatment of infections in this population[44,45].

Additionally, there is a need to urgently scale up the training of both laboratory and pharmacy staff in antifungal/antibiotic stewardship at health facilities where laboratory investigations are available[46]. This is critical in communicating with clinicians, who are the cornerstone in the proper management of patients with infections, especially about the use of these fungal drugs. There is a need for regular antifungal resistance surveys to establish evidence-based and locally relevant resistance data that would help create guidelines to improve clinical practice[47-49].

The implementation and development of the 2022 WHO fungal priority pathogens list to guide research, development, and public health action for containment of antifungal resistance through inter-continental, regional, and sub-regional-wide surveillance programs as a health systems approach has met with several challenges[1,50]. In low- and middle-income countries, implementing the strategy has proven difficult, because the human and financial resources as well as mycological expertise are insufficient[51,52]. In addition, it is difficult to obtain appropriate sample sizes for an accurate representation of resistance patterns. Novel approaches to antimicrobial surveillance are therefore needed for low-resource settings, which include the development of surveillance programs utilizing smaller sample sizes to provide locally relevant AMR patterns and to encourage appropriate empirical antimicrobial therapy[53-55]. Also, the development of new point-of-care diagnostic tools able to detect antifungal resistance resistance cost-effectively will improve patient management and limit the emergence of drug resistance[56,57].

Lastly, of the 413 publications identified, we considered only 11 dues to the lack of standardization and quality of the methodology and reporting. This highlights the scarcity of good-quality data that could allow stakeholders to assess the real burden of antifungal resistance. The susceptibility data reported was very inconsistent and less from low mid-income countries of West Africa, perhaps due to the limited access to mycology laboratories and expertise[58,59]. Thus, better-standardized research protocols are needed to evaluate the emergence of antifungal resistance in different local settings to obtain comparable results and implement befitting interventions[60,61]. Although many papers reported susceptibility data from small ad-hoc laboratory surveillance projects, formal surveillance and data linkage to clinical outcomes were lacking[62-

[64]. Furthermore, susceptibility was reported very inconsistently, making comparisons over time or between geographic areas difficult. Susceptibility data were less common from low- and middle-income countries, likely due to limited access to medical mycology laboratories in resource-limited settings[65].

CONCLUSION

This review highlights the concerning prevalence of resistance to commonly used antifungal drugs in West Africa. The emergence of multi-drug resistance poses a significant and growing threat in the region, potentially rendering multiple antifungal therapies ineffective for treating fungal infections. Single isolates exhibiting resistance to more than one antifungal drug, including those from different classes, further complicate this challenge. To address this growing public health concern, West African countries should prioritize the implementation of comprehensive antimicrobial stewardship programs. These programs should not only focus on combating bacterial resistance but also encompass strategies to tackle the rising threat of antifungal resistance. Key areas for intervention include strengthening pharmacy management practices, implementing robust laboratory quality control measures, expanding mycological investigations, and disseminating standardized fungal susceptibility profiles.

ACKNOWLEDGMENT

We would like to thank Dr. Jacob Stanley Iramiot for his critical review of the manuscript. No external funding was received for this research.

AUTHORS' CONTRIBUTION

Conceptualization: Abraham Bwalhuma Muhindo, Adamu Almustapha Aliero
Data curation: Abraham Bwalhuma Muhindo, Adamu Almustapha Aliero, Festo Mwebaze Syalhasha, Ibrahim Ntulume, Emmanuel Eilu, Martin Odoki, Joe Mutebi
Formal analysis: Abraham Bwalhuma Muhindo, Adamu Almustapha Aliero, Festo Mwebaze Syalhasha, Ibrahim Ntulume, Emmanuel Eilu, Martin Odoki, Joe Mutebi
Funding acquisition: -
Investigation: Abraham Bwalhuma Muhindo, Adamu Almustapha Aliero, Festo Mwebaze Syalhasha, Ibrahim Ntulume, Emmanuel Eilu, Martin Odoki, Joe Mutebi
Methodology: Abraham Bwalhuma Muhindo, Adamu Almustapha Aliero
Project administration: Abraham Bwalhuma Muhindo, Adamu Almustapha Aliero, Festo Mwebaze Syalhasha, Ibrahim Ntulume, Emmanuel Eilu, Martin Odoki, Joe Mutebi
Resources: Abraham Bwalhuma Muhindo, Adamu Almustapha Aliero, Festo Mwebaze Syalhasha, Ibrahim Ntulume, Emmanuel Eilu, Martin Odoki, Joe Mutebi
Software: -
Supervision: Abraham Bwalhuma Muhindo, Adamu Almustapha Aliero, Festo Mwebaze Syalhasha, Ibrahim Ntulume, Emmanuel Eilu, Martin Odoki, Joe Mutebi
Validation: Abraham Bwalhuma Muhindo, Adamu Almustapha Aliero, Festo Mwebaze Syalhasha, Ibrahim Ntulume, Emmanuel Eilu, Martin Odoki, Joe Mutebi
Visualization: Abraham Bwalhuma Muhindo, Adamu Almustapha Aliero, Festo Mwebaze Syalhasha, Ibrahim Ntulume, Emmanuel Eilu, Martin Odoki, Joe Mutebi
Writing - original draft: Abraham Bwalhuma Muhindo, Adamu Almustapha Aliero

Writing - review & editing: Abraham Bwalhuma Muhindo, Adamu Almustapha Aliero, Festo Mwebaze Syalhasha, Ibrahim Ntulume, Emmanuel Eilu, Martin Odoki, Joe Mutebi

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Antimicrobial Resistance Division Control of Neglected Tropical Diseases World Health Organization. WHO fungal priority pathogens list to guide research, development and public health action. Geneva: World Health Organization; 2022.

2. Papon N, Bougnoux ME, d'Enfert C. Tracing the Origin of Invasive Fungal Infections. Trends Microbiol. 2020;28(4):240-2. Doi: 10.1016/j.tim.2020.01.007; PMID: 32044130

3. Firacative C. Invasive fungal disease in humans: are we aware of the real impact? Mem Inst Oswaldo Cruz. 2020;115:e200430. DOI: 10.1590/0074-02760200430; PMCID: PMC7546207; PMID: 33053052

4. Lamoth F, Lockhart SR, Berkow EL, Calandra T. Changes in the epidemiological landscape of invasive candidiasis. J Antimicrob Chemother. 2018;73(suppl_1):i4-13. DOI: 10.1093/jac/dkx444; PMID: 29304207

5. Vitiello A, Ferrara F, Boccellino M, Ponzo A, Cimmino C, Comberiati E, et al. Antifungal Drug Resistance: An Emergent Health Threat. Biomedicines. 2023;11(4):1063. DOI: 10.3390/biomedicines11041063; PMCID: PMC10135621; PMID: 37189681

6. Fisher MC, Alastruey-Izquierdo A, Berman J, Bicanic T, Bignell EM, Bowyer P, et al. Tackling the emerging threat of antifungal resistance to human health. Nat Rev Microbiol. 2022;20(9):557-71. DOI: 10.1038/s41579-022-00720-1; PMCID: PMC8962932; PMID: 35352028

7. Ioannidis K, Papachristos A, Skarlatinis I, Kiospe F, Sotiriou S, Papadogeorgaki E, et al. Do we need to adopt antifungal stewardship programmes?. Eur J Hosp Pharm. 2020;27(1):14-8. DOI: 10.1136/ejhpharm-2017-001467; PMCID: PMC6992971; PMID: 32064083

8. Osherov N, Kontoyiannis DP. The anti-Aspergillus drug pipeline: Is the glass half full or empty? Med Mycol. 2017;55(1):118-24. DOI: 10.1093/mmy/myw060; PMID: 27562862

9. Hoenigl M, Sprute R, Egger M, Arastehfar A, Cornely OA, Krause R, et al. The Antifungal Pipeline: Fosmanogepix, Ibrexafungerp, Olorofim, Opelconazole, and Rezafungin. Drugs. 2021;81(15):1703-29. DOI: 10.1007/s40265-021-01611-0; PMCID: PMC8501344; PMID: 34626339

10. Perfect JR. The antifungal pipeline: a reality check. Nat Rev Drug Discov. 2017;16(9):603-16. DOI: 10.1038/nrd.2017.46; PMCID: PMC5760994; PMID: 28496146

11. Denning DW, Bromley MJ. Infectious Disease. How to bolster the antifungal pipeline. Science. 2015;347(6229):1414-6. DOI: 10.1126/science.aaa6097; PMID: 25814567

12. Rayens E, Norris KA. Prevalence and Healthcare Burden of Fungal Infections in the United States, 2018. Open Forum Infect Dis. 2022;9(1):ofab593. DOI: 10.1093/ofid/ofab593; PMCID: PMC8754384; PMID: 35036461

13. Köhler JR, Hube B, Puccia R, Casadevall A, Perfect JR. Fungi that Infect Humans. Microbiol Spectr. 2017;5(3):10. DOI: 10.1128/microbiolspec.FUNK-0014-2016. PMID: 28597822

14. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. Sci Transl Med. 2012;4(165):165rv13. DOI: 10.1126/scitranslmed.3004404; PMID: 23253612

15. Bailly S, Maubon D, Fournier P, Pelloux H, Schwebel C, Chapuis C, et al. Impact of antifungal prescription on relative distribution and susceptibility of Candida spp. - Trends over 10 years. J Infect. 2016;72(1):103-11. DOI: 10.1016/j.jinf.2015.09.041; PMID: 26518058

16. Lakoh S, Orefuwa E, Kamara MN, Jiba DF, Kamara JB, Kpaka S, et al. The burden of serious fungal infections in Sierra Leone: a national estimate. Ther Adv Infect Dis. 2021;8:20499361211027996. DOI: 10.1177/20499361211027996; PMCID: PMC8252340; PMID: 34262759

17. Hoenigl M, Seidel D, Sprute R, Cunha C, Oliverio M, Goldman GH, Ibrahim AS, Carvalho A. COVID-19-associated fungal infections. Nat Microbiol. 2022;7(8):1127-1140. DOI: 10.1038/s41564-022-01172-2; PMCID: PMC9362108; PMID: 35918423

18. Tang KWK, Millar BC, Moore JE. Antimicrobial Resistance (AMR). Br J Biomed Sci. 2023;80:11387. DOI: 10.3389/bjbs.2023.11387; PMCID: PMC10336207; PMID: 37448857

19. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eleventh Edition. CLSI document M02–A11. Wayne: Clinical and Laboratory Standards Institute; 2012.

20. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: 30th Informational Supplement. CLSI document M100-S22. Wayne, Clinical and Laboratory Standards Institute; 2020.

21. Njunda AL, Nsagha DS, Assob JC, Kamga HL, Teyim P. In vitro Antifungal Susceptibility Patterns of Candida Albicans from HIV and AIDS Patients Attending the Nylon Health District Hospital in Douala, Cameroon. J Public Health Afr. 2012;3(1):e2. DOI: 10.4081/jphia.2012.e2; PMCID: PMC5345446; PMID: 28299076

22. Njunda LA, Assob JCN, Nsagha SD. Oral and urinary colonization of Candida species in HIV/AIDS patients in Cameroon. Basic Sci Med. 2013;2(1):1–8. DOI: 10.5923/j.medicine.20130201.01

23. Abrantes PMDS, McArthur CP, Africa CWJ. Multi-drug resistant oral Candida species isolated from HIV-positive patients in South Africa and Cameroon. Diagn Microbiol Infect Dis. 2014;79(2):222-7. DOI: 10.1016/j.diagmicrobio.2013.09.016; PMID: 24726686

24. Nweze EI, Ogbonnaya UL. Oral Candida isolates among HIV-infected subjects in Nigeria. J Microbiol Immunol Infect. 2011;44(3):172-7. DOI: 10.1016/j.jmii.2011.01.028; PMID: 21524610

25. Feglo PK, Narkwa P. Prevalence and antifungal susceptibility patterns of yeast isolates at the Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana. British Microbiol Res J. 2012;2(1):10–22. DOI: 10.9734/BMRJ/2012/861

26. David O, Ajayi YO, bablola TO. Virulence Factors and Antifungal Resistance in Dermatophytes Associated with Dandruff among University Students and a Tertiary Institution in Nigeria. Egypt Acad J Biol Sci G Microbiol. 2020; 12(2):1-8. DOI: 10.21608/eajbsg.2020.105965

27. Ungokore HY, Ehinmidu JO, Onaolapo JA, Olonitola OS. In Vitro Antifungal Susceptibility Profiles of Dermatophytes Isolates from Tinea Capitis in Northwest, Nigeria. Infect Epidemiol Microbiol. 2021;7(3):347-56. DOI: 10.52547/iem.7.4.347

28. Dongmo W, Kechia F, Tchuenguem R, Nangwat C, Yves I, Kuiate JR, et al. In Vitro Antifungal Susceptibility of Environmental Isolates of Cryptococcus spp. from the West Region of Cameroon. Ethiop J Health Sci. 2016;26(6):555-60. DOI: 10.4314/ejhs.v26i6.8; PMCID: PMC5389075; PMID: 28450771

29. Ayodele EH, Charles N, Abayomi F. Prevalence, identification and antifungal susceptibility of dermatophytes causing tinea capitis in a locality of north central nigeria. Afr J Infect Dis. 2020;15(1):1-9. DOI: 10.21010/ajid.v15i1.1; PMCID: PMC8047286; PMID: 33884353

30. Djohan V, Angora KE, Vanga-Bosson AH, Konaté A, Kassi FK, Yavo W, et al. [In vitro susceptibility of vaginal Candida albicans to antifungal drugs in Abidjan (Ivory Coast)]. J Mycol Med. 2012;22(2):129-33. DOI: 10.1016/j.mycmed.2011.11.005; PMID: 23518014

31. Eba M, Njunda AL, Mouliom RN, Kwenti ET, Fuh AN, Nchanji GT, et al. Onychomycosis in diabetic patients in Fako Division of Cameroon: prevalence, causative agents, associated factors and antifungal sensitivity patterns. BMC Res Notes. 2016;9(1):494. DOI: 10.1186/s13104-016-2302-1; PMCID: PMC5120551; PMID: 27876085

32. Maheronnaghsh M, Fatahinia M, Dehghan P, Teimoori A. Identification of Candida Species and Antifungal Susceptibility in Cancer Patients with Oral Lesions in Ahvaz, Southern West of Iran. Adv Biomed Res. 2020;9:50. DOI: 10.4103/abr.abr_214_19; PMCID: PMC7792871; PMID: 33457333

33. Arastehfar A, Wickes BL, Ilkit M, Pincus DH, Daneshnia F, Pan W, et al. Identification of Mycoses in Developing Countries. J Fungi. 2019;5(4):90. DOI: 10.3390/jof5040090; PMCID: PMC6958481; PMID: 31569472

34. Soriano A, Honore PM, Puerta-Alcalde P, Garcia-Vidal C, Pagotto A, Gonçalves-Bradley DC, et al. Invasive candidiasis: current clinical challenges and unmet needs in adult populations. J Antimicrob Chemother. 2023;78(7):1569-85. DOI: 10.1093/jac/dkad139; PMCID: PMC10320127; PMID: 37220664

35. Figueiredo TP, de Lucas RC, Cazzaniga RA, França CN, Segato F, Taglialegna R, et al. Antifungal Susceptibility Testing and Genotyping Characterization of Cryptococcus neoformans and gattii Isolates from HIV-Infected Patients of Ribeirão Preto, São Paulo, Brazil. Rev Inst Med Trop Sao Paulo. 2016;58:69. DOI: 10.1590/s1678-9946201658069; PMCID: PMC5048640; PMID: 27680174

36. May RC, Stone NR, Wiesner DL, Bicanic T, Nielsen K. Cryptococcus: from environmental saprophyte to global pathogen. Nat Rev Microbiol. 2016;14(2):106-17. DOI: 10.1038/nrmicro.2015.6; PMCID: PMC5019959; PMID: 26685750

37. Medina N, Alastruey-Izquierdo A, Mercado D, Denning DW, Arathoon E, Rodriguez-Tudela JL. Diagnostic Mycology Laboratories Should Have a Central Role for the Management of Fungal Disease. J Fungi. 2022;8(12):1285. DOI: 10.3390/jof8121285; PMCID: PMC9787327; PMID: 36547618

38. Mudenda S, Matafwali SK, Mukosha M, Daka V, Chabalenge B, Chizimu J, et al. Antifungal resistance and stewardship: a knowledge, attitudes and practices survey among pharmacy students at the University of Zambia; findings and implications. JAC Antimicrob Resist. 2023;5(6):dlad141. DOI: 10.1093/jacamr/dlad141; PMCID: PMC10733812; PMID: 38130703

39. Oladele R, Otu AA, Olubamwo O, Makanjuola OB, Ochang EA, Ejembi J, et al. Evaluation of knowledge and awareness of invasive fungal infections amongst resident doctors in Nigeria. Pan Afr Med J. 2020;36:297. DOI: 10.11604/pamj.2020.36.297.23279; PMCID: PMC7572690; PMID: 33117491

40. Johnson MD, Lewis RE, Ashley ESD, Ostrosky-Zeichner L, Zaoutis T, Thompson GR, et al. Core Recommendations for Antifungal Stewardship: A Statement of the Mycoses Study Group Education and Research Consortium. J Infect Dis. 2020;222(Suppl 3):S175-98. DOI: 10.1093/infdis/jiaa394; PMCID: PMC7403757; PMID: 32756879

41. Rabaan AA, Sulaiman T, Al-Ahmed SH, Buhaliqah ZA, Buhaliqah AA, AlYuosof B, et al. Potential Strategies to Control the Risk of Antifungal Resistance in Humans: A Comprehensive Review. Antibiotics. 2023;12(3):608. DOI: 10.3390/antibiotics12030608; PMCID: PMC10045400; PMID: 36978475

42. Kimbowa IM, Ocan M, Eriksen J, Nakafeero M, Obua C, Lundborg CS, et al. Characteristics of antimicrobial stewardship programmes in hospitals of Uganda. PLoS One. 2022;17(5):e0268032. DOI: 10.1371/journal.pone.0268032; PMCID: PMC9089898; PMID: 35536856

43. Adebisi YA, Alaran A, Badmos A, Bamisaiye AO, Emmanuella N, Etukakpan AU, et al. How West African countries prioritize health. Trop Med Health. 2021;49(1):87. DOI: 10.1186/s41182-021-00380-6; PMCID: PMC8547726; PMID: 34702370

44. Fang W, Wu J, Cheng M, Zhu X, Du M, Chen C, et al. Diagnosis of invasive fungal infections: challenges and recent developments. J Biomed Sci. 2023;30(1):42. DOI: 10.1186/s12929-023-00926-2; PMCID: PMC10278348; PMID: 37337179

45. Karajacob AS, Azizan NB, Al-Maleki ARM, Goh JPE, Loke MF, Khor HM, et al. Candida species and oral mycobiota of patients clinically diagnosed with oral thrush. PLoS One. 2023;18(4):e0284043. DOI: 10.1371/journal.pone.0284043; PMCID: PMC10109505; PMID: 37068057

46. Basu S, Copana R, Morales Jr R, Anugulreungkitt S, Puthanakit T, Maramba-Lazarte C, et al. Keeping It Real: Antibiotic Use Problems and Stewardship Solutions in Low- and Middle-income Countries. Pediatr Infect Dis J. 2022;41(3S):S18-25. DOI: 10.1097/inf.0000000000003321; PMCID: PMC8815843; PMID: 35134036

47. Osset-Trénor P, Pascual-Ahuir A, Proft M. Fungal Drug Response and Antimicrobial Resistance. J Fungi. 2023;9(5):565. DOI: 10.3390/jof9050565; PMCID: PMC10219139; PMID: 37233275

48. Li PKT, Chow KM, Cho Y, Fan S, Figueiredo AE, Harris T, et al. ISPD peritonitis guideline recommendations: 2022 update on prevention and treatment. Perit Dial Int. 2022;42(2):110-53. DOI: 10.1177/08968608221080586; PMID: 35264029

49. Wattal C, Chakrabarti A, Oberoi JK, Donnelly JP, Barnes RA, Sherwal BL, et al. Issues in antifungal stewardship: an opportunity that should not be lost. J Antimicrob Chemother. 2017;72(4):969-74. DOI: 10.1093/jac/dkw506; PMID: 27999053

50. Fisher MC, Denning DW. The WHO fungal priority pathogens list as a game-changer. Nat Rev Microbio. 2023;21(4):211-2. DOI: 10.1038/s41579-023-00861-x; PMCID: PMC9901396; PMID: 36747091

51. AMaghrabi RS, Al-Musawi T, Albaksami O, Subhi AL, Fakih RE, Stone NR. Challenges in the Management of Invasive Fungal Infections in the Middle East: Expert Opinion to Optimize Management Using a Multidisciplinary Approach. Cureus. 2023;15(8):e44356. DOI: 10.7759/cureus.44356; PMCID: PMC10539715; PMID: 37779746

52. Evans L, Rhodes A, Alhazzani W, Antonelli M, Coopersmith CM, French C, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. Intensive Care Med. 2021;47(11):1181-247. DOI: 10.1007/s00134-021-06506-y; PMCID: PMC8486643; PMID: 34599691

53. World Health Organization. WHO Global Strategy for Containment of Antimicrobial Resistance. Geneva; World Health Organization: 2001.

54. Pezzani MD, Mazzaferri F, Compri M, Galia L, Mutters NT, Kahlmeter G, et al. Linking antimicrobial resistance surveillance to antibiotic policy in healthcare settings: the COMBACTE-Magnet EPI-Net COACH project. J Antimicrob Chemother. 2020;75(Suppl 2):ii2-19. DOI: 10.1093/jac/dkaa425; PMCID: PMC7719409; PMID: 33280049

55. Seale AC, Gordon NC, Islam J, Peacock SJ, Scott JAG. AMR Surveillance in low and middle-income settings - A roadmap for participation in the Global Antimicrobial Surveillance System (GLASS). Wellcome Open Res. 2017;2:92. DOI: 10.12688/wellcomeopenres.12527.1; PMCID: PMC5645727; PMID: 29062918

56. McComick TS, Ghannoum M. Time to Think Antifungal Resistance Increased Antifungal Resistance Exacerbates the Burden of Fungal Infections Including Resistant Dermatomycoses. Pathog Immun. 2024;8(2):158-76. DOI: 10.20411/pai.v8i2.656; PMCID: PMC10939368; PMID: 38486922

57. Gow NAR, Johnson C, Berman J, Coste AT, Cuomo CA, Perlin DS, et al. The importance of antimicrobial resistance in medical mycology. Nat Commun. 2022;13:5352. DOI: 10.1038/s41467-022-32249-5

58. Bongomin F, Ekeng BE, Kwizera R, Salmanton-García J, Kibone W, van Rhijn N, et al. Fungal diseases in Africa: Closing the gaps in diagnosis and treatment through implementation research and advocacy. J Mycol Med. 2023;33(4):101438. DOI: 10.1016/j.mycmed.2023.101438; PMCID: PMC11103624; PMID: 38358796

59. Bahati F, Mcknight J, Swaleh F, Malaba R, Karimi L, Ramadhan M, et al. Reporting of diagnostic and laboratory tests by general hospitals as an indication of access to diagnostic laboratory services in Kenya. PLoS One. 2022;17(4):e0266667. DOI: 10.1371/journal.pone.0266667; PMCID: PMC8992978; PMID: 35395040

60. Stover KR, Hawkins BK, Keck JM, Barber KE, Cretella DA. Antifungal resistance, combinations and pipeline: oh my! Drugs Context. 2023;12:2023-7-1. DOI: 10.7573/dic.2023-7-1; PMCID: PMC10653594; PMID: 38021410

61. Beardsley J, Halliday CL, Chen SCA, Sorrell TC. Responding to the emergence of antifungal drug resistance: perspectives from the bench and the bedside. Future Microbiol. 2018;13(10):1175-91. DOI: 10.2217/fmb-2018-0059; PMCID: PMC6190174; PMID: 30113223

62. Niu X, Al-Hatmi AMS, Vitale RG, Lackner M, Ahmed SA, Verweij PE, et al. Evolutionary trends in antifungal resistance: a meta-analysis. Microbiol Spectr. 2024;12(4):e0212723. DOI: 10.1128/spectrum.02127-23; PMCID: PMC10986544; PMID: 38445857

63. Wulandari A, Hapsari R, Mujahidah, Sari D, Puspitasari I, Pramukarso DT. Antifungal susceptibility profile of Candida spp. causing candidemia in an Indonesian tertiary hospital. J Clin Microbiol Infect Dis. 2021;1(2):28-32. DOI: 10.51559/jcmid.v1i2.5

64. Seale AC, Hutchison C, Fernandes S, Stoesser N, Kelly H, Lowe B, et al. Supporting surveillance capacity for antimicrobial resistance: Laboratory capacity strengthening for drug resistant infections in low and middle income countries. Wellcome Open Res. 2017;2:91. DOI: 10.12688/wellcomeopenres.12523.1; PMCID: PMC5686477; PMID: 29181453

65. Fernandes R, Sabino R, Cunha C, Cornely OA, Carvalho A, Salmanton-García J, et al. Multicentric Study on the Clinical Mycology Capacity and Access to Antifungal Treatment in Portugal. Mycopathologia. 2024;189(1):15. DOI: 10.1007/s11046-024-00830-9; PMCID: PMC10808446; PMID: 38265528

Borneo Journal of Pharmacy
Vol 7 Issue 2 May 2024
Pages 198 – 205

https://journal.umpr.ac.id/index.php/bjop/article/view/6857
DOI: https://doi.org/10.33084/bjop.v7i2.6857
e-ISSN: 2621-4814

Research Article

Bromelain-Extracted of Virgin Coconut Oil: Physical and Chemical Stability in Different Temperature During the Storage

Sabtanti Harimurti [1*]
Dyani Primasari Sukamdi [1]
Hari Widada [1]
Hasna Fadia Sari [1]
Azura Amid [2]

[1] School of Pharmacy, Universitas Muhammadiyah Yogyakarta, Bantul, Special Region of Yogyakarta, Indonesia
[2] International Institute for Halal Research and Training (INHART), International Islamic University Malaysia, Kuala Lumpur, Federal Territory of Kuala Lumpur, Malaysia

*email: sabtanti@umy.ac.id; phone: +6285643842082

Keywords:
Ambient
Elevated temperature
Stability
Storage
VCO

Abstract

The bromelain-enzymatic reaction is applied in virgin coconut oil (VCO) production. Hydrolysis of the emulator by bromelain enzyme in coconut milk during fermentation maximized further the separation of oil and water. In the higher demand for VCO in many industries, the VCO stability during transportation and storage must be evaluated. The research aims to evaluate the physical and chemical stability of the effect of temperature in the storage. VCO's physical and chemical stability was evaluated under two different temperature and storage periods: an elevated temperature of 50°C for 10 days and room temperature (27-30°C) for 50 days. The storage was conducted in a clear glass bottle. The evaluation was based on physical and chemical stability tests before and after storage, including organoleptic, pH changes, density, viscosity, acid number, peroxide number, and saponification number. Based on the data, the quality of the VCO after storing at 50°C for 10 days and 27-30°C for 50 days was found to be changed for pH, specific gravity, viscosity, acid number, peroxide number, saponification number, while for the appearance was found to be no changes. The VCO was very sensitive to environmental effects. Therefore, it is necessary to find the best storage chamber and temperature for stabilizing the VCO.

Received: March 17th, 2024
1st Revised: May 14th, 2024
Accepted: May 21st, 2024
Published: May 30th, 2024

INTRODUCTION

Indonesia, a Southeast Asian nation characterized by its tropical climate, boasts a rich diversity of plant life. Among these readily available plants is the coconut (*Cocos nucifera*)[1]. Notably, all parts of the *C. nucifera* palm find utility, from the leaves and roots to the stems and fruit[2]. *Cocos nucifera* fruit, in particular, serves as the primary source of virgin coconut oil (VCO)[3,4]. Virgin coconut oil, renowned for its applications in both daily consumption and pharmaceutical contexts, undergoes various processing techniques to yield a product characterized by low water content, minimal free fatty acids, a clear appearance, a distinct aroma, and a shelf life exceeding 12 months[5-7]. Virgin coconut oil production utilizes various methods, including traditional, enzymatic, thermal, acidification, centrifugation, and inducement techniques[8-10] Among these, the enzymatic process offers distinct advantages.

Bromelain, an enzyme extracted from pineapples (*Ananas comosus*), plays a crucial role in this method. It accelerates the breakdown of the *C. nucifera* milk emulsion system by hydrolyzing peptide bonds, leading to oil separation[11,12]. *Ananas comosus*, a widely cultivated tropical fruit, is a rich source of bromelain, particularly in its yellow flesh[13]. While *A. comosus* are known for their high water content (approximately 90%) and essential minerals like potassium, calcium, iodine, sulfur, and chlorine[14], bromelain emerges as the key component for VCO production due to its efficient oil separation properties.

Virgin coconut oil has gained significant popularity due to its perceived health benefits. Consequently, ensuring the consistent delivery of high-quality VCO to consumers is paramount[15]. However, the quality of VCO can deteriorate during

How to cite: Harimurti S, Sukamdi DP, Widada H, Sari HF, Amid A. Bromelain-Extracted of Virgin Coconut Oil: Physical and Chemical Stability in Different Temperature During the Storage. Borneo J Pharm. 2024;7(2):198-205. doi:10.33084/bjop.v7i2.6857

transportation and storage[16]. During transport, exposure to elevated temperatures exceeding 50°C can occur within vehicles[17]. Similarly, storage conditions are often inconsistent, leading to temperature fluctuations[18]. Several studies have documented that unstable storage temperatures contribute to a decline in VCO quality[19,20]. Therefore, research investigating the stability of VCO produced using the bromelain enzymatic method is crucial to establishing accurate expiration dates. This research should encompass a battery of stability parameters, including organoleptic tests (color, odor, and taste), changes in pH, specific gravity, viscosity, acid number, peroxide number, and saponification number.

MATERIALS AND METHODS

Materials

The instruments used include equipment for VCO production and quality analysis. Virgin coconut oil production equipment typically included blenders, spatulas, chambers, analytical balances, measuring cylinders, and clear glass bottles. Quality analysis equipment used in this study included Erlenmeyer flasks, beakers, measuring flasks, conical flasks, porcelain cups, glass funnels, weighing bottles, analytical balances, stirrers, ovens, pH meters, pycnometers, cooling baths, and Brookfield viscometers.

Cocos nucifera and *A. comosus* were purchased from the Gamping traditional market in Yogyakarta, Indonesia. The scientific identification of these raw materials was confirmed by the Biological Education Laboratory, Faculty of Science and Applied Technology, Universitas Ahmad Dahlan, Yogyakarta (reference numbers: 454/Lab.Bio/B/XI/2023 for *A. comosus* and 474/Lab.Bio/B/XII/2023 for *C. nucifera*). For the quality analysis of VCO, analytical grade chemicals were used without further purification. These chemicals included distilled water, 95% ethanol, standardized NaOH, oxalic acid, standardized KOH, 0.5 N alcoholic KOH, phenolphthalein indicator, HCl, acetic acid, chloroform, sodium thiosulfate, potassium dichromate, and starch powder. All analytical grade chemicals were purchased from Merck, Germany.

Methods

Preparation of VCO

Raw materials were weighed according to the experimental design. Grated *C. nucifera* flesh was added to distilled water at a 1 : 1 ratio. The mixture was then homogenized and filtered to obtain *C. nucifera* milk. The *C. nucifera* milk was stored in a transparent container at room temperature to allow for separation into cream and skim layers. After separation, the top layer (*C. nucifera* cream) was carefully collected using a sterile pipette to avoid contamination from the lower skim layer (water). Fresh *A. comosus* fruits were peeled, washed, and blended using a sanitized blender. The homogenized mixture was then filtered to obtain clarified *A. comosus* juice.

Cocos nucifera cream and *A. comosus* juice were mixed at a 10 : 1 ratio and homogenized to ensure a uniform mixture. The homogenized mixture was stored in a transparent container at room temperature for 24 hours to allow for phase separation into three distinct layers: water (bottom layer), VCO (middle layer), and residue (top layer). The VCO layer was carefully separated using a sterile straw inserted into the middle of the container. The collected VCO was then analyzed for fatty acid composition using gas chromatography-mass spectrometry (GC-MS). Fatty acid composition analysis was performed using a GC-MS system equipped with a 30-meter DB-5MS column. Helium gas served as the mobile phase at a flow rate of 33.2 mL/minute. The injection port temperature was set to 300°C, and the initial column temperature was maintained at 70°C.

Data analysis

This study evaluated the physical and chemical stability of VCO in two storage conditions: room temperature (27-30°C) for 50 days and high temperature (50°C) for 10 days. The stability test was determined through various tests, including:

1. Organoleptic evaluation: This involved assessing color, odor, and taste of the VCO samples.
2. pH measurement: A pH meter was used to measure changes in the acidity or alkalinity of the VCO during storage.
3. Specific gravity determination: A pycnometer was employed to measure the density of the VCO samples.
4. Viscosity measurement: A Brookfield viscometer was used to assess changes in the viscosity of the VCO over time.
5. Chemical analysis: This included determination of acid number, peroxide number, and saponification number, following standard methods[21]. It's important to note that all analyses were performed at room temperature.

RESULTS AND DISCUSSION

Our previous study[22] has demonstrated that incorporating bromelain extract from *A. comosus* juice into the processing of VCO can significantly increase yield. In these studies, 1000 mL of *C. nucifera* milk yielded an average of 300 mL of VCO when combined with 100 mL of *A. comosus* juice. The bromelain enzyme in *A. comosus* juice is hypothesized to hydrolyze proteins within the *C. nucifera* milk during fermentation. This process disrupts the emulsion of oil and water in the *C. nucifera* milk, potentially maximizing oil extraction and leading to a higher VCO yield compared to traditional methods without bromelain supplementation.

Gas chromatography is a well-established technique for analyzing fatty acid methyl esters (FAMEs) in oils. It separates components in a mixture based on their interactions with the mobile (gas carrier) and stationary phases within the GC column[23]. The method offers rapid separation, typically within seconds, as the gas flow carries vaporized samples through the column. The separated components are detected, and a chromatogram (a graphical representation of detector response versus time) is generated. The specific characteristics of the GC instrument, including column dimensions (length and diameter) and operating conditions (gas flow rate and oven temperature), influence the separation and retention times of the FAMEs[24].

Our GC analysis of the VCO sample identified several fatty acids. These included caproic acid (retention time: 7.3 minutes), caprylic acid (14.2 minutes), capric acid (20.6 minutes), lauric acid (26.3 minutes, highest peak), myristic acid (31.2 minutes), palmitic acid (35.7 minutes), linoleic acid (39.1 minutes), oleic acid (39.2 minutes), and stearic acid (39.7 minutes). The chromatogram is presented in **Figure 1**, and **Table I** summarizes the identified fatty acids and their corresponding retention times. As evident from the highest peak in **Figure 1** (peak number 4), lauric acid was the most abundant fatty acid in the VCO sample. The peak height in a chromatogram generally corresponds to the relative concentration of the component in the sample. This finding aligns with Indonesian National Standard (*Standar Nasional Indonesia*; SNI) 7381-2022 for VCO, which specify lauric acid as the predominant fatty acid[21].

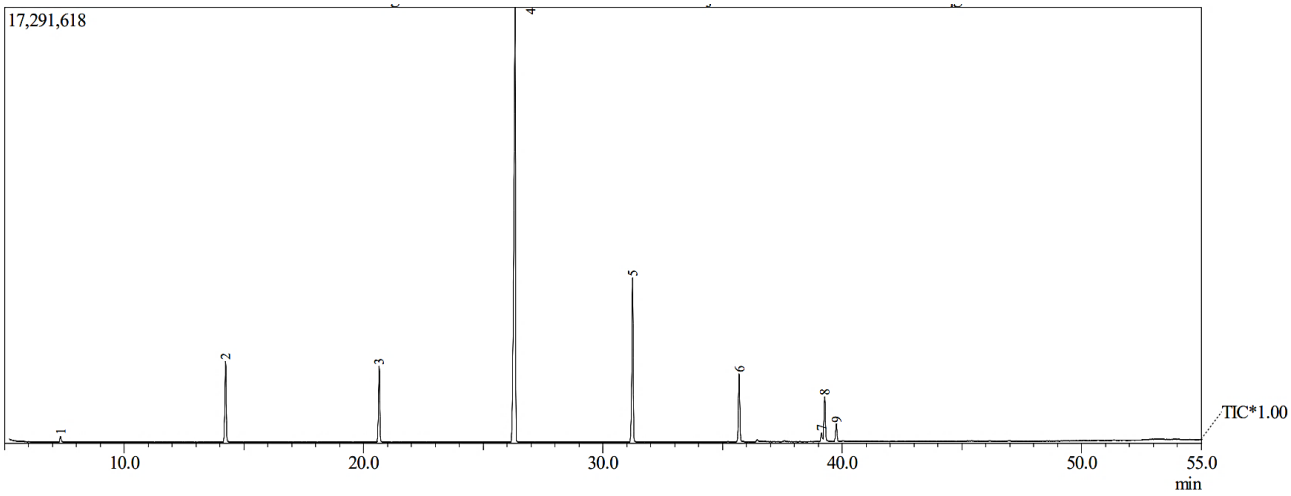


Figure 1. GC-MS Chromatogram of bromelain-extracted VCO.

Table I. Summarized fatty acids of bromelain-extracted VCO.

Peak	Rt (minutes)	Fatty acid
1	7.3	Caproic acid
2	14.2	Caprylic acid
3	20.6	Capric acid
4	26.3	Lauric acid
5	31.2	Myristic acid
6	35.7	Palmitic acid
7	39.1	Linoleic acid
8	39.2	Oleic acid
9	39.7	Stearic acid

The stability of the emulgel formulations was assessed through organoleptic evaluation, pH measurement, specific gravity determination, viscosity measurement, acid value testing, peroxide value testing, and saponification value testing. **Table II** summarizes the changes observed in these stability indicators during storage at room temperature (25-27°C) for 50 days and at 50°C for 10 days. Organoleptic evaluation was performed to assess the color, odor, and taste of the VCO samples. Fresh VCO is characterized by a clear yellowish color, a pleasant *C. nucifera* odor, and a savory *C. nucifera* taste[21]. The VCO produced in this study maintained these characteristics throughout the storage period, with no significant changes observed in color, odor, or taste between the VCO before treatment and the samples stored for ten days at 50°C, 50 days at room temperature (27-30°C), or both. The resulting taste still tastes savory *C. nucifera* without a stale taste even though it has been in extreme temperatures and has been stored for a long time. These findings indicate that the VCO formulation exhibits good stability in terms of organoleptic properties.

The pH of VCO was measured to assess its stability during storage. A pH meter was used to determine the initial pH of VCO, which was 5.857. Virgin coconut oil samples stored at 50°C for 10 days exhibited a slight decrease in pH to 5.590. Similarly, samples stored at room temperature for 50 days showed a decrease in pH to 5.598. These observations suggest a potential decrease in VCO stability during storage, particularly at elevated temperatures. The decrease in pH may be attributed to the hydrolysis of triglycerides in VCO into free fatty acids, which are acidic in nature[20]. Further studies are needed to investigate the extent of free fatty acid formation and its impact on other quality parameters of VCO during storage.

Specific gravity, defined as the ratio of a substance's density to that of water at the same temperature, is an indicator of oil quality. The standard range for VCO specific gravity is 0.9150-0.9244 g/cm^3[21]. Our analysis revealed a specific gravity of 0.9235 g/cm^3 for the VCO before treatment. This value falls within the established acceptable range. However, a significant decrease in specific gravity was observed after storage, with values of 0.8939 and 0.9023 g/cm^3 for samples stored at 50°C for 10 days and at room temperature for 50 days, respectively. This decrease suggests potential instability during storage. The observed decrease in specific gravity during storage aligns with the notion that hydrolysis reactions are accelerated at higher temperatures[25]. These reactions break down triglycerides, releasing glycerol and free fatty acids. Glycerol has a higher density than most common fatty acids found in VCO. Therefore, as hydrolysis progresses, the overall density of the oil decreases, leading to a lower specific gravity[26]. This highlights the importance of proper storage conditions to maintain VCO quality.

Viscosity, a measure of a fluid's resistance to flow, was determined using a Brookfield viscometer. This instrument utilizes a rotating spindle at a specific speed to measure the resistance of the sample[27]. The appropriate spindle selection depends on the sample's viscosity; higher spindle numbers correspond to smaller physical dimensions and are suitable for testing thicker, more viscous materials[28]. In our study, three key factors influenced viscosity measurements: spindle speed (RPM), torque range (scale), and spindle number[27]. The viscosity of the untreated VCO was 13.8 cP. VCO stored at 50°C for 10 days and at room temperature for 50 days exhibited viscosities of 13.6 and 13.5 cP, respectively. These observations suggest a decrease in VCO viscosity during storage, potentially indicating a form of instability. The observed decrease in viscosity may be attributed to the hydrolysis process occurring during storage.

The acid number, expressed as mg of base (NaOH) required to neutralize free fatty acids present in 1 g of oil, serves as an indicator of oil quality. Higher acid numbers reflect increased free fatty acid content, which can arise from hydrolysis, heating, enzymatic reactions, or physical processing[19]. In this study, untreated VCO exhibited an acid number of 0.48 mg NaOH/g. Notably, storage at elevated (50°C for 10 days) and room temperatures (for 50 days) resulted in modest increases in acid number to 0.72 and 0.56 mg NaOH/g, respectively. While these increases suggest a slight decrease in VCO stability during storage, the overall changes were relatively small. These findings are consistent with previous studies[29,30], which report that VCO generally exhibits good stability due to its high content of lauric acid, a saturated fatty acid resistant to hydrolysis. However, prolonged storage at elevated temperatures can accelerate hydrolysis, leading to a gradual increase in free fatty acid content and acid number.

Peroxide number is a well-established indicator of oil damage caused by oxidation. It reflects the concentration of peroxides formed during the oxidation process, expressed as milliequivalents (meq) of active oxygen per kg of oil. As observed in this study, an increase in peroxide number indicates ongoing oxidation within the oil. The free radical chain reaction initiated by the interaction between oxygen and double bonds in the fatty acids progressively generates peroxides[31,32]. However, a low peroxide number doesn't necessarily imply minimal oxidation. The fatty acid composition of the oil also influences its

peroxide number[33]. The peroxide number of untreated VCO in our study was 1.8 meq O_2/kg. Storage at 50°C for 10 days increased the peroxide number to 5.6 meq O_2/kg, and storage at room temperature for 50 days resulted in a peroxide number of 4.8 meq O_2/kg. Notably, both storage conditions (room temperature for 50 days and 50°C for 10 days) yielded peroxide number values exceeding SNI 7381-2022 for VCO (20 meq O_2/kg)[21]. This observation aligns with the established notion that higher storage temperatures accelerate oil oxidation, leading to increased peroxide numbers[34]. Our findings suggest that storing VCO in a cool environment is crucial to maintaining its quality and preventing excessive oxidation.

The saponification number, which reflects the amount of KOH required to saponify 1 g of oil, is inversely proportional to the oil's molecular weight. This means that oils with higher saponification numbers contain shorter fatty acid chains. Shorter chain fatty acids are generally considered more resistant to oxidation and hydrolysis, contributing to increased oil stability and reduced susceptibility to rancidity[35]. Our study investigated the changes in the saponification number of VCO following storage at different temperatures. The initial saponification number of the VCO was 258.06 mg KOH/g. After storage at 50°C for 10 days, the saponification number decreased to 246.84 mg KOH/g. Similarly, storage at room temperature (27-30°C) for 50 days resulted in a decrease in the saponification number to 240.66 mg KOH/g. These observations are consistent with the established relationship between saponification number and oil stability. The decrease in saponification number following storage suggests a potential breakdown of triglycerides in the VCO, likely due to hydrolysis or oxidation reactions. This breakdown could result in the formation of shorter chain fatty acids, contributing to the observed decrease in saponification number[36]. However, further investigation is needed to confirm the specific nature of the ongoing chemical reactions during storage.

Table II. Summarized value of stability test.

Description	Value		
	Initial	**Storage at 25-27°C for 50 days**	**Storage at 50°C for 10 days**
Smell, taste, and color	Specific *C. nucifera* smell, oily, and yellowish white	Specific *C. nucifera* smell, oily, and yellowish white	Specific *C. nucifera* smell, oily, and yellowish white
pH	5.857±0.024	5.598±0.011	5.59±0.01
Specific gravity (g/cm^3)	0.924±0.013	0.902±0.03	0.894±0.024
Viscosity (Cp)	13.8±0.076	13.6±0.038	13.6±0.076
Acid number (mg NaOH/g)	0.48±0.01	0.56±0.005	0.72±0.015
Peroxide number (meq O_2/kg)	1.8±0.017	4.8±0.05	5.6±0.066
Saponification number (mg KOH/g)	258.06±0.106	240.66±0.131	246.84±0.045

CONCLUSION

This study investigated the influence of storage temperature and duration on the quality of VCO. The results revealed significant changes (pH, specific gravity, viscosity, acid number, peroxide number, and saponification number) in VCO stored at 50°C for 10 days compared to those stored at room temperature (27-30°C) for 50 days. Notably, the appearance of the VCO remained unchanged across all storage conditions. These findings suggest that VCO is highly sensitive to elevated temperatures. Further research is warranted to identify optimal storage conditions that effectively preserve the quality of VCO.

ACKNOWLEDGMENT

Funds for this research came from a grant from Universitas Muhammadiyah Yogyakarta with No. 16/R-LRI/I/2023.

AUTHORS' CONTRIBUTION

Conceptualization: Sabtanti Harimurti
Data curation: Sabtanti Harimurti, Hasna Fadia Sari
Formal analysis: Sabtanti Harimurti, Hasna Fadia Sari
Funding acquisition: Sabtanti Harimurti

Investigation: Sabtanti Harimurti, Hasna Fadia Sari
Methodology: Sabtanti Harimurti, Hasna Fadia Sari
Project administration: Dyani Primasari Sukamdi, Hari Widada
Resources: Sabtanti Harimurti, Hasna Fadia Sari
Software: Sabtanti Harimurti, Hasna Fadia Sari
Supervision: Dyani Primasari Sukamdi, Hari Widada
Validation: Hari Widada, Azura Amid
Visualization: Sabtanti Harimurti
Writing - original draft: Sabtanti Harimurti
Writing - review & editing: Hari Widada, Azura Amid

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Pawera L, Khomsan A, Zuhud EAM, Hunter D, Ickowitz A, Polesny Z. Wild Food Plants and Trends in Their Use: From Knowledge and Perceptions to Drivers of Change in West Sumatra, Indonesia. Foods. 2020;9(9):1240. DOI: 10.3390/foods9091240; PMCID: PMC7555794; PMID: 32899857

2. Maluin FN, Hussein MZ, Idris AS. An Overview of the Oil Palm Industry: Challenges and Some Emerging Opportunities for Nanotechnology Development. Agronomy. 2020;10(3):356. 2020 Mar;10(3):356. DOI: 10.3390/agronomy10030356

3. Dacasin AB, Diagono DMB, So PKN, Bautista VRL, Bucu MLB, Grano RVR De, et al. The potential use of virgin coconut oil as an adjunctive treatment for COVID-19: A review. J Pharmacogn Phytochem. 2021;10(6):37-49. DOI: 10.22271/phyto.2021.v10.i6a.14254

4. Patil U, Benjakul S. Coconut Milk and Coconut Oil: Their Manufacture Associated with Protein Functionality. J Food Sci. 2018;83(8):2019–27. DOI: 10.1111/1750-3841.14223; PMID: 30004125

5. Hosny KM, Alhakamy NA, Sindi AM, Khallaf RA. Coconut Oil Nanoemulsion Loaded with a Statin Hypolipidemic Drug for Management of Burns: Formulation and In Vivo Evaluation. Pharmaceutics. 2020;12(11):1061. DOI: 10.3390/pharmaceutics12111061; PMCID: PMC7695003; PMID: 33171816

6. Rohman A. Infrared spectroscopy for quantitative analysis and oil parameters of olive oil and virgin coconut oil: A review. Int J Food Prop. 2017;20(7):1447–56. DOI: 10.1080/10942912.2016.1213742

7. Risakotta MYS, Salamena GA, Loupatty G. Physicochemical characteristics analysis of virgin coconut oil from immersion and fermentation methods. AIP Conf Proceed. 2021;2360(1):050004. DOI: 10.1063/5.0059691

8. Ng YJ, Tham PE, Khoo KS, Cheng CK, Chew KW, Show PL. A comprehensive review on the techniques for coconut oil extraction and its application. Bioprocess Biosyst Eng. 2021;44(9):1807-18. DOI: 10.1007/s00449-021-02577-9; PMCID: PMC8132276; PMID: 34009462

9. Mujdalipah S. Pengaruh Ragi Tradisional Indonesia dalam Proses Fermentasi Santan Terhadap Karakteristik Rendemen, Kadar Air, dan Kadar Asam Lemak Bebas Virgin Coconut Oil (VCO). Edufortech. 2016;1(1):10-5. DOI: 10.17509/edufortech.v1i1.3969

10. Soetikno JS. Prospects of Coconut Biodiversity for Safe, Healthy, and Sustainable Edible Oil Discovery. In: Sangeetha J, Thangadurai D, Ching GH, Islam S, editors. Biodiversity and Conservation: Characterization and Utilization of Plants, Microbes and Natural Resources for Sustainable Development and Ecosystem Management. New York: Apple Academic Press; 2019. p.151–206. DOI: 10.1201/9780429425790

11. Adi S, Prayitno SA. The Physical and Chemical Properties of Virgin Coconut Oil (VCO) Product Obtained Through Fermentation and Enzymatic. Food Sci Technol J. 2019;2(1):1–6. DOI: 10.25139/fst.v2i1.1590

12. Rahmalia I, Kusumayanti H. The Optimization of Addition of Bromelain Enzyme Catalyst on the Fermentation of Coconut Milk to VCO (Virgin Coconut Oil) Using Tempeh Yeast. J Voc Stud Appl Res. 2021;3(2):31-7. DOI: 10.14710/jvsar.v3i2.11949

13. Alarcon RT, Lamb KJ, Bannach G, North M. Opportunities for the Use of Brazilian Biomass to Produce Renewable Chemicals and Materials. ChemSusChem. 2021;14(1):169–88. DOI: 10.1002/cssc.202001726; PMID: 32975380

14. Santos DI, Martins CF, Amaral RA, Brito L, Saraiva JA, Vicente AA, et al. Pineapple (Ananas comosus L.) By-Products Valorization: Novel Bio Ingredients for Functional Foods. Molecules. 2021;26(11):3216. DOI: 10.3390/molecules26113216; PMCID: PMC8198275; PMID: 34072026

15. Mohammed NK, Samir ZT, Jassim MA, Saeed SK. Effect of different extraction methods on physicochemical properties, antioxidant activity, of virgin coconut oil. Mater Today Proceed. 2021;42(4):2000–5. DOI: 10.1016/j.matpr.2020.12.248

16. Srivastava Y, Semwal AD. A study on monitoring of frying performance and oxidative stability of virgin coconut oil (VCO) during continuous/prolonged deep fat frying process using chemical and FTIR spectroscopy. J Food Sci Technol. 2015;52(2):984–91. DOI: 10.1007/s13197-013-1078-8; PMCID: PMC4325013; PMID: 25694709

17. Mitchell MA, Kettlewell PJ. Physiological stress and welfare of broiler chickens in transit: solutions not problems! Poult Sci. 2998;77(12):1803-14. DOI: 10.1093/ps/77.12.1803; PMID: 9872583

18. Dimzon IKD, Valde MF, Santos JER, Garrovillas MJM, Dejarme HM, Remollo JMW, et al. Physico-Chemical and Microbiological Parameters in the Deterioration of Virgin Coconut Oil. Philipp J Sci. 2011;140(1):89–103.

19. Srivastava Y, Semwal AD, Sajeevkumar VA, Sharma GK. Melting, crystallization and storage stability of virgin coconut oil and its blends by differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR). J Food Sci Technol. 2017;54(1):45–54. DOI: 10.1007/s13197-016-2427-1; PMCID: PMC5305700; PMID: 28242902

20. Villarino CBJ, Basinang ARP, Velasquez MMM, Pagulayan JMDG, Ong PKA, Lizada MaCC. Descriptive Aroma Changes in Selected Philippine Virgin Coconut Oil (VCO) during Storage at Elevated Temperatures. Proceedings. 2021;70(1):111. DOI: 10.3390/foods_2020-07736

21. Standar Nasional Indonesia. SNI 7381:2022 - Minyak Kelapa Virgin (VCO). Jakarta: Badan Standardisasi Nasional; 2022.

22. Harimurti S, Susanawati, Sukamdi DP, Krisridwany A, Widada H, Nadhifa N, et al. Green Technology on The Virgin Coconut Oil Production Using Enzyme from Pineapple Waste. Indones J Pharm. 2022;33(3):412-21. DOI: 10.22146/ijp.1133

23. Miksusanti M, Herlina H, Annuria FN, Zulhijjah Z. Patch Film from Celullose Derivative Incorporating with Virgin Coconut Oil and its Physical and Antibacterial Properties. Key Eng Mater. 2020;840(1):351–9. DOI: 10.4028/www.scientific.net/KEM.840.351

24. Gruber B, David F, Sandra P. Capillary gas chromatography-mass spectrometry: Current trends and perspectives. Trends Analyt Chem. 2020;124:115475. DOI: 10.1016/j.trac.2019.04.007

25. Roni KA, Rifdah R, Melani A, Reformis AAI, Sri SM. Making Virgin Coconut Oil (VCO) With Enzymatic Method Using Pineapple Hump Extract. Int J Sci Technol Manag. 2022;3(3):685–9. DOI: 10.46729/ijstm.v3i3.516

26. Dayrit FM, Dimzon IKD, Valde MF, Santos JER, Garrovillas MJM, Villarino BJ. Quality characteristics of virgin coconut oil: Comparisons with refined coconut oil. Pure Appl Chem. 2011;83(9):1789-99. DOI: 10.1351/PAC-CON-11-04-01

27. Faizal WM, Ghazali NNN, Khor CY, Badruddin IA, Zainon MZ, Yazid AA, et al. Computational fluid dynamics modelling of human upper airway: A review. Comput Methods Programs Biomed. 2020;196:105627. DOI: 10.1016/j.cmpb.2020.105627; PMCID: PMC7318976; PMID: 32629222

28. Sieben M, Hanke R, Büchs J. Contact-free determination of viscosity in multiple parallel samples. Sci Rep. 2019;9(1):8335. DOI: 10.1038/s41598-019-44859-z; PMCID: PMC6554296; PMID: 31171822

29. Deen A, Visvanathan R, Wickramarachchi D, Marikkar N, Nammi S, Jayawardana BC, et al. Chemical composition and health benefits of coconut oil: an overview. J Sci Food Agric. 2021;101(6):2182-93. DOI: 10.1002/jsfa.10870; PMID: 33022082

30. Ghani NAA, Channip AA, Hwa PCH, Ja'afar F, Yasin HM, Usman A. Physicochemical properties, antioxidant capacities, and metal contents of virgin coconut oil produced by wet and dry processes. Food Sci Nutr. 2018;6(5):1298-306. DOI: 10.1002/fsn3.671; PMCID: PMC6060898; PMID: 30065831

31. Jia W, Wu X, Kang X. Integrated the embedding delivery system and targeted oxygen scavenger enhances free radical scavenging capacity. Food Chem X. 2023;17:100558. DOI: 10.1016/j.fochx.2022.100558; PMCID: PMC9943856; PMID: 36845467

32. Juita, Dlugogorski BZ, Kennedy EM, Mackie JC. Roles of peroxides and unsaturation in spontaneous heating of linseed oil. Fire Saf J. 2013;61:108-15. DOI: 10.1016/j.firesaf.2013.07.005

33. Nurhasanah S, Wulandari N, Munarso SJ, Hariyadi P. Stabilitas Oksidasi Lipida Terstruktur Berbasis Minyak Kelapa dan Minyak Kelapa Sawit. Buletin Palma. 2017;18(2):53–62.

34. Liu K, Liu Y, Chen F. Effect of storage temperature on lipid oxidation and changes in nutrient contents in peanuts. Food Sci Nutr. 2019;7(7):2280-90. DOI: 10.1002/fsn3.1069; PMCID: PMC6657719; PMID: 31367356

35. Flores M, Avendaño V, Bravo J, Valdés C, Forero-Doria O, Quitral V, et al. Edible Oil Parameters during Deterioration Processes. Int J Food Sci. 2021;2021:7105170. DOI: 10.1155/2021/7105170; PMCID: PMC8463213; PMID: 34568484

36. Vidal NP, Adigun OA, Pham TH, Mumtaz A, Manful C, Callahan G, et al. The Effects of Cold Saponification on the Unsaponified Fatty Acid Composition and Sensory Perception of Commercial Natural Herbal Soaps. Molecules. 2018;23(9):2356. DOI: 10.3390/molecules23092356; PMCID: PMC6225244; PMID: 30223479

Borneo Journal of Pharmacy
Vol 7 Issue 2 May 2024
Pages 206 – 214

https://journal.umpr.ac.id/index.php/bjop/article/view/6872
DOI: https://doi.org/10.33084/bjop.v7i2.6872
e-ISSN: 2621-4814

Research Article

Ethnobotanical Study and Hedonic Evaluation with Cost Analysis of Banana (*Musa paradisiaca* L.) Stem Serum Preparation as an Anti-Aging Solution

Fitria Megawati*

Ni Luh Kade Arman Anita Dewi

Ni Putu Dewi Agustini

I Putu Satria Antara

Ni Luh Firda Ekayanti

Ni Wayan Darmayanti

Department of Pharmacy, Universitas Mahasaraswati Denpasar, Denpasar, Bali, Indonesia

*email: fitriamega83@unmas.ac.id; phone: +6281229913010

Keywords:
Cost
Hedonic
Musa paradisiaca

Abstract

In skincare, particularly facial serums, the utilization of natural ingredients is crucial in addressing various skin issues, notably combating the effects of free radicals that contribute to wrinkles. This study explores the potential of banana (*Musa paradisiaca* L.) stem extract as an active ingredient in serum formulation due to its rich anthocyanin content known for antioxidant properties. Hedonic testing and cost analysis serve as initial steps in the marketing mix of a product. This research aims to assess the hedonic preferences of various *M. paradisiaca* stem serum formulations and analyze the production costs. A descriptive research method employing survey and observational techniques was utilized for data collection and descriptive analysis. Hedonic testing was conducted to gauge the personal preferences of panelists toward *M. paradisiaca* stem serum formulations. Three formulations (F1 with 4%, F2 with 8%, and F3 with 12% *M. paradisiaca* extract) were evaluated by 40 panelists. Cost analysis of serum production employed quantitative descriptive analysis, computing the cost per unit using a variable costing method. The hedonic evaluation results showed that F1 formulation was highly preferred (79.3%), followed by F2 (73.2%), and F3 (66.8%). Cost analysis using the variable costing method revealed a total production cost of IDR 614,000.00 for 10 packages of *M. paradisiaca* stem serum formulation, translating to an approximate unit price of IDR 61,400.00.

Received: March 20th, 2024
1st Revised: May 14th, 2024
Accepted: May 21st, 2024
Published: May 30th, 2024

INTRODUCTION

The human skin, serving as the body's outermost barrier, is constantly exposed to environmental stressors. Chief among these are ultraviolet (UV) radiation and pollutants, both of which can inflict significant damage[1]. Furthermore, daily activities can contribute to the development of skin concerns, particularly in the facial area[2]. Improper facial cleansing and skincare routines can lead to the accumulation of dead skin cells, potentially hindering collagen production and ultimately accelerating the formation of wrinkles and fine lines[3].

Antioxidants have emerged as promising tools in the fight against skin aging and damage[4]. These molecules act by neutralizing free radicals, a class of highly reactive species containing unpaired electrons in their outer shells. Free radicals' inherent instability compels them to seek stability by stealing electrons from surrounding molecules, thereby causing cellular damage and contributing to various skin concerns[5]. By donating electrons and stabilizing free radicals, antioxidants offer protection against this cellular damage[6].

How to cite: Megawati F, Dewi NLKAA, Agustini NPD, Antara IPS, Ekayanti NLF, Darmayanti NW. Ethnobotanical Study and Hedonic Evaluation with Cost Analysis of Banana (Musa paradisiaca L.) Stem Serum Preparation as an Anti-Aging Solution. Borneo J Pharm. 2024;7(2):206-214. doi:10.33084/bjop.v7i2.6872

Natural antioxidants have gained significant interest for their potential benefits in topical skincare formulations. Topical application of antioxidants can offer protection against UVA radiation, a key contributor to skin darkening and premature aging[7]. These antioxidants can also help combat the damaging effects of UVB radiation, including wrinkles and inflammation[8]. Moreover, serums formulated with natural ingredients are generally considered safer for consumers compared to synthetic alternatives[9]. This study investigates the development of an antioxidant serum derived from banana (*Musa paradisiaca*) extracts as a potential option to shield the skin from free radicals and their associated signs of aging.

Musa paradisiaca thrives in tropical climates, making Indonesia a prime location for its cultivation[10]. Interestingly, *M. paradisiaca* stems hold potential as a natural antibiotic, and their use in wound healing is a traditional practice among some Indonesian communities[11]. Scientific studies have corroborated these traditional practices, demonstrating the effectiveness of *M. paradisiaca* stem extracts in inhibiting the growth of pathogenic bacteria like *Staphylococcus aureus*[12,13]. Building upon Dewi *et al*.[14] on formulating *M. paradisiaca* stem extract into serum preparations and evaluating their physical and antioxidant properties, this study aims to investigate two key aspects. Firstly, we assess the organoleptic properties of the serum preparation, including taste, aroma, and color, to gauge its acceptability among potential users. Secondly, we analyze the production costs associated with *M. paradisiaca* stem serum preparations.

MATERIALS AND METHODS

Materials

Musa paradisiaca stems were obtained from East Duda Village, Selat, Karangasem, Bali, Indonesia (**Figure 1**). The botanical identification was confirmed by the Plant Conservation Center of the Indonesian Institute of Sciences (LIPI), Eka Bedugul Botanical Gardens (voucher specimen number B-337/IPH.7/AP/XII/2020). Other materials used in this study included 70% ethanol (Brataco®), xanthan gum, glycerin, potassium sorbate, sodium benzoate, distilled water, and DPPH (2,2-diphenyl-1-picrylhydrazyl). The following equipment was used in this research included rotary evaporator, glassware (Pyrex®), flannel cloth, gram scale (accuracy 0.1 g), digital analytical balance (accuracy 0.0001 g), and glass jar. A questionnaire was developed to assess the level of acceptance of different *M. paradisiaca* stem serum formulations by participants. Additionally, the questionnaire aimed to gather information regarding production costs.

Figure 1. *Musa paradisiaca.*

Methods

Preparation of extracts and phytochemical screening

Musa paradisiaca stems were thoroughly sorted and chopped into small pieces. The chopped *M. paradisiaca* stem was then subjected to maceration extraction using 70% ethanol as the solvent. The maceration process was carried out for three cycles of 24 hours each, with occasional stirring. The macerate was stored in a light-protected environment throughout the extraction period. Following each maceration cycle, the mixture was filtered using a flannel cloth to separate the filtrate

(extract) from the residue. The remaining residue was then re-extracted with fresh 70% ethanol using the same maceration procedure. The combined filtrates from all maceration cycles were concentrated using a rotary evaporator under vacuum until a thick extract was obtained.

Preparation of serum

An oil-in-water (O/W) type serum formulation was prepared as detailed in **Table I**. Xanthan gum was dispersed in 20 parts of distilled water under constant stirring until an emulsion base formed. Glycerin was then gradually added to the mixture with continuous stirring. Potassium sorbate, sodium benzoate, and *M. paradisiaca* stem extract were incorporated sequentially into the mixture with continued slow stirring until a homogeneous product was obtained. Finally, distilled water was added to reach a final volume of 100 mL while stirring continuously. The homogeneous serum (**Figure 2**) was then stored in a pre-prepared container.

Table I. *Musa paradisiaca* stem serum formulation.

Material Name	Function	Concentration (%)		
		F1	F2	F3
Musa paradisiaca stem extract	Active ingredients	4	8	12
Xanthan gum	Thickener	0.5	0.5	0.5
Glycerin	Humectant	10	10	10
Potassium sorbate	Preservative	0.1	0.1	0.1
Sodium benzoate	Preservative	0.1	0.1	0.1
Distilled water	Solvent	Ad 100	Ad 100	Ad 100

a **b** **c**

Figure 2. *Musa paradisiaca* stem serum F1 (**a**), F2 (**b**), and F3 (**c**).

Serum evaluation

The physical properties of the *M. paradisiaca* stem extract serum were assessed using the following methods: organoleptic test, transferred volume test, pH test, and homogeneity test[15], as described in detail below.

Organoleptic test: The organoleptic properties of *M. paradisiaca* stem serum preparations were assessed visually. This evaluation included observations of consistency, color, and smell.

Transferred volume test: The transferred volume test is performed to ensure that the *M. paradisiaca* stem extract serum retains its designated dosage volume (100 mL) after transfer from its original container. This test verifies that at least 95% of the prepared serum volume can be effectively transferred. The test involves transferring the entire *M. paradisiaca* stem extract serum preparation to a measuring cup. The transferred volume is then measured, and the percentage of the initial volume is calculated.

pH test: The acidity of the *M. paradisiaca* stem extract serum was determined using a pH meter. Five milliliters of the extract serum were transferred to a clean, dry beaker. The pH meter electrode was immersed into the sample solution, ensuring good contact without stirring. The pH reading was allowed to stabilize and recorded. The electrode was then rinsed thoroughly with deionized water and patted dry with a lint-free tissue before further measurements.

Homogeneity test: The homogeneity of *M. paradisiaca* stem extract serum was assessed visually. A small amount of the extract serum was placed on two clean glass slides. The presence or absence of coarse particles on both slides was evaluated to

determine homogeneity. The extract serum was considered homogeneous if no coarse particles were observed on either slide. Conversely, the presence of coarse particles on either slide indicated inhomogeneity.

Hedonic test sampling technique

This study employed a non-probability sampling approach, specifically utilizing a combination of incidental sampling and quota sampling techniques. Incidental sampling involved recruiting participants who met the inclusion criteria on an opportunistic basis. These criteria included being between the ages of 20 and 30 years old, identifying as male or female, having a self-reported healthy body condition, and expressing willingness to participate. Quota sampling ensured that the final sample composition reflected pre-determined proportions based on gender (female/male). A total of 50 participants were recruited using this combined approach. All participants provided written informed consent after being thoroughly briefed about the study's objectives, procedures, and potential risks/benefits. This study received ethical approval from the Health Research Ethics Committee at STIKES Bina Usada Bali (approval No. 052/EA/KEPK-BUB-2024).

Hedonic test data collection technique

A questionnaire was used to collect data from this study's participants. The questionnaire, accessible via a Google Form link (https://forms.gle/yPKt8TBMe7UtJxPi6), was distributed to a sample of 50 panelists at Universitas Mahasaraswati Denpasar. A production cost analysis was conducted for each *M. paradisiaca* stem serum formula. This involved identifying and analyzing each cost component associated with the production of each formula. The analysis considered the market price of similar serums (IDR 100,000.00) as a reference point.

Data analysis

Hedonic testing was conducted to evaluate the sensory acceptability of the *M. paradisiaca* stem serum product by a panel of 50 assessors. Panelists evaluated the color, taste, and aroma of the serum using a standardized checklist with a four-point hedonic scale: highly preferred (4), preferred (3), less preferred (2), and not preferred (1). The data were tabulated according to frequency distribution and analyzed descriptively using percentages. Results are presented in both narrative and chart formats. Production cost analysis employed quantitative technical methods, including calculations, interviews, record-keeping, and observations. This process aimed to determine the cost of producing the *M. paradisiaca* stem serum preparation.

RESULTS AND DISCUSSION

Organoleptic evaluation revealed that all *M. paradisiaca* stem extract serum formulations (F1, F2, and F3) displayed a consistent appearance: a slightly thick, brown liquid with a characteristic *M. paradisiaca* stem extract odor. This coloration and aroma are inherent properties of the *M. paradisiaca* stem extract itself. Visual assessment of homogeneity confirmed the absence of coarse particles in all formulations, indicating good physical stability. The pH of each formulation was measured to ensure compatibility with the skin's natural acidic range (pH 4.5-6.5). All formulations exhibited a pH of 6, suggesting minimal potential for skin irritation. Finally, a transferred volume test was conducted to assess the delivered dosage volume from each formulation. All formulations (F1, F2, and F3) achieved transferred volumes exceeding the 95% minimum requirement, with values of 98%, 98%, and 97%, respectively. Previous studies from Syarifah *et al.*[16] showed similar results for serum from *M. paradisiaca* fruit skin, indicating the stability of serum produced from *M. paradisiaca* parts. Detailed results for all evaluations are presented in **Table II**.

Table II. Organoleptic test results of *M. paradisiaca* stem serum.

Test parameters	F1	F2	F3
Consistency	Slightly thick liquid	Slightly thick liquid	Slightly thick liquid
Color	Light brown	Dark brown	Dark brown
Smell	Characteristic smell of extract	Characteristic smell of extract	Characteristic smell of extract
Transferred volume (%)	98	98	97
pH	6	6	6
Homogeneity	Homogeneous	Homogeneous	Homogeneous

Panelists evaluated the sensory attributes (aroma, texture, stickiness, and viscosity) of *M. paradisiaca* stem serum formulations (F1, F2, and F3) using a scoring. Formulations received consistently high scores (4) for aroma, texture, and

stickiness across all groups, indicating overall panelist satisfaction with these aspects. Formulations F1 and F2 received high viscosity scores (4), suggesting a preference for their consistency compared to F3. Regarding color, panelists showed a mild preference for F1 (score 3) compared to F2 and F3. As Palmer and Schloss reported[17], people have a tendency towards products with their favorite colors. In this case, the color of the *M. paradisiaca* stem extract may not be liked by the panelists. Overall, formulations F1 and F2 were favored by the panelists based on the combined hedonic scores, as shown in **Table III**.

Table III. Hedonic test results of *M. paradisiaca* stem serum.

Formulas	Aroma	Viscosity	Texture	Color	Sticky effect	Whole products
F1	4	4	4	3	4	4
F2	4	4	4	2	4	4
F3	4	3	4	2	4	3

Note: 4: highly preferred; 3: preferred; 2: less preferred; 1: not preferred

Panelist satisfaction with various parameters of the *M. paradisiaca* stem extract serum formulations was assessed using a hedonic test (**Table IV**). Panelists indicated a high degree of satisfaction (96%) with the aroma across all formulations. Basically, aroma is one of the main factors in panelists' preference for topical products such as serum[18]. However, responses regarding viscosity, texture, color, and stickiness revealed a preference for formulation F1 compared to F2 and F3. The concentration of *M. paradisiaca* stem extract significantly impacted the color of the serum preparations. The hedonic test results from 50 panelists evaluating five parameters suggest that panelists found F1 to be more aesthetically pleasing than F2 and F3. This difference in color preference can be attributed to the varying concentrations of the extract across the formulations[19]. The F1, containing the lowest concentration of *M. paradisiaca* stem extract (4%), exhibited a lighter brown color compared to F2 (8% extract) and F3 (12% extract). In the context of facial serums, a lighter color is generally considered more desirable[20,21]. Therefore, higher concentrations of the extract, while potentially increasing the product's efficacy, may lead to a less visually appealing[22].

Table IV. Level of likeness for *M. paradisiaca* stem serum.

Formulas	Aroma (%)	Viscosity (%)	Texture (%)	Color (%)	Sticky effect (%)	Whole products (%)
F1	96	96	98	80	98	93
F2	96	96	96	62	96	88
F3	96	83	94	46	96	83

Determining the production cost of *M. paradisiaca* stem serum is crucial for establishing a sustainable and commercially viable product. Production cost directly influences the selling price, which is a key factor influencing market competitiveness[23]. Three primary cost components are considered in production cost analysis: raw materials, labor, and factory overhead[24]. Accurate recording and classification of these costs are essential. For this study, we employed variable costing, a method that considers only variable production costs. This approach is particularly suited for small-scale production[25], such as the research-oriented production of *M. paradisiaca* stem serum preparations employed here. Variable costs fluctuate in direct proportion to the volume of production and include raw materials, direct labor, and variable factory overhead costs. By utilizing variable costing, we can gain a more accurate understanding of the cost associated with each unit of serum produced[26].

Raw materials, the unprocessed components used in a product's manufacture, represent a significant cost factor in production processes. They are directly incorporated into the final product and can be physically identified within it[26]. A detailed breakdown of the raw material costs for the *M. paradisiaca* stem serum formulations is presented in **Table V**.

Table V. Raw material costs for 10 bottles of *M. paradisiaca* stem serum.

Raw materials	Quantity (g) /100 mL	Price (g/L)	Total cost @1 bottle (IDR)	Total cost 10 bottles (IDR)
Musa paradisiaca stem	4	5000	20,000.00	200,000.00
Xanthan gum	0.5	1000	200.00	2,000.00
Glycerin	10	100	1,000.00	10,000.00
Potassium sorbate	0.1	300	30.00	300.00
Sodium benzoate	0.1	200	20.00	200.00
Distilled water	Ad 100	5000	500.00	5,000.00
Total			**21,750.00**	**217,500.00**

Direct labor costs are those directly attributable to the production process and can be readily traced back to the finished product (as detailed in **Table VI**). These costs are typically calculated based on factors such as working hours, working days, or product units[27]. In this study, direct labor costs encompassed laboratory rental and plant identification expenses. Utilizing a rented laboratory space was necessary due to the small-scale nature of the research. Additionally, costs associated with plant identification were incurred to ensure the precise identity of the plant materials used.

Table VI. Direct labor costs for 10 bottles of *M. paradisiaca* stem serum.

Direct labor	Costs (IDR)
Laboratory rental	75,000.00
Determination	240,000.00
Total	**315,000.00**

Factory overhead costs encompass indirect manufacturing expenses incurred during production, excluding direct labor costs and raw materials[28]. In this study, factory overhead costs included expenses associated with packaging materials (drop bottles for serum, dosage packaging) and graphic design (sticker design). A detailed breakdown of the factory overhead costs for the *M. paradisiaca* stem serum formulations is presented in **Table VII**.

Table VII. Factory overhead costs for 10 bottles of *M. paradisiaca* stem serum.

Factory overhead	Costs (IDR)
Bottle	22,500.00
Preparation packaging	35,000.00
Sticker design	25,000.00
Total	**82,500.00**

The total cost of producing *M. paradisiaca* stem serum preparations, as detailed in **Tables V** to **VII**, was IDR 614,000.00. Using a variable costing approach, the cost per bottle of *M. paradisiaca* stem serum in a 10-bottle batch translates to IDR 61,400.00 per 10 mL bottle. A separate consumer survey (**Table VIII**) revealed that the preferred price for a bottle of *M. paradisiaca* stem serum was IDR 55,000.00, which falls below the calculated production cost. This discrepancy between production cost and consumer preference highlights the need for further cost reduction strategies or the exploration of methods to enhance the perceived value of the M. *paradisiaca* stem serum product[29].

Table VIII. Survey results of 86 respondents in determining the price of *M. paradisiaca* stem serum per 10 mL bottle.

The desired price for a 10 mL bottle	Voters (n (%))
50,000.00	18 (21)
55,000.00	42 (49)
60,000.00	8 (9)
65,000.00	14 (16)
70,000.00	4 (5)
Total	**86 (100)**

CONCLUSION

In conclusion, the sensory evaluation results demonstrated a strong preference for F1 compared to F2 and F3. Panelists rated F1 highest (80% satisfaction) for aroma, viscosity, texture, color, and stickiness. The addition of higher extract concentrations in F2 and F3 appears to have negatively impacted these sensory attributes. The variable costing method revealed a total production cost of IDR 614,000.00 for 10 bottles of *M. paradisiaca* stem serum preparations, translating to an estimated price per product of IDR 61,400.00.

ACKNOWLEDGMENT

Thank you to all parties who have helped with this research and to Universitas Mahasaraswati Denpasar for funding with contract No K.098/B.01.0l/LPPM-UNMAS/V/2021.

AUTHORS' CONTRIBUTION

Conceptualization: Fitria Megawati, Ni Luh Kade Arman Anita Dewi
Data curation: Fitria Megawati
Formal analysis: Fitria Megawati, Ni Luh Kade Arman Anita Dewi
Funding acquisition: Fitria Megawati, Ni Luh Kade Arman Anita Dewi
Investigation: Fitria Megawati, Ni Luh Kade Arman Anita Dewi
Methodology: Fitria Megawati
Project administration: Fitria Megawati
Resources: Ni Putu Dewi Agustini
Software: -
Supervision: Ni Putu Dewi Agustini, I Putu Satria Antara, Ni Luh Firda Ekayanti, Ni Wayan Darmayanti
Validation: Fitria Megawati
Visualization: -
Writing - original draft: Fitria Megawati
Writing - review & editing: Fitria Megawati, Ni Luh Kade Arman Anita Dewi, Ni Putu Dewi Agustini

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. D'Orazio J, Jarrett S, Amaro-Ortiz A, Scott T. UV radiation and the skin. Int J Mol Sci. 2013;14(6):12222-48. DOI: 10.3390/ijms140612222; PMCID: PMC3709783; PMID: 23749111

2. Ganceviciene R, Liakou AI, Theodoridis A, Makrantonaki E, Zouboulis CC. Skin anti-aging strategies. Dermatoendocrinol. 2012;4(3):308-19. DOI: 10.4161/derm.22804; PMCID: PMC3583892; PMID: 23467476

3. Rodan K, Fields K, Majewski G, Falla T. Skincare Bootcamp: The Evolving Role of Skincare. Plast Reconstr Surg Glob Open. 2016;4(12 Suppl Anatomy and Safety in Cosmetic Medicine: Cosmetic Bootcamp):e1152. DOI: 10.1097/gox.0000000000001152; PMCID: PMC5172479; PMID: 28018771

4. Bjørklund G, Shanaida M, Lysiuk R, Butnariu M, Peana M, Sarac I, et al. Natural Compounds and Products from an Anti-Aging Perspective. Molecules. 2022;27(20):7084. DOI: 10.3390/molecules27207084; PMCID: PMC9610014; PMID: 36296673

5. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem. 2015;30(1):11-26. DOI: 10.1007/s12291-014-0446-0; PMCID: PMC4310837; PMID: 25646037

6. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010;4(8):118-26. DOI: 10.4103/0973-7847.70902; PMCID: PMC3249911; PMID: 22228951

7. Michalak M. Plant-Derived Antioxidants: Significance in Skin Health and the Ageing Process. Int J Mol Sci. 2022;23(2):585. DOI: 10.3390/ijms23020585; PMCID: PMC8776015; PMID: 35054770

8. Moskwa J, Bronikowska M, Socha K, Markiewicz-Żukowska R. Vegetable as a Source of Bioactive Compounds with Photoprotective Properties: Implication in the Aging Process. Nutrients. 2023;15(16):3594. DOI: 10.3390/nu15163594; PMCID: PMC10459432; PMID: 37630784

9. Najm AA, Salih SA, Fazry S, Law D. Moderated mediation role of ethnicity on natural skin care products purchasing intention model among multicultural consumers. PLoS One. 2024;19(3):e0300376. DOI: 10.1371/journal.pone.0300376; PMCID: PMC10956806; PMID: 38512877

10. Justine AK, Kaur N, Savita, Pati PK. Biotechnological interventions in banana: current knowledge and future prospects. Heliyon. 2022;8(11):e11636. DOI: 10.1016/j.heliyon.2022.e11636; PMCID: PMC9676558; PMID: 36419664

11. Amanah NK, Mashudi S, Munawaroh S, Azzarin AW, karimah FN, Gunawan F. Exploring the Efficacy of Musa Cavendish Stem Extract (Mucase) as a Novel Wound Dressing: A Comparative Study With Sofratulle®. Cureus. 2024;16(2):e54411. DOI: 10.7759/cureus.54411; PMCID: PMC10950323; PMID: 38505455

12. Karuppiah P, Mustaffa M. Antibacterial and antioxidant activities of Musa sp. leaf extracts against multidrug resistant clinical pathogens causing nosocomial infection. Asian Pac J Trop Biomed. 2013;3(9):737-42. DOI: 10.1016/s2221-1691(13)60148-3; PMCID: PMC3757284; PMID: 23998016

13. Wibowo FS, Prasetyaningrum E. Pemanfaatan Ekstrak Batang Tanaman Pisang (Musa Paradisiacal) Sebagai Obat Antiacne Dalam Sediaan Gel Antiacne. J Ilmu Farmasi Farmasi Klinik. 2015;12(1):38–46. DOI: 10.31942/jiffk.v12i1.1401

14. Dewi NLKAA, Yuda PESK, Suarnata IGA, Sasadara MMV. Uji In Vivo Tahap Preklinis Terhadap Ekstrak Batang Pisang (Musa Paradisiaca L.) Sebagai Antiinflamasi Topikal. J Riset Kefarmasian Indones. 2021;3(2):138–51. DOI: 10.33759/jrki.v3i2.129

15. Suhaela J, Nadjamuddin M, Amar MI, Wahyuni W. Formulasi dan Uji Aktivitas Antioksidan dan SPF (Sun Protection Factor) Serum Ekstrak Etanol Kulit Pisang Raja (Musa paradisiaca L). J Sains Kesehatan. 2023;5(6):915-24. DOI: 10.25026/jsk.v5i6.2118

16. Syarifah A, Budiman A, Nazilah SA. Formulation and Antioxidant Activity of Serum Gel of Ethyl Acetate Fraction from Musa x paradisiaca L. In: Wardaningsih S, Tamayo MDB, Thanee S, Natason A, Poblete MLO, Martin NM, editors. Proceedings of the 4th International Conference on Sustainable Innovation 2020–Health Science and Nursing (ICoSIHSN 2020). Paris: Atlantis Press; 2020. p.310-5. DOI: 10.2991/ahsr.k.210115.066

17. Palmer SE, Schloss KB. An ecological valence theory of human color preference. Proc Natl Acad Sci U S A. 2010;107(19):8877-82. DOI: 10.1073/pnas.0906172107; PMCID: PMC2889342; PMID: 20421475

18. Feron G, Ayed C, Qannari EM, Courcoux P, Laboure H, Guichard E. Understanding aroma release from model cheeses by a statistical multiblock approach on oral processing. PLoS One. 2014;9(4):e93113. DOI: 10.1371/journal.pone.0093113; PMCID: PMC3972224; PMID: 24691625

19. Wang H, Chen Y, Wang L, Liu Q, Yang S, Wang C. Advancing herbal medicine: enhancing product quality and safety through robust quality control practices. Front Pharmacol. 2023;14:1265178. DOI: 10.3389/fphar.2023.1265178; PMCID: PMC10561302; PMID: 37818188

20. Garre A, Narda M, Valderas-Martinez P, Piquero J, Granger C. Antiaging effects of a novel facial serum containing L-Ascorbic acid, proteoglycans, and proteoglycan-stimulating tripeptide: ex vivo skin explant studies and in vivo clinical studies in women. Clin Cosmet Investig Dermatol. 2018;11:253-63. DOI: 10.2147/ccid.s161352; PMCID: PMC5985795; PMID: 29881301

21. Stephen ID, Smith MJL, Stirrat MR, Perrett DI. Facial Skin Coloration Affects Perceived Health of Human Faces. Int J Primatol. 2009;30(6):845-57. DOI: 10.1007/s10764-009-9380-z; PMCID: PMC2780675; PMID: 19946602

22. Ponphaiboon J, Krongrawa W, Aung WW, Chinatangkul N, Limmatvapirat S, Limmatvapirat C. Advances in Natural Product Extraction Techniques, Electrospun Fiber Fabrication, and the Integration of Experimental Design: A

Comprehensive Review. Molecules. 2023;28(13):5163. DOI: 10.3390/molecules28135163; PMCID: PMC10343563; PMID: 37446825

23. Artami LAP, Sudiartha IK, Karma IGM. Calculation of the Cost of Production in Determining the Selling Price of Kelle Soap Products Sari Amertha Aan. J Appl Sci Account Fin Tax. 2021;4(2):173-8. DOI: 10.31940/jasafint.v4i2.173-178

24. Thomas D. Costs, Benefits, and Adoption of Additive Manufacturing: A Supply Chain Perspective. Int J Adv Manuf Technol. 2016;85(5-8):1857-76. DOI: 10.1007/s00170-015-7973-6; PMCID: PMC5524380; PMID: 28747809

25. Špacírová Z, Epstein D, García-Mochón L, Rovira J, Lima AOdL, Espín J. A general framework for classifying costing methods for economic evaluation of health care. Eur J Health Econ. 2020;21(4):529-42. DOI: 10.1007/s10198-019-01157-9; PMCID: PMC8149350; PMID: 31960181

26. Triwidatin Y. The Influence of Raw Materials and Direct Labor Costs on Profit Margin Ratio in UMKM Agan Cibinong, Bogor. J Fin Bus Digit. 2022;1(4):311-24. DOI: 10.55927/jfbd.v1i4.2172

27. Zhenjing G, Chupradit S, Ku KY, Nassani AA, Haffar M. Impact of Employees' Workplace Environment on Employees' Performance: A Multi-Mediation Model. Front Public Health. 2022;10:890400. DOI: 10.3389/fpubh.2022.890400; PMCID: PMC9136218; PMID: 35646787

28. Lismawati. Factory Overhead Cost Budget. Multidisc Output Res Actual Int Issue J. 2023;2(4):190-5. DOI: 10.54443/morfai.v3i2.850

29. Luo B, Li L, Sun Y. Understanding the Influence of Consumers' Perceived Value on Energy-Saving Products Purchase Intention. Front Psychol. 2022;12:640376. DOI: 10.3389/fpsyg.2021.640376; PMCID: PMC8844988; PMID: 35178004

Borneo Journal of Pharmacy
Vol 7 Issue 2 May 2024
Pages 206 – 214

https://journal.umpr.ac.id/index.php/bjop/article/view/6872
DOI: https://doi.org/10.33084/bjop.v7i2.6872
e-ISSN: 2621-4814

Research Article

Ethnobotanical Study and Hedonic Evaluation with Cost Analysis of Banana (*Musa paradisiaca* L.) Stem Serum Preparation as an Anti-Aging Solution

Fitria Megawati*

Ni Luh Kade Arman Anita Dewi

Ni Putu Dewi Agustini

I Putu Satria Antara

Ni Luh Firda Ekayanti

Ni Wayan Darmayanti

Department of Pharmacy, Universitas Mahasaraswati Denpasar, Denpasar, Bali, Indonesia

*email: fitriamega83@unmas.ac.id; phone: +6281229913010

Keywords:
Cost
Hedonic
Musa paradisiaca

Abstract

In skincare, particularly facial serums, the utilization of natural ingredients is crucial in addressing various skin issues, notably combating the effects of free radicals that contribute to wrinkles. This study explores the potential of banana (*Musa paradisiaca* L.) stem extract as an active ingredient in serum formulation due to its rich anthocyanin content known for antioxidant properties. Hedonic testing and cost analysis serve as initial steps in the marketing mix of a product. This research aims to assess the hedonic preferences of various *M. paradisiaca* stem serum formulations and analyze the production costs. A descriptive research method employing survey and observational techniques was utilized for data collection and descriptive analysis. Hedonic testing was conducted to gauge the personal preferences of panelists toward *M. paradisiaca* stem serum formulations. Three formulations (F1 with 4%, F2 with 8%, and F3 with 12% *M. paradisiaca* extract) were evaluated by 40 panelists. Cost analysis of serum production employed quantitative descriptive analysis, computing the cost per unit using a variable costing method. The hedonic evaluation results showed that F1 formulation was highly preferred (79.3%), followed by F2 (73.2%), and F3 (66.8%). Cost analysis using the variable costing method revealed a total production cost of IDR 614,000.00 for 10 packages of *M. paradisiaca* stem serum formulation, translating to an approximate unit price of IDR 61,400.00.

Received: March 20th, 2024
1st Revised: May 14th, 2024
Accepted: May 21st, 2024
Published: May 30th, 2024

INTRODUCTION

The human skin, serving as the body's outermost barrier, is constantly exposed to environmental stressors. Chief among these are ultraviolet (UV) radiation and pollutants, both of which can inflict significant damage[1]. Furthermore, daily activities can contribute to the development of skin concerns, particularly in the facial area[2]. Improper facial cleansing and skincare routines can lead to the accumulation of dead skin cells, potentially hindering collagen production and ultimately accelerating the formation of wrinkles and fine lines[3].

Antioxidants have emerged as promising tools in the fight against skin aging and damage[4]. These molecules act by neutralizing free radicals, a class of highly reactive species containing unpaired electrons in their outer shells. Free radicals' inherent instability compels them to seek stability by stealing electrons from surrounding molecules, thereby causing cellular damage and contributing to various skin concerns[5]. By donating electrons and stabilizing free radicals, antioxidants offer protection against this cellular damage[6].

How to cite: Megawati F, Dewi NLKAA, Agustini NPD, Antara IPS, Ekayanti NLF, Darmayanti NW. Ethnobotanical Study and Hedonic Evaluation with Cost Analysis of Banana (Musa paradisiaca L.) Stem Serum Preparation as an Anti-Aging Solution. Borneo J Pharm. 2024;7(2):206-214. doi:10.33084/bjop.v7i2.6872

Natural antioxidants have gained significant interest for their potential benefits in topical skincare formulations. Topical application of antioxidants can offer protection against UVA radiation, a key contributor to skin darkening and premature aging[7]. These antioxidants can also help combat the damaging effects of UVB radiation, including wrinkles and inflammation[8]. Moreover, serums formulated with natural ingredients are generally considered safer for consumers compared to synthetic alternatives[9]. This study investigates the development of an antioxidant serum derived from banana (*Musa paradisiaca*) extracts as a potential option to shield the skin from free radicals and their associated signs of aging.

Musa paradisiaca thrives in tropical climates, making Indonesia a prime location for its cultivation[10]. Interestingly, *M. paradisiaca* stems hold potential as a natural antibiotic, and their use in wound healing is a traditional practice among some Indonesian communities[11]. Scientific studies have corroborated these traditional practices, demonstrating the effectiveness of *M. paradisiaca* stem extracts in inhibiting the growth of pathogenic bacteria like *Staphylococcus aureus*[12,13]. Building upon Dewi *et al*.[14] on formulating *M. paradisiaca* stem extract into serum preparations and evaluating their physical and antioxidant properties, this study aims to investigate two key aspects. Firstly, we assess the organoleptic properties of the serum preparation, including taste, aroma, and color, to gauge its acceptability among potential users. Secondly, we analyze the production costs associated with *M. paradisiaca* stem serum preparations.

MATERIALS AND METHODS

Materials

Musa paradisiaca stems were obtained from East Duda Village, Selat, Karangasem, Bali, Indonesia (**Figure 1**). The botanical identification was confirmed by the Plant Conservation Center of the Indonesian Institute of Sciences (LIPI), Eka Bedugul Botanical Gardens (voucher specimen number B-337/IPH.7/AP/XII/2020). Other materials used in this study included 70% ethanol (Brataco®), xanthan gum, glycerin, potassium sorbate, sodium benzoate, distilled water, and DPPH (2,2-diphenyl-1-picrylhydrazyl). The following equipment was used in this research included rotary evaporator, glassware (Pyrex®), flannel cloth, gram scale (accuracy 0.1 g), digital analytical balance (accuracy 0.0001 g), and glass jar. A questionnaire was developed to assess the level of acceptance of different *M. paradisiaca* stem serum formulations by participants. Additionally, the questionnaire aimed to gather information regarding production costs.

Figure 1. *Musa paradisiaca.*

Methods

Preparation of extracts and phytochemical screening

Musa paradisiaca stems were thoroughly sorted and chopped into small pieces. The chopped *M. paradisiaca* stem was then subjected to maceration extraction using 70% ethanol as the solvent. The maceration process was carried out for three cycles of 24 hours each, with occasional stirring. The macerate was stored in a light-protected environment throughout the extraction period. Following each maceration cycle, the mixture was filtered using a flannel cloth to separate the filtrate

(extract) from the residue. The remaining residue was then re-extracted with fresh 70% ethanol using the same maceration procedure. The combined filtrates from all maceration cycles were concentrated using a rotary evaporator under vacuum until a thick extract was obtained.

Preparation of serum

An oil-in-water (O/W) type serum formulation was prepared as detailed in **Table I**. Xanthan gum was dispersed in 20 parts of distilled water under constant stirring until an emulsion base formed. Glycerin was then gradually added to the mixture with continuous stirring. Potassium sorbate, sodium benzoate, and *M. paradisiaca* stem extract were incorporated sequentially into the mixture with continued slow stirring until a homogeneous product was obtained. Finally, distilled water was added to reach a final volume of 100 mL while stirring continuously. The homogeneous serum (**Figure 2**) was then stored in a pre-prepared container.

Table I. *Musa paradisiaca* stem serum formulation.

Material Name	Function	Concentration (%)		
		F1	F2	F3
Musa paradisiaca stem extract	Active ingredients	4	8	12
Xanthan gum	Thickener	0.5	0.5	0.5
Glycerin	Humectant	10	10	10
Potassium sorbate	Preservative	0.1	0.1	0.1
Sodium benzoate	Preservative	0.1	0.1	0.1
Distilled water	Solvent	Ad 100	Ad 100	Ad 100

a **b** **c**

Figure 2. *Musa paradisiaca* stem serum F1 (**a**), F2 (**b**), and F3 (**c**).

Serum evaluation

The physical properties of the *M. paradisiaca* stem extract serum were assessed using the following methods: organoleptic test, transferred volume test, pH test, and homogeneity test[15], as described in detail below.

Organoleptic test: The organoleptic properties of *M. paradisiaca* stem serum preparations were assessed visually. This evaluation included observations of consistency, color, and smell.

Transferred volume test: The transferred volume test is performed to ensure that the *M. paradisiaca* stem extract serum retains its designated dosage volume (100 mL) after transfer from its original container. This test verifies that at least 95% of the prepared serum volume can be effectively transferred. The test involves transferring the entire *M. paradisiaca* stem extract serum preparation to a measuring cup. The transferred volume is then measured, and the percentage of the initial volume is calculated.

pH test: The acidity of the *M. paradisiaca* stem extract serum was determined using a pH meter. Five milliliters of the extract serum were transferred to a clean, dry beaker. The pH meter electrode was immersed into the sample solution, ensuring good contact without stirring. The pH reading was allowed to stabilize and recorded. The electrode was then rinsed thoroughly with deionized water and patted dry with a lint-free tissue before further measurements.

Homogeneity test: The homogeneity of *M. paradisiaca* stem extract serum was assessed visually. A small amount of the extract serum was placed on two clean glass slides. The presence or absence of coarse particles on both slides was evaluated to

determine homogeneity. The extract serum was considered homogeneous if no coarse particles were observed on either slide. Conversely, the presence of coarse particles on either slide indicated inhomogeneity.

Hedonic test sampling technique

This study employed a non-probability sampling approach, specifically utilizing a combination of incidental sampling and quota sampling techniques. Incidental sampling involved recruiting participants who met the inclusion criteria on an opportunistic basis. These criteria included being between the ages of 20 and 30 years old, identifying as male or female, having a self-reported healthy body condition, and expressing willingness to participate. Quota sampling ensured that the final sample composition reflected pre-determined proportions based on gender (female/male). A total of 50 participants were recruited using this combined approach. All participants provided written informed consent after being thoroughly briefed about the study's objectives, procedures, and potential risks/benefits. This study received ethical approval from the Health Research Ethics Committee at STIKES Bina Usada Bali (approval No. 052/EA/KEPK-BUB-2024).

Hedonic test data collection technique

A questionnaire was used to collect data from this study's participants. The questionnaire, accessible via a Google Form link (https://forms.gle/yPKt8TBMe7UtJxPi6), was distributed to a sample of 50 panelists at Universitas Mahasaraswati Denpasar. A production cost analysis was conducted for each *M. paradisiaca* stem serum formula. This involved identifying and analyzing each cost component associated with the production of each formula. The analysis considered the market price of similar serums (IDR 100,000.00) as a reference point.

Data analysis

Hedonic testing was conducted to evaluate the sensory acceptability of the *M. paradisiaca* stem serum product by a panel of 50 assessors. Panelists evaluated the color, taste, and aroma of the serum using a standardized checklist with a four-point hedonic scale: highly preferred (4), preferred (3), less preferred (2), and not preferred (1). The data were tabulated according to frequency distribution and analyzed descriptively using percentages. Results are presented in both narrative and chart formats. Production cost analysis employed quantitative technical methods, including calculations, interviews, record-keeping, and observations. This process aimed to determine the cost of producing the *M. paradisiaca* stem serum preparation.

RESULTS AND DISCUSSION

Organoleptic evaluation revealed that all *M. paradisiaca* stem extract serum formulations (F1, F2, and F3) displayed a consistent appearance: a slightly thick, brown liquid with a characteristic *M. paradisiaca* stem extract odor. This coloration and aroma are inherent properties of the *M. paradisiaca* stem extract itself. Visual assessment of homogeneity confirmed the absence of coarse particles in all formulations, indicating good physical stability. The pH of each formulation was measured to ensure compatibility with the skin's natural acidic range (pH 4.5-6.5). All formulations exhibited a pH of 6, suggesting minimal potential for skin irritation. Finally, a transferred volume test was conducted to assess the delivered dosage volume from each formulation. All formulations (F1, F2, and F3) achieved transferred volumes exceeding the 95% minimum requirement, with values of 98%, 98%, and 97%, respectively. Previous studies from Syarifah *et al.*[16] showed similar results for serum from *M. paradisiaca* fruit skin, indicating the stability of serum produced from *M. paradisiaca* parts. Detailed results for all evaluations are presented in **Table II**.

Table II. Organoleptic test results of *M. paradisiaca* stem serum.

Test parameters	F1	F2	F3
Consistency	Slightly thick liquid	Slightly thick liquid	Slightly thick liquid
Color	Light brown	Dark brown	Dark brown
Smell	Characteristic smell of extract	Characteristic smell of extract	Characteristic smell of extract
Transferred volume (%)	98	98	97
pH	6	6	6
Homogeneity	Homogeneous	Homogeneous	Homogeneous

Panelists evaluated the sensory attributes (aroma, texture, stickiness, and viscosity) of *M. paradisiaca* stem serum formulations (F1, F2, and F3) using a scoring. Formulations received consistently high scores (4) for aroma, texture, and

stickiness across all groups, indicating overall panelist satisfaction with these aspects. Formulations F1 and F2 received high viscosity scores (4), suggesting a preference for their consistency compared to F3. Regarding color, panelists showed a mild preference for F1 (score 3) compared to F2 and F3. As Palmer and Schloss reported[17], people have a tendency towards products with their favorite colors. In this case, the color of the *M. paradisiaca* stem extract may not be liked by the panelists. Overall, formulations F1 and F2 were favored by the panelists based on the combined hedonic scores, as shown in **Table III**.

Table III. Hedonic test results of *M. paradisiaca* stem serum.

Formulas	Aroma	Viscosity	Texture	Color	Sticky effect	Whole products
F1	4	4	4	3	4	4
F2	4	4	4	2	4	4
F3	4	3	4	2	4	3

Note: 4: highly preferred; 3: preferred; 2: less preferred; 1: not preferred

Panelist satisfaction with various parameters of the *M. paradisiaca* stem extract serum formulations was assessed using a hedonic test (**Table IV**). Panelists indicated a high degree of satisfaction (96%) with the aroma across all formulations. Basically, aroma is one of the main factors in panelists' preference for topical products such as serum[18]. However, responses regarding viscosity, texture, color, and stickiness revealed a preference for formulation F1 compared to F2 and F3. The concentration of *M. paradisiaca* stem extract significantly impacted the color of the serum preparations. The hedonic test results from 50 panelists evaluating five parameters suggest that panelists found F1 to be more aesthetically pleasing than F2 and F3. This difference in color preference can be attributed to the varying concentrations of the extract across the formulations[19]. The F1, containing the lowest concentration of *M. paradisiaca* stem extract (4%), exhibited a lighter brown color compared to F2 (8% extract) and F3 (12% extract). In the context of facial serums, a lighter color is generally considered more desirable[20,21]. Therefore, higher concentrations of the extract, while potentially increasing the product's efficacy, may lead to a less visually appealing[22].

Table IV. Level of likeness for *M. paradisiaca* stem serum.

Formulas	Aroma (%)	Viscosity (%)	Texture (%)	Color (%)	Sticky effect (%)	Whole products (%)
F1	96	96	98	80	98	93
F2	96	96	96	62	96	88
F3	96	83	94	46	96	83

Determining the production cost of *M. paradisiaca* stem serum is crucial for establishing a sustainable and commercially viable product. Production cost directly influences the selling price, which is a key factor influencing market competitiveness[23]. Three primary cost components are considered in production cost analysis: raw materials, labor, and factory overhead[24]. Accurate recording and classification of these costs are essential. For this study, we employed variable costing, a method that considers only variable production costs. This approach is particularly suited for small-scale production[25], such as the research-oriented production of *M. paradisiaca* stem serum preparations employed here. Variable costs fluctuate in direct proportion to the volume of production and include raw materials, direct labor, and variable factory overhead costs. By utilizing variable costing, we can gain a more accurate understanding of the cost associated with each unit of serum produced[26].

Raw materials, the unprocessed components used in a product's manufacture, represent a significant cost factor in production processes. They are directly incorporated into the final product and can be physically identified within it[26]. A detailed breakdown of the raw material costs for the *M. paradisiaca* stem serum formulations is presented in **Table V**.

Table V. Raw material costs for 10 bottles of *M. paradisiaca* stem serum.

Raw materials	Quantity (g) /100 mL	Price (g/L)	Total cost @1 bottle (IDR)	Total cost 10 bottles (IDR)
Musa paradisiaca stem	4	5000	20,000.00	200,000.00
Xanthan gum	0.5	1000	200.00	2,000.00
Glycerin	10	100	1,000.00	10,000.00
Potassium sorbate	0.1	300	30.00	300.00
Sodium benzoate	0.1	200	20.00	200.00
Distilled water	Ad 100	5000	500.00	5,000.00
Total			**21,750.00**	**217,500.00**

Direct labor costs are those directly attributable to the production process and can be readily traced back to the finished product (as detailed in **Table VI**). These costs are typically calculated based on factors such as working hours, working days, or product units[27]. In this study, direct labor costs encompassed laboratory rental and plant identification expenses. Utilizing a rented laboratory space was necessary due to the small-scale nature of the research. Additionally, costs associated with plant identification were incurred to ensure the precise identity of the plant materials used.

Table VI. Direct labor costs for 10 bottles of *M. paradisiaca* stem serum.

Direct labor	Costs (IDR)
Laboratory rental	75,000.00
Determination	240,000.00
Total	**315,000.00**

Factory overhead costs encompass indirect manufacturing expenses incurred during production, excluding direct labor costs and raw materials[28]. In this study, factory overhead costs included expenses associated with packaging materials (drop bottles for serum, dosage packaging) and graphic design (sticker design). A detailed breakdown of the factory overhead costs for the *M. paradisiaca* stem serum formulations is presented in **Table VII**.

Table VII. Factory overhead costs for 10 bottles of *M. paradisiaca* stem serum.

Factory overhead	Costs (IDR)
Bottle	22,500.00
Preparation packaging	35,000.00
Sticker design	25,000.00
Total	**82,500.00**

The total cost of producing *M. paradisiaca* stem serum preparations, as detailed in **Tables V** to **VII**, was IDR 614,000.00. Using a variable costing approach, the cost per bottle of *M. paradisiaca* stem serum in a 10-bottle batch translates to IDR 61,400.00 per 10 mL bottle. A separate consumer survey (**Table VIII**) revealed that the preferred price for a bottle of *M. paradisiaca* stem serum was IDR 55,000.00, which falls below the calculated production cost. This discrepancy between production cost and consumer preference highlights the need for further cost reduction strategies or the exploration of methods to enhance the perceived value of the M. *paradisiaca* stem serum product[29].

Table VIII. Survey results of 86 respondents in determining the price of *M. paradisiaca* stem serum per 10 mL bottle.

The desired price for a 10 mL bottle	Voters (n (%))
50,000.00	18 (21)
55,000.00	42 (49)
60,000.00	8 (9)
65,000.00	14 (16)
70,000.00	4 (5)
Total	**86 (100)**

CONCLUSION

In conclusion, the sensory evaluation results demonstrated a strong preference for F1 compared to F2 and F3. Panelists rated F1 highest (80% satisfaction) for aroma, viscosity, texture, color, and stickiness. The addition of higher extract concentrations in F2 and F3 appears to have negatively impacted these sensory attributes. The variable costing method revealed a total production cost of IDR 614,000.00 for 10 bottles of *M. paradisiaca* stem serum preparations, translating to an estimated price per product of IDR 61,400.00.

ACKNOWLEDGMENT

Thank you to all parties who have helped with this research and to Universitas Mahasaraswati Denpasar for funding with contract No K.098/B.01.0l/LPPM-UNMAS/V/2021.

AUTHORS' CONTRIBUTION

Conceptualization: Fitria Megawati, Ni Luh Kade Arman Anita Dewi
Data curation: Fitria Megawati
Formal analysis: Fitria Megawati, Ni Luh Kade Arman Anita Dewi
Funding acquisition: Fitria Megawati, Ni Luh Kade Arman Anita Dewi
Investigation: Fitria Megawati, Ni Luh Kade Arman Anita Dewi
Methodology: Fitria Megawati
Project administration: Fitria Megawati
Resources: Ni Putu Dewi Agustini
Software: -
Supervision: Ni Putu Dewi Agustini, I Putu Satria Antara, Ni Luh Firda Ekayanti, Ni Wayan Darmayanti
Validation: Fitria Megawati
Visualization: -
Writing - original draft: Fitria Megawati
Writing - review & editing: Fitria Megawati, Ni Luh Kade Arman Anita Dewi, Ni Putu Dewi Agustini

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. D'Orazio J, Jarrett S, Amaro-Ortiz A, Scott T. UV radiation and the skin. Int J Mol Sci. 2013;14(6):12222-48. DOI: 10.3390/ijms140612222; PMCID: PMC3709783; PMID: 23749111

2. Ganceviciene R, Liakou AI, Theodoridis A, Makrantonaki E, Zouboulis CC. Skin anti-aging strategies. Dermatoendocrinol. 2012;4(3):308-19. DOI: 10.4161/derm.22804; PMCID: PMC3583892; PMID: 23467476

3. Rodan K, Fields K, Majewski G, Falla T. Skincare Bootcamp: The Evolving Role of Skincare. Plast Reconstr Surg Glob Open. 2016;4(12 Suppl Anatomy and Safety in Cosmetic Medicine: Cosmetic Bootcamp):e1152. DOI: 10.1097/gox.0000000000001152; PMCID: PMC5172479; PMID: 28018771

4. Bjørklund G, Shanaida M, Lysiuk R, Butnariu M, Peana M, Sarac I, et al. Natural Compounds and Products from an Anti-Aging Perspective. Molecules. 2022;27(20):7084. DOI: 10.3390/molecules27207084; PMCID: PMC9610014; PMID: 36296673

5. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem. 2015;30(1):11-26. DOI: 10.1007/s12291-014-0446-0; PMCID: PMC4310837; PMID: 25646037

6. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010;4(8):118-26. DOI: 10.4103/0973-7847.70902; PMCID: PMC3249911; PMID: 22228951

7. Michalak M. Plant-Derived Antioxidants: Significance in Skin Health and the Ageing Process. Int J Mol Sci. 2022;23(2):585. DOI: 10.3390/ijms23020585; PMCID: PMC8776015; PMID: 35054770

8. Moskwa J, Bronikowska M, Socha K, Markiewicz-Żukowska R. Vegetable as a Source of Bioactive Compounds with Photoprotective Properties: Implication in the Aging Process. Nutrients. 2023;15(16):3594. DOI: 10.3390/nu15163594; PMCID: PMC10459432; PMID: 37630784

9. Najm AA, Salih SA, Fazry S, Law D. Moderated mediation role of ethnicity on natural skin care products purchasing intention model among multicultural consumers. PLoS One. 2024;19(3):e0300376. DOI: 10.1371/journal.pone.0300376; PMCID: PMC10956806; PMID: 38512877

10. Justine AK, Kaur N, Savita, Pati PK. Biotechnological interventions in banana: current knowledge and future prospects. Heliyon. 2022;8(11):e11636. DOI: 10.1016/j.heliyon.2022.e11636; PMCID: PMC9676558; PMID: 36419664

11. Amanah NK, Mashudi S, Munawaroh S, Azzarin AW, karimah FN, Gunawan F. Exploring the Efficacy of Musa Cavendish Stem Extract (Mucase) as a Novel Wound Dressing: A Comparative Study With Sofratulle®. Cureus. 2024;16(2):e54411. DOI: 10.7759/cureus.54411; PMCID: PMC10950323; PMID: 38505455

12. Karuppiah P, Mustaffa M. Antibacterial and antioxidant activities of Musa sp. leaf extracts against multidrug resistant clinical pathogens causing nosocomial infection. Asian Pac J Trop Biomed. 2013;3(9):737-42. DOI: 10.1016/s2221-1691(13)60148-3; PMCID: PMC3757284; PMID: 23998016

13. Wibowo FS, Prasetyaningrum E. Pemanfaatan Ekstrak Batang Tanaman Pisang (Musa Paradisiacal) Sebagai Obat Antiacne Dalam Sediaan Gel Antiacne. J Ilmu Farmasi Farmasi Klinik. 2015;12(1):38–46. DOI: 10.31942/jiffk.v12i1.1401

14. Dewi NLKAA, Yuda PESK, Suarnata IGA, Sasadara MMV. Uji In Vivo Tahap Preklinis Terhadap Ekstrak Batang Pisang (Musa Paradisiaca L.) Sebagai Antiinflamasi Topikal. J Riset Kefarmasian Indones. 2021;3(2):138–51. DOI: 10.33759/jrki.v3i2.129

15. Suhaela J, Nadjamuddin M, Amar MI, Wahyuni W. Formulasi dan Uji Aktivitas Antioksidan dan SPF (Sun Protection Factor) Serum Ekstrak Etanol Kulit Pisang Raja (Musa paradisiaca L). J Sains Kesehatan. 2023;5(6):915-24. DOI: 10.25026/jsk.v5i6.2118

16. Syarifah A, Budiman A, Nazilah SA. Formulation and Antioxidant Activity of Serum Gel of Ethyl Acetate Fraction from Musa x paradisiaca L. In: Wardaningsih S, Tamayo MDB, Thanee S, Natason A, Poblete MLO, Martin NM, editors. Proceedings of the 4th International Conference on Sustainable Innovation 2020–Health Science and Nursing (ICoSIHSN 2020). Paris: Atlantis Press; 2020. p.310-5. DOI: 10.2991/ahsr.k.210115.066

17. Palmer SE, Schloss KB. An ecological valence theory of human color preference. Proc Natl Acad Sci U S A. 2010;107(19):8877-82. DOI: 10.1073/pnas.0906172107; PMCID: PMC2889342; PMID: 20421475

18. Feron G, Ayed C, Qannari EM, Courcoux P, Laboure H, Guichard E. Understanding aroma release from model cheeses by a statistical multiblock approach on oral processing. PLoS One. 2014;9(4):e93113. DOI: 10.1371/journal.pone.0093113; PMCID: PMC3972224; PMID: 24691625

19. Wang H, Chen Y, Wang L, Liu Q, Yang S, Wang C. Advancing herbal medicine: enhancing product quality and safety through robust quality control practices. Front Pharmacol. 2023;14:1265178. DOI: 10.3389/fphar.2023.1265178; PMCID: PMC10561302; PMID: 37818188

20. Garre A, Narda M, Valderas-Martinez P, Piquero J, Granger C. Antiaging effects of a novel facial serum containing L-Ascorbic acid, proteoglycans, and proteoglycan-stimulating tripeptide: ex vivo skin explant studies and in vivo clinical studies in women. Clin Cosmet Investig Dermatol. 2018;11:253-63. DOI: 10.2147/ccid.s161352; PMCID: PMC5985795; PMID: 29881301

21. Stephen ID, Smith MJL, Stirrat MR, Perrett DI. Facial Skin Coloration Affects Perceived Health of Human Faces. Int J Primatol. 2009;30(6):845-57. DOI: 10.1007/s10764-009-9380-z; PMCID: PMC2780675; PMID: 19946602

22. Ponphaiboon J, Krongrawa W, Aung WW, Chinatangkul N, Limmatvapirat S, Limmatvapirat C. Advances in Natural Product Extraction Techniques, Electrospun Fiber Fabrication, and the Integration of Experimental Design: A

Comprehensive Review. Molecules. 2023;28(13):5163. DOI: 10.3390/molecules28135163; PMCID: PMC10343563; PMID: 37446825

23. Artami LAP, Sudiartha IK, Karma IGM. Calculation of the Cost of Production in Determining the Selling Price of Kelle Soap Products Sari Amertha Aan. J Appl Sci Account Fin Tax. 2021;4(2):173-8. DOI: 10.31940/jasafint.v4i2.173-178

24. Thomas D. Costs, Benefits, and Adoption of Additive Manufacturing: A Supply Chain Perspective. Int J Adv Manuf Technol. 2016;85(5-8):1857-76. DOI: 10.1007/s00170-015-7973-6; PMCID: PMC5524380; PMID: 28747809

25. Špacírová Z, Epstein D, García-Mochón L, Rovira J, Lima AOdL, Espín J. A general framework for classifying costing methods for economic evaluation of health care. Eur J Health Econ. 2020;21(4):529-42. DOI: 10.1007/s10198-019-01157-9; PMCID: PMC8149350; PMID: 31960181

26. Triwidatin Y. The Influence of Raw Materials and Direct Labor Costs on Profit Margin Ratio in UMKM Agan Cibinong, Bogor. J Fin Bus Digit. 2022;1(4):311-24. DOI: 10.55927/jfbd.v1i4.2172

27. Zhenjing G, Chupradit S, Ku KY, Nassani AA, Haffar M. Impact of Employees' Workplace Environment on Employees' Performance: A Multi-Mediation Model. Front Public Health. 2022;10:890400. DOI: 10.3389/fpubh.2022.890400; PMCID: PMC9136218; PMID: 35646787

28. Lismawati. Factory Overhead Cost Budget. Multidisc Output Res Actual Int Issue J. 2023;2(4):190-5. DOI: 10.54443/morfai.v3i2.850

29. Luo B, Li L, Sun Y. Understanding the Influence of Consumers' Perceived Value on Energy-Saving Products Purchase Intention. Front Psychol. 2022;12:640376. DOI: 10.3389/fpsyg.2021.640376; PMCID: PMC8844988; PMID: 35178004

Borneo Journal of Pharmacy
Vol 7 Issue 2 May 2024
Pages 215 – 223

https://journal.umpr.ac.id/index.php/bjop/article/view/6554
DOI: https://doi.org/10.33084/bjop.v7i2.6554
e-ISSN: 2621-4814

Mini Review

Diabetic Foot Ulcers: Impact on Quality of Life and Instruments for Its Measurement

Khairunisa Qomariyanti [1*]

Rani Sauriasari [2]

Ratu Ayu Dewi Sartika [3]

[1] Master Program of Pharmaceutical Sciences, Universitas Indonesia, Depok, West Java, Indonesia
[2] Department of Pharmaceutical Sciences, Universitas Indonesia, Depok, West Java, Indonesia
[3] Department of Public Health, Universitas Indonesia, Depok, West Java, Indonesia

*email: nisa.qomariyanti@gmail.com; phone: +6281229913010

Keywords:
Diabetes
Diabetic foot ulcers
Instruments
Quality of life

Abstract

Diabetic foot ulcers (DFUs) are a major complication of diabetes mellitus, significantly impacting patients' quality of life (QoL) due to the heightened risk of infection and amputation. Pharmacists play a crucial role in managing diabetes and its complications, and assessing QoL can be a valuable tool for monitoring treatment success and medication effectiveness. This review explores instruments used to measure QoL in patients with DFUs, encompassing both general and disease-specific tools. We examine the impact of DFUs on QoL and discuss various theoretical frameworks used to understand this complex relationship.

Received: January 9th, 2024
1st Revised: April 22nd, 2024
Accepted: May 2nd, 2024
Published: May 30th, 2024

INTRODUCTION

Diabetes mellitus (DM) is a global health crisis, with an estimated 537 million people affected in 2021[1]. This number is projected to rise significantly in the coming decades. One of the most concerning complications of DM is diabetic foot ulcers (DFUs). These ulcers dramatically increase the risk of infection, amputation, and disability, leading to poor quality of life (QoL) and premature mortality[2]. Up to 34% of diabetic patients will experience foot ulcers during their lifetime[3].

The underlying mechanisms of DFU development are not fully understood. Impaired immune function in diabetic individuals significantly increases their susceptibility to wound infections[4]. Studies have shown that DFU development is associated with a five-fold increase in mortality within the first year and a forty-two percent mortality rate within five years[5]. Additionally, patients with DFUs experience increased morbidity, lower health-related QoL, and poorer psychosocial well-being[6].

Recognizing the significant impact of DFUs on QoL, healthcare professionals are increasingly emphasizing the importance of QoL assessment and monitoring as an essential outcome measure in diabetes care. A patient's QoL can significantly influence their self-care behaviors, which in turn, affect their diabetes control[7]. Pharmaceutical services play a crucial role in achieving positive treatment outcomes and improving patient QoL. Pharmacist-implemented pharmaceutical care programs, often in collaboration with other healthcare professionals, have been established worldwide to enhance clinical outcomes and health-related quality of life (HRQoL)[8].

Quality of life can be measured using various instruments, categorized as generic or disease-specific. Generic instruments, like the SF-36 and EQ-5D, assess QoL as a multidimensional concept encompassing cultural, social, psychological, and physical health aspects[9]. Disease-specific instruments, also known as HRQoL measures, focus on specific areas of health and

How to cite: Qomariyanti K, Sauriasari R, Sartika RAD. Diabetic Foot Ulcers: Impact on Quality of Life and Instruments for Its Measurement. Borneo J Pharm. 2024;7(2):215-23. doi:10.33084/bjop.v7i2.6554

QoL relevant to a particular disease or treatment[10]. This review explores various theories on DFU development, their impact on QoL, and compares different QoL measurement instruments.

DEFINITION OF DIABETIC FOOT ULCERS

Diabetic foot ulcers represent a significant complication of diabetes mellitus, encompassing a spectrum of pathological conditions. The most common manifestation, a full-thickness skin breakdown extending into the dermis, is termed an ulcer[11]. However, DFUs can also present without overt skin compromise, manifesting as infections like cellulitis or osteomyelitis. Diabetic foot ulcers can be further categorized as acute or chronic. Chronic DFUs are particularly concerning, as they exhibit impaired healing and are strongly linked to adverse outcomes such as amputation[12]. Any wound persisting unhealed beyond four weeks warrants heightened clinical attention due to the increased risk of amputation[13].

PATHOPHYSIOLOGY OF DIABETIC FOOT ULCERS

Diabetic foot ulcers are a debilitating complication affecting a significant portion of the diabetic population. Several risk factors are well-established as predisposing individuals to DFU development, including poor glycemic control, peripheral neuropathy, peripheral vascular disease, and immunosuppression[14]. Peripheral neuropathy, in particular, leads to a series of events that contribute to ulcer formation. It causes intrinsic muscle atrophy, resulting in functional anatomical changes like hammer toe formation. These deformities create "high-pressure zones" on the plantar surface of the foot, particularly at the metatarsal heads (**Figure 1**)[15]. Repetitive microtrauma goes unnoticed due to decreased sensation and proprioception, further promoting skin injury. Additionally, atrophy and dislocation of the protective plantar fat pad can occur, leaving the underlying tissue vulnerable to ulceration and infection[16].

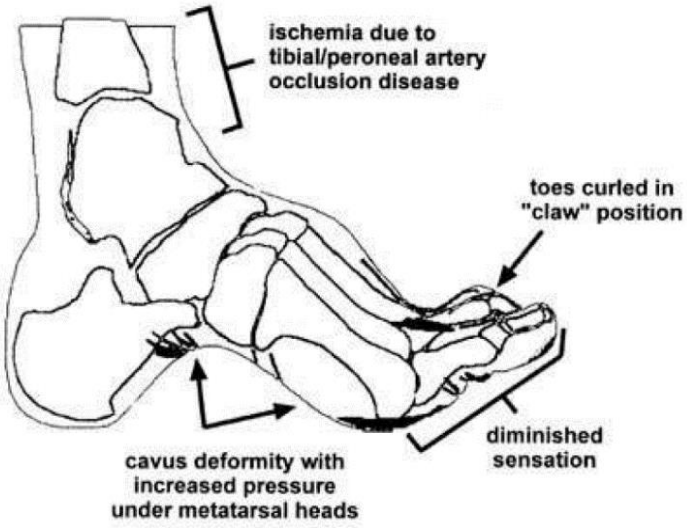


Figure 1. Mechanisms associated with the diabetic foot[15].

Inadequate foot care practices further exacerbate the risk. Failure to use moisturizing creams or neglecting to promptly address early signs of dermal trauma (redness, blisters) can lead to ulceration and potentially, invasive soft tissue infections. Continued ambulation on an injured foot without prompt intervention allows tissue damage to progress[17]. Notably, the presence of neuropathy, foot deformity, or a history of toe amputation dramatically increases the risk of ulceration (by 32 times). In the most severe cases, the destructive process of trauma and infection can penetrate the deep fascia, allowing the spread of infection into the midfoot muscles, joints, and along tendon sheaths. This highlights the critical role of early intervention in preventing the devastating consequences of DFUs, including a significant contribution to lower extremity amputations in diabetic patients[15,18].

MANAGEMENT OF DIABETIC FOOT ULCERS

Diabetic foot ulcers are a serious complication affecting millions of people with diabetes worldwide. These chronic wounds can significantly impact a patient's QoL and pose a major healthcare burden. Effective management of DFUs requires a multidisciplinary approach that addresses the underlying causes, promotes wound healing, and prevents future complications. This includes optimizing blood sugar control, offloading pressure from the wound, proper wound care techniques, and potentially addressing vascular issues. Early intervention and a comprehensive treatment plan are crucial to minimize tissue damage, prevent amputation, and improve patient outcomes[19]. According to Giazcomozzi *et al.*[20], management of DFUs explained in **Figure 2**.

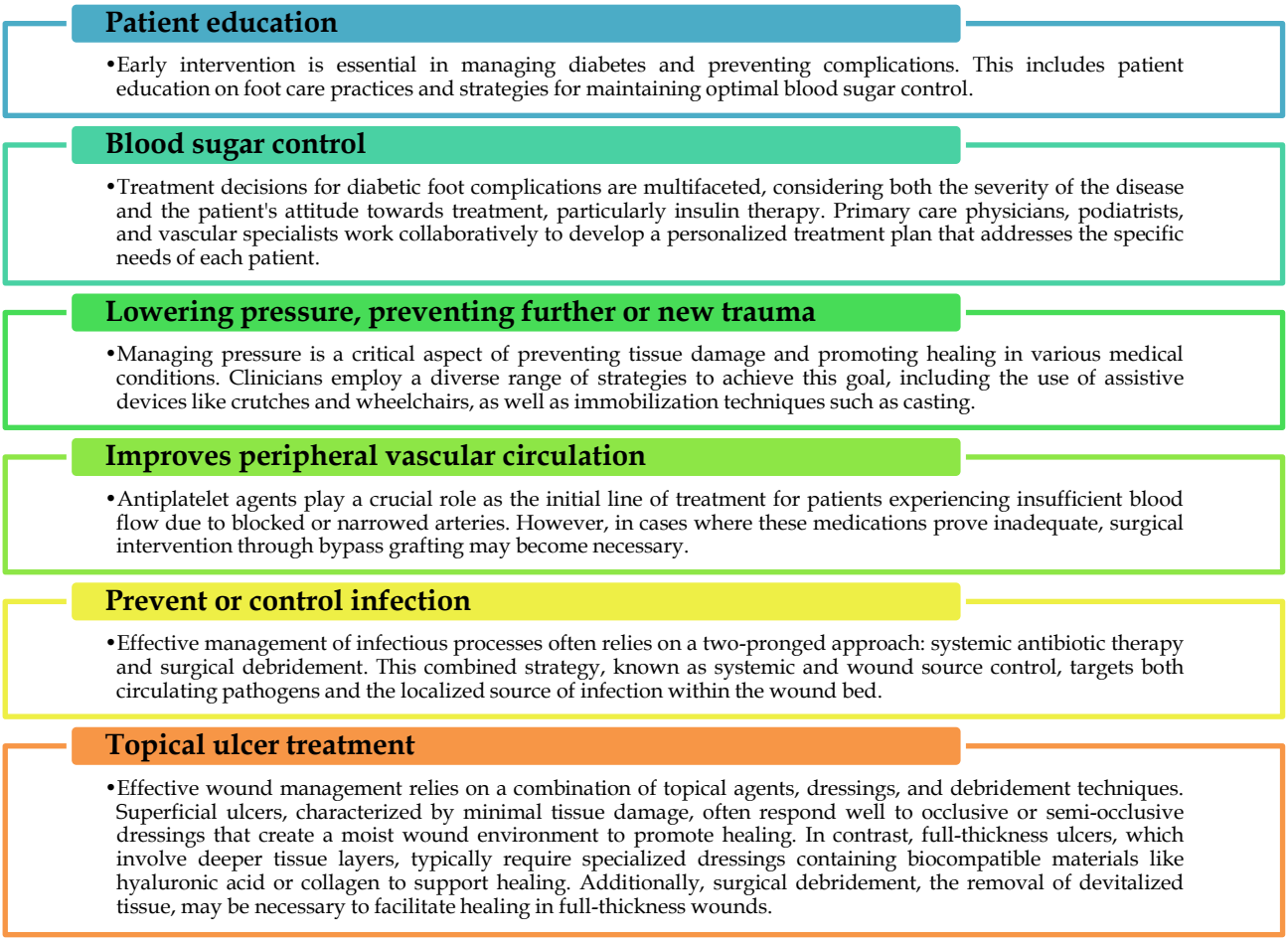


Figure 2. Management of DFUs[20].

DEFINITION OF QUALITY OF LIFE

The concept of QoL goes beyond simply being alive. It encompasses a person's physical and mental well-being, their social connections, and their ability to live a fulfilling life according to their own values and goals. Quality of life is a subjective experience, meaning it varies greatly from person to person. Understanding and measuring QoL is crucial in various fields, including healthcare, social policy, and development studies. By examining factors that contribute to a good QoL, we can work towards improving individual and societal well-being[21]. The World Health Organization defines QoL as a person's perception of their position in life in the context of the value systems and culture in which they live and about their goals, expectations, standards, and concerns[22]. Nemcová *et al.*[23] describes QoL as the patient's level of physical and psychosocial well-being, independence, life satisfaction, and the experience of feelings of success in various areas of daily life.

IMPACT OF DIABETIC FOOT ULCERS ON QUALITY OF LIFE

The presence of DFUs significantly impacts patients' HRQoL. Patients with DFUs experience a multitude of challenges, including disability, reduced mobility, and difficulty performing daily activities. These limitations have a profound negative effect on their physical, social, and psychological well-being[24,25]. Compared to diabetics without DFUs, individuals with these ulcers report higher levels of depression, lower life satisfaction, and poorer psychosocial adjustment to their illness[26].

The assessment of HRQoL is crucial for establishing evidence-based treatment protocols for DFUs[27]. Decreased mobility, a direct consequence of DFUs, significantly hinders patients' ability to perform daily tasks and participate in leisure activities[28]. Studies like Alrub *et al*.[29] support this notion, demonstrating that patients with DFUs have lower scores on physical and mental health components of HRQoL assessment scales.

Diabetic foot ulcers pose a significant physical and psychological burden on patients. Beyond the risk of lower limb amputation (nerve damage or deformity) and recurrent ulceration (39% in the first year, decreasing to 18% and 12.8% in the second and third years, respectively), DFUs can lead to permanent disability, particularly when complicated by infection[30].

The emotional toll of DFUs is substantial. Patients often experience fear of amputation and re-ulceration, contributing to negative mood and sleep disturbances[31]. Proper wound care is crucial to prevent amputation, but the associated costs can be a significant source of stress[32]. Job loss, reported in up to 50% of DFU patients, further diminishes self-esteem, particularly among younger individuals[6]. Compared to diabetic patients without DFUs, those with DFUs experience higher levels of anger, frustration, depression, and powerlessness[33,34].

The financial burden of DFUs is substantial. Baroroh *et al*.[35] reported that the average monthly cost of managing type 2 diabetes with complications ranged from IDR 128,143 to IDR 1,174,342, with DFU medication costs alone reaching IDR 127,094 per day. Inpatient management focused on infection control with antibiotics, often injectables, further inflates medication costs.

QUALITY OF LIFE MEASUREMENT INSTRUMENTS

Diabetic Foot Ulcer Scale-Short Form (DFS-SF)

The diabetic foot ulcer scale (DFS) is a comprehensive instrument for assessing QoL in individuals with DFUs. It consists of 58 items categorized into 11 domains encompassing various aspects of well-being, such as leisure time, physical health, daily activities, and emotional well-being[36]. A shorter version, the diabetic foot ulcer scale-short form (DFS-SF), was developed with 29 items grouped into six subscales: leisure, daily life dependence, negative emotions, physical health, wound concerns, and wound care burden[37]. Both the DFS and DFS-SF have been psychometrically evaluated, demonstrating good validity and reliability. Each item utilizes a 5-point Likert scale, ranging from "not at all" or "never" (1) to "a lot," "always," or "very often" (5).

The DFS-SF's adaptability and validity have been established across diverse populations. For instance, a Brazilian study prospectively adapted and validated the DFS-SF for the Spanish-speaking population[38]. Similar validations have been conducted for Polish[39], Greek[40], Chinese[41], Dutch[42], Korean[43], and Indian[44]. These translated versions consistently demonstrate good psychometric properties, highlighting the instrument's versatility for cross-cultural use.

Cardiff Wound Impact Scale (CWIS)

The Cardiff wound impact schedule (CWIS) is a validated tool specifically designed to assess HRQoL in individuals with chronic wounds, such as leg ulcers and DFUs[45]. This 47-item questionnaire comprises four scales:

1. Demographic and clinical characteristics (3 items)
2. Global HRQoL (1 item)
3. Satisfaction with HRQoL (1 item)
4. Impact of the wound on lifestyle (42 items)

The last scale delves deeper into three key domains:

1. Social life (14 items): explores stress (7 items) and experiences (7 items) related to the wound.
2. Well-being (7 items)

3. Physical symptoms and everyday living (24 items): further divided into stress (12 items) and experience (12 items) associated with the wound.

All three domains utilize a 5-point Likert scale ranging from "not at all" to "always" for scoring. The total CWIS score ranges from 0 (indicating poorer HRQoL) to 100 (indicating higher HRQoL)[46].

36-Item Short-Form Health Survey (SF-36)

The 36-item short-form health survey (SF-36)[47] is a widely used generic instrument for assessing patient health status. This multicultural scale comprises 36 questions categorized into eight domains: physical functioning (PF; 10 items), general health (GH; 5 items), role limitations due to physical health (RP; 4 items), bodily pain (BP; 2 items), social functioning (SF; 2 items), vitality (VT; 4 items), role limitations due to emotional problems (RE; 3 items), and mental health (MH; 5 items). Each domain is scored on a 0-100 scale, with higher scores indicating better health.

EuroQoL 5D Health Utility Index (EQ-5D)

The EuroQoL 5D health utility index (EQ-5D) instrument is a widely used and validated tool for assessing HRQoL in individuals with various chronic conditions[48,49]. It evaluates patients' physical, mental, and social functioning across five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension has three levels of severity: no problems, some problems, and extreme problems. A single index score (EQ-5D index value) is derived by combining these dimensions using established value sets, such as UK weights. Additionally, the EQ-5D incorporates a visual analog scale (VAS) where participants rate their current health status on a 0 (worst) to 100 (best) scale[38]. Three versions of the EQ-5D exist: EQ-5D-3L, EQ-5D-5L, and EQ-5D-y. The 5-level version (EQ-5D-5L) was introduced in 2009 by the EuroQol Group to enhance the instrument's sensitivity and reduce ceiling effects compared to the earlier 3-level version[50].

COMPARISON BETWEEN INSTRUMENTS MEASURING QUALITY OF LIFE

Two main types of instruments are used to measure QoL: generic questionnaires and disease-specific questionnaires. Generic instruments assess the impact of various health conditions on overall health across different domains. They are particularly useful when patients have multiple chronic conditions or experience side effects from medications. Conversely, disease-specific questionnaires focus solely on aspects relevant to a particular disease, allowing for more sensitive detection of changes in QoL resulting from treatment or intervention[51].

A study by Yordanova *et al*.[52] compared the EQ-5D and SF-36 instruments, finding the EQ-5D to be less responsive to variations in patient health status compared to the SF-36. Macioch *et al*.[39] reported that the Polish translation and validation of the DFS-SF instrument demonstrated superior psychometric performance compared to the SF-36. While the CWIS is not specifically designed for diabetic foot ulcers (DFUs), it can effectively discriminate between healed and unhealed ulcers. Additionally, Jeffcoate *et al*.[53] demonstrated the CWIS's sensitivity to wound healing in a randomized clinical trial evaluating different dressing types for DFUs. The heightened sensitivity of disease-specific instruments like the DFS-SF compared to generic instruments can be attributed, in part, to the extensive use of generic instruments in past QoL studies[38].

CONCLUSION

Diabetic foot ulcers significantly impact patients' QoL and are a major concern due to the increased risk of infection and morbidity. While established standards of care exist, assessing therapeutic outcomes beyond clinical success is crucial. This includes evaluating patient QoL and cost-effectiveness through pharmacoeconomic studies using cost-utility analysis (CUA). Our review highlights the trade-offs between generic and disease-specific patient-reported outcome measures (PROMs) used to assess QoL. Generic instruments offer the advantage of facilitating comparisons across diverse populations, conditions, and interventions. However, they may be less sensitive to specific aspects of a patient's experience. Conversely, disease-specific instruments demonstrate increased clinical sensitivity and responsiveness to changes in a patient's condition. However, their applicability is limited to specific patient groups and conditions.

ACKNOWLEDGMENT

The authors gratefully acknowledge the financial support provided by the Directorate of Research and Development, Universitas Indonesia, under the *Publikasi Terindeks Internasional* (PUTI) Grant 2024 (Grant number: NKB-39/UN2.RST/HKP.05.00/2024).

AUTHORS' CONTRIBUTION

Conceptualization: Rani Sauriasari, Ratu Ayu Dewi Sartika
Data curation: -
Formal analysis: -
Funding acquisition: -
Investigation: -
Methodology: -
Project administration: -
Resources: -
Software: -
Supervision: -
Validation: -
Visualization: -
Writing - original draft: Khairunisa Qomariyanti
Writing - review & editing: Khairunisa Qomariyanti, Rani Sauriasari, Ratu Ayu Dewi Sartika

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Magliano DJ, Boyko EJ; IDF Diabetes Atlas 10th edition scientific committee. IDF Diabetes Atlas. 10th edition. Brussels: International Diabetes Federation; 2021. PMID: 35914061

2. Dayya D, O'Neill OJ, Huedo-Medina TB, Habib N, Moore J, Iyer K. Debridement of Diabetic Foot Ulcers. Adv Wound Care. 2022;11(12):666–86. DOI: 10.1089/wound.2021.0016; PMCID: PMC9527061; PMID: 34376065

3. Jiang P, Li Q, Luo Y, Luo F, Che Q, Lu Z, et al. Current status and progress in research on dressing management for diabetic foot ulcer. Front Endocrinol. 2023;14:1221705. DOI: 10.3389/fendo.2023.1221705; PMCID: PMC10470649; PMID: 37664860

4. Chen CY, Wu RW, Hsu MC, Hsieh CJ, Chou MC. Adjunctive Hyperbaric Oxygen Therapy for Healing of Chronic Diabetic Foot Ulcers: A Randomized Controlled Trial. J Wound Ostomy Continence Nurs. 2017;44(6):536–545. DOI: 10.1097/won.0000000000000374; PMID: 28968346

5. Everett E, Mathioudakis N. Update on Management of Diabetic Foot Ulcers. Ann N Y Acad Sci. 2018;1411(1):153–65. DOI: 10.1111/nyas.13569; PMCID: PMC5793889; PMID: 29377202

6. Jayalakshmi MS, Thenmozhi P, Vijayaragavan R. Impact of chronic wound on quality of life among diabetic foot ulcer patients in a selected hospital of Guwahati, Assam, India. Ayu. 2020;41(1):19-23. DOI: 10.4103/ayu.ayu_33_20; PMCID: PMC8415236; PMID: 34566380

7. Sriram S, Chack LE, Ramasamy R, Ghasemi A, Ravi TK, Sabzghabaee AM. Impact of Pharmaceutical Care on Quality of Life in Patients with Type 2 Diabetes Mellitus. J Res Med Sci. 2011;16(Suppl 1):412–8. PMCID: PMC3252774; PMID: 22247727

8. Sallom H, Abdi A, Halboup AM, Başgut B. Evaluation of pharmaceutical care services in the Middle East Countries: a review of studies of 2013-2020. BMC Public Health. 2023;23(1):1364. DOI: 10.1186/s12889-023-16199-1; PMCID: PMC10351150; PMID: 37461105

9. Németh G. Health related quality of life outcome instruments. Eur Spine J. 2006;15 (Suppl 1):S44-51. DOI: 10.1007/s00586-005-1046-8; PMCID: PMC3454556; PMID: 16320032

10. Kiling IY, Kiling-Bunga BN. Pengukuran dan Faktor Kualitas Hidup pada Orang Usia Lanjut. J Health Behav Sci. 2019;1(3):149–65. DOI: 10.35508/jhbs.v1i3.2095

11. Jais S. Various Types of Wounds That Diabetic Patients Can Develop: A Narrative Review. Clin Pathol. 2023;16:2632010X231205366. DOI: 10.1177/2632010X231205366

12. Raja JM, Maturana MA, Kayali S, Khouzam A, Efeovbokhan N. Diabetic foot ulcer: A comprehensive review of pathophysiology and management modalities. World J Clin Cases. 2023;11(8):1684-94. DOI: 10.12998/wjcc.v11.i8.1684; PMCID: PMC10037283; PMID: 36970004

13. Frykberg RG, Banks J. Challenges in the Treatment of Chronic Wounds. Adv Wound Care. 2015;4(9):560-82. DOI: 10.1089/wound.2015.0635; PMCID: PMC4528992; PMID: 26339534

14. Lim JZM, Ng NSL, Thomas C. Prevention and Treatment of Diabetic Foot Ulcers. J R Soc Med. 2017;110(3):104–9. DOI: 10.1177/0141076816688346; PMCID: PMC5349377; PMID: 28116957

15. Bandyk DF. The Diabetic Foot: Pathophysiology, Evaluation, and Treatment. Semin Vasc Surg. 2018;31(2–4):43–8. DOI: 10.1053/j.semvascsurg.2019.02.001; PMID: 30876640

16. Nowak NC, Menichella DM, Miller R, Paller AS. Cutaneous innervation in impaired diabetic wound healing. Transl Res. 2021;236:87-108. DOI: 10.1016/j.trsl.2021.05.003; PMCID: PMC8380642; PMID: 34029747

17. Sari Y, Yusuf S, Haryanto H, Sumeru A, Saryono S. The barriers and facilitators of foot care practices in diabetic patients in Indonesia: A qualitative study. Nurs Open. 2022;9(6):2867-77. DOI: 10.1002/nop2.993; PMCID: PMC9584460; PMID: 34411445

18. Wang X, Yuan CX, Xu B, Yu Z. Diabetic foot ulcers: Classification, risk factors and management. World J Diabetes. 2022;13(12):1049-65. DOI: 10.4239/wjd.v13.i12.1049; PMCID: PMC9791567; PMID: 36578871

19. Edmonds M, Manu C, Vas P. The current burden of diabetic foot disease. J Clin Orthop Trauma. 2021;17:88-93. DOI: 10.1016/j.jcot.2021.01.017; PMCID: PMC7919962; PMID: 33680841

20. Giazcomozzi C, Sartor CD, Telles R, Uccioli L, Sacco ICN. Ulcer-risk Classification and Plantar Pressure Distribution in Patients with Diabetic Polyneuropathy: Exploring the Factors that can Lead to Foot Ulceration. Ann Ist Super Sanità. 2018;54(4):284–93. DOI: 10.4415/ann_18_04_04; PMID: 30575564

21. Aqtam I, Ayed A, Zaben K. Quality of Life: Concept Analysis. Saudi J Nurs Health Care. 2023;6(1):10-5. DOI: 10.36348/sjnhc.2023.v06i01.003

22. Beslerová S, Dzuričková J. Quality of Life Measurements in EU Countries. Proced Econ Financ. 2014;12:37–47. DOI: 10.1016/s2212-5671(14)00318-9

23. Nemcová J, Hlinková E, Farský I, Žiaková K, Jarošová D, Zeleníková R, et al. Quality of Life in Patients with Diabetic Foot Ulcer in Visegrad Countries. J Clin Nurs. 2017;26(9–10):1245–56. DOI: 10.1111/jocn.13508; PMID: 27539540

24. Coffey L, Mahon C, Gallagher P. Perceptions and experiences of diabetic foot ulceration and foot care in people with diabetes: A qualitative meta-synthesis. Int Wound J. 2019;16(1):183–210. DOI: 10.1111/iwj.13010; PMCID: PMC7949356; PMID: 30393976

25. Vedhara K, Beattie A, Metcalfe C, Roche S, Weinman J, Cullum N, et al. Development and preliminary evaluation of a psychosocial intervention for modifying psychosocial risk factors associated with foot re-ulceration in diabetes. Behac Res Ther. 2012;50(5):323–32. DOI: 10.1016/j.brat.2012.02.013; PMID: 22459731

26. Crocker RM, Palmer KNB, Marrero DG, Tan TW. Patient perspectives on the physical, psycho-social, and financial impacts of diabetic foot ulceration and amputation. J Diabetes Complications. 2021;35(8):107960. DOI: 10.1016/j.jdiacomp.2021.107960; PMCID: PMC8316286; PMID: 34059410

27. Jaksa PJ, Mahoney JL. Quality of life in patients with diabetic foot ulcers: Validation of the Cardiff Wound Impact Schedule in a Canadian population. Int Wound J. 2010;7(6):502–7. DOI: 10.1111/j.1742-481X.2010.00733.x; PMCID: PMC7951786; PMID: 20860554

28. Goodridge D, Trepman E, Embil JM. Health-related quality of life in diabetic patients with foot ulcers: literature review. J Wound Ostomy Continence Nurs. 2005;32(6):368–77. DOI: 10.1097/00152192-200511000-00007; PMID: 16301902

29. Alrub AA, Hyassat D, Khader YS, Bani-Mustafa R, Younes N, Ajlouni K. Factors Associated with Health-Related Quality of Life among Jordanian Patients with Diabetic Foot Ulcer. J Diabetes Res. 2019;2019:4706720. DOI: 10.1155/2019/4706720; PMCID: PMC6360050; PMID: 30800685

30. Khunkaew S, Fernandez R, Sim J. Health-related quality of life among adults living with diabetic foot ulcers: a meta-analysis. Qual Life Res. 2019;28(6):1413–27. DOI: 10.1007/s11136-018-2082-2; PMID: 30565072

31. Muzakkir, Yunding M, Yunding J, Harli K. The Relationship of Psychological Stress on Diabetic Wound Healing Processes: A Literature Review. J Pharm Negat Results. 2022;13(S01):879–82. DOI: 10.47750/pnr.2022.13.s01.106

32. Kurdi F, Kholis AH, Hidayah N, Fitriasari M. Stress Pasien Dengan Ulkus Kaki Diabetikum Di Al Hijrah Wound Care Center Jombang. J Ilmiah Keperawatan Sci J Nurs. 2020;6(1):128–36. DOI: 10.33023/jikep.v6i1.577

33. Sari Y, Purnawan I, Taufik A, Sumeru A. Quality of Life and Associated Factors in Indonesian Diabetic Patients with Foot Ulcers. Nurse Media J Nurs. 2018;8(1):13-24. DOI: 10.14710/nmjn.v8i1.16815

34. Utami DT, Karim D, Agrina. Faktor-faktor yang Mempengaruhi Kualitas Hidup Pasien Diabetes Mellitus dengan Ulkus Diabetikum. J Online Mahasiswa Program Studi Ilmu Keperawatan Universitas Riau. 2014;1(2):1–7.

35. Baroroh F, Solikah WY, Urfiyya QA. Analisis Biaya Terapi Diabetes Melitus Tipe 2 Di Rumah Sakit PKU Muhammadiyah Bantul Yogyakarta. J Farmasi Sains Praktis. 2016;1(2):11–22. DOI: 10.31603/pharmacy.v1i2.230

36. Abetz L, Sutton M, Brady L, McNulty P, Gagnon DD. The Diabetic Foot Ulcer Scale (DFS): a quality of life instrument for use in clinical trials. Pract Diabetes Int. 2002;19(6):167–75. DOI: 10.1002/pdi.356

37. MAPI Research Trust. DFS-SF Information Booklet, 1st Edition (Issue February). Lyon: MAPI Research Trust; 2011.

38. Martinez-Gonzalez D, Dòria M, Martínez-Alonso M, Alcubierre N, Valls J, Verdú-Soriano J, et al. Adaptation and Validation of The Diabetic Foot Ulcer Scale-Short Form in Spanish Subjects. J Clin Med. 2020;9(8):2497. DOI: 10.3390/jcm9082497; PMCID: PMC7465700; PMID: 32756508

39. Macioch T, Sobol E, Krakowiecki A, Mrozikiewicz-Rakowska B, Kasprowicz M, Hermanowski T. Health related quality of life in patients with diabetic foot ulceration - translation and Polish adaptation of Diabetic Foot Ulcer Scale short form. Health Qual Life Outcomes. 2017;15(1):15. DOI: 10.1186/s12955-017-0587-y; PMCID: PMC5251239; PMID: 28109278

40. Kontodimopoulos N, Veniou A, Tentolouris N, Niakas D. Validity and Reliability of the Greek Version of the Diabetic Foot Ulcer Scale-Short Form (DFS-SF). Hormones. 2016;15(3):394–403. DOI: 10.14310/horm.2002.1682; PMID: 27394704

41. Ma L, Ma W, Lin S, Li Y, Ran X. Adaptation and Validation of the Diabetic Foot Ulcer Scale-Short Form Scale for Chinese Diabetic Foot Ulcers Individuals. Int J Environ Res Public Health. 2022;19(21):14568. DOI: 10.3390/ijerph192114568; PMCID: PMC9659257; PMID: 36361446

42. Rezaie W, Lusendi F, Doggen K, Matricali G, Nobels F. Health-related Quality of Life in Patients with Diabetic Foot Ulceration: Study Protocol for Adaptation and Validation of Patient-reported Outcome Measurements (PROMs) in Dutch-speaking Patients. BMJ Open. 2019;9(12):e034491. DOI: 10.1136/bmjopen-2019-034491; PMCID: PMC7008415; PMID: 31874898

43. Lee YN. Translation and Validation of the Korean Version of the Diabetic Foot Ulcer Scale-Short Form. Int Wound J. 2019;16(Suppl 1):3–12. DOI: 10.1111/iwj.13025; PMCID: PMC7948823; PMID: 30793855

44. Raju BN, Mateti UV, Mohan R, D'Souza C, Shastry CS, D'Souza N. Transcultural adaptation of the Malayalam version of the diabetic foot ulcer scale-short form. Clin Epidemiol Glob Health. 2022;18:101190. DOI: 10.1016/j.cegh.2022.101190

45. Price P, Harding K. Cardiff Wound Impact Schedule: the development of a condition-specific questionnaire to assess health-related quality of life in patients with chronic wounds of the lower limb. Int Wound J. 2004;1(1):10–7. DOI: 10.1111/j.1742-481x.2004.00007.x; PMCID: PMC7951606; PMID: 16722893

46. Granado-Casas M, Martinez-Gonzalez D, Martínez-Alonso M, Dòria M, Alcubierre N, Valls J, et al. Psychometric validation of the cardiff wound impact schedule questionnaire in a spanish population with diabetic foot ulcer. J Clin Med. 2021;10(17):4023. DOI: 10.3390/jcm10174023; PMCID: PMC8432453; PMID: 34501471

47. Abbasi-Ghahramanloo A, Soltani-Kermanshahi M, Mansori K, Khazaei-Pool M, Sohrabi M, Baradaran HR, et al. Comparison of sf-36 and whoqol-bref in measuring quality of life in patients with type 2 diabetes. Int J Gen Med. 2020;13:497–506. DOI: 10.2147/IJGM.S258953; PMCID: PMC7434519; PMID: 32884330

48. Lee WJ, Song KH, Noh JH, Choi YJ, Jo MW. Health-related quality of life using the EuroQol 5D questionnaire in Korean patients with type 2 diabetes. J Korean Med Sci. 2012;27(3):255–60. DOI: 10.3346/jkms.2012.27.3.255; PMCID: PMC3286771; PMID: 22379335

49. Matter-Walstra K, Klingbiel D, Szucs T, Pestalozzi BC, Schwenkglenks M. Using the EuroQol EQ-5D in Swiss cancer patients, which value set should be applied? Pharmacoeconomics.2014;32(6):591–9. DOI:10.1007/s40273-014-0151-0; PMID: 24671924

50. Abedini MR, Bijari B, Miri Z, Shakhs FS, Abbasi A. The quality of life of the patients with diabetes type 2 using EQ-5D-5 L in Birjand. Health Qual Life Outcomes. 2020;18(1):18. DOI: 10.1186/s12955-020-1277-8; PMCID: PMC6990543; PMID: 32000785

51. Kheir NM, van Mil JWF, Shaw JP, Sheridan JL. Health-related Quality of Life in Pharmaceutical Care - Targeting an Outcome That Matters. Pharm World Sci. 2004;26(3):125–8. DOI: 10.1023/b:phar.0000026811.37414.4f; PMID: 15230357

52. Yordanova S, Petkova V, Petrova G, Dimitrov M, Naseva E, Dimitrova M, et al. Comparison of health-related quality-of-life measurement instruments in diabetic patients. Biotechnol Biotechnol Equip. 2014;28(4):769–74. DOI: 10.1080/13102818.2014.935572; PMCID: PMC4434102; PMID: 26019561

53. Jeffcoate WJ, Price PE, Phillips CJ, Game FL, Mudge E, Davies S, et al. Randomised controlled trial of the use of three dressing preparations in the management of chronic ulceration of the foot in diabetes. Health Technol Assess. 2009;13(54):1–86. DOI: 10.3310/hta13540; PMID: 19922726

Borneo Journal of Pharmacy
Vol 7 Issue 2 May 2024
Pages 224 – 232

https://journal.umpr.ac.id/index.php/bjop/article/view/4909
DOI: https://doi.org/10.33084/bjop.v7i2.4909
e-ISSN: 2621-4814

Research Article

Trend in the Utilization of Antipsychotics in the National Health Coverage Era in Indonesia: A Cross-Sectional Study

Julaeha [1*]

Verra Yuliana [2]

Josephine Paramita Ayuningtyas [3]

[1] Research Centre for Preclinical and Clinical Medicine, National Research and Innovation Agency of the Republic of Indonesia, Central Jakarta, Special Capital Region of Jakarta, Indonesia
[2] Pharmacy Department, Menur National Mental Hospital, Surabaya, East Java, Indonesia
[3] East Java Provincial Health Service, Surabaya, East Java, Indonesia

*email: jula002@brin.go.id; phone: +6281392932832

Keywords:
Antipsychotics
Cost
Drug utilization study
Mental health
Schizophrenia

Abstract

The utilization pattern of antipsychotics has undergone significant changes since the introduction of atypical antipsychotics. Currently, medication for patients with schizophrenia predominantly uses atypical antipsychotics rather than typical antipsychotics. This study aimed to present the updated utilization pattern of antipsychotics among Indonesians. A cross-sectional study was conducted in 2019-2020 at the National Mental Hospital in Indonesia. Data were collected from medication-used reports from either inpatients or outpatients. A descriptive analysis was conducted to present the pattern and the annual total cost for each antipsychotic used. The pattern of typical antipsychotics used from 2019 to 2020 was likely to decline. The total cost estimated for typical antipsychotics in 2019 was IDR 475 million, and IDR 420 million in 2020. Trifluoperazine 5 mg was the most commonly typical antipsychotic used, followed by chlorpromazine 100 mg and haloperidol 5 mg. Eventually, the pattern of atypical antipsychotics used was likely to increase. The total cost was estimated at IDR 3.2 billion in 2019 and IDR 3.8 billion in 2020. Risperidone 2 mg was the most commonly atypical antipsychotic used, followed by clozapine 25 mg and risperidone 3 mg. This study proves the trend toward increased atypical antipsychotics used. Accordingly, the cost of schizophrenia treatment was elevated.

Received: April 2nd, 2023
1st Revised: November 7th, 2023
2nd Revised: February 1st, 2024
Accepted: March 13th, 2024
Published: May 30th, 2024

INTRODUCTION

Mental disorders represent a significant global health burden, accounting for nearly one-third of all years lived with disability (YLDs) according to the World Health Organization (WHO)[1]. Antipsychotics have become a cornerstone of treatment for various mental health conditions, particularly schizophrenia[2]. The introduction of chlorpromazine in the 1950s revolutionized schizophrenia treatment, followed by the development of additional antipsychotics. This led to a rise in antipsychotic prescribing for a broader range of mental disorders[3].

Two main classes of antipsychotics are typically used: typical and atypical. Typical antipsychotics, such as haloperidol, chlorpromazine, and trifluoperazine, effectively manage positive symptoms of schizophrenia but can cause extrapyramidal side effects (EPS)[4]. Atypical antipsychotics, including clozapine, risperidone, quetiapine, olanzapine, and aripiprazole, offer broader symptom control (positive and negative) with a lower risk of EPS[5]. While atypical antipsychotic monotherapy is currently preferred for schizophrenia due to its improved side effect profile and positive impact on quality of life, combination or polypharmacy regimens are still commonly used[2,6]. Polypharmacy can lead to issues like non-adherence, increased drug-drug interactions, and adverse effects, including metabolic problems and higher medication costs[7].

How to cite: Julaeha, Yuliana V, Ayuningtyas JP. Trend in the Utilization of Antipsychotics in the National Health Coverage Era in Indonesia: A Cross-Sectional Study. Borneo J Pharm. 2024;7(2):224-32. doi:10.33084/bjop.v7i2.4909

Schizophrenia is one of nine chronic diseases covered by Indonesia's national health program[8]. The economic burden of schizophrenia is significant, with estimates suggesting annual costs of 100 billion USD for rehospitalizations and 290 USD per patient due to non-adherence[9]. In Indonesia alone, the annual cost of illness per schizophrenia patient is estimated at 32 million IDR[10].

As defined by the WHO, drug utilization refers to "the marketing, distribution, prescription, and use of drugs in society with special emphasis on the resulting medical, social, and economic consequences"[11,12]. Drug utilization studies provide valuable insights into medication prescribing patterns and their use in disease management. Given the widespread use of antipsychotics and the potential economic and health-related consequences associated with polypharmacy, this study aims to describe and evaluate the utilization patterns and costs of antipsychotics in the treatment of mental disorders.

MATERIALS AND METHODS

Materials

Data on antipsychotic usage patterns and annual costs were obtained from the Pharmacy Department of Menur Mental Hospital, Surabaya, East Java, Indonesia. This study was designed to adhere to the ethical principles outlined in the Declaration of Helsinki[13]. The study protocol was approved by the Ethics Committee of Menur Mental Hospital, Surabaya, East Java, Indonesia (reference number: 070/7556/305/2019).

Methods

A cross-sectional study was conducted from 2019 to 2020 to collect data on medication use among inpatients and outpatients at the National Mental Hospital in Indonesia. The study included patients with a diagnosed mental disorder, regardless of their inpatient or outpatient status. Data were collected retrospectively from pharmacy department reports spanning the two-year period.

Data analysis

This study employed a descriptive analysis to investigate the epidemiology of mental disorders, antipsychotic medication utilization patterns, and associated costs within the hospital setting. We collected data on the following epidemiology patterns of mental disorders, percentage of typical and atypical antipsychotics used, pattern of long-acting injection (LAI) use, and annual total cost for each antipsychotic used.

RESULTS AND DISCUSSION

We analyzed antipsychotic utilization data retrieved from the Pharmacy Department's usage reports for a two-year period, January 2019 to December 2020. Schizophrenia was the most frequently diagnosed mental health disorder in tertiary referral hospitals, accounting for 64.87% of cases in 2019 and 48.52% in 2020. These findings suggest that a substantial proportion of antipsychotic medications dispensed are likely used for schizophrenia treatment.

Patterns of antipsychotics use

Schizophrenia emerged as the most prevalent mental health disorder among both inpatients and outpatients in the present study, followed by disorders stemming from brain damage and dysfunction. These findings align with previous research by Sweileh *et al.*[3], Patted *et al.*[4], and Thakkar *et al.*[14], who identified schizophrenia as a leading indication for antipsychotic medication. As illustrated in **Figure 1**, the use of atypical antipsychotics steadily increased from 57% in 2019 to 63% in 2020. Conversely, the use of typical antipsychotics exhibited a declining trend, dropping from 43% to 37% over the same period.

Our study revealed a rising trend in the use of atypical antipsychotics for schizophrenia treatment. This suggests a shift in treatment practices, with atypical medications becoming the preferred first-line therapy. These findings contrast with studies from Palestine and Sudan, where typical antipsychotics reportedly comprised 85.7% and 93.9% of prescribed medications, respectively[3,15]. This difference aligns with observations from other studies conducted in India, the USA, Japan, and Canada, which documented widespread use of atypical antipsychotics for mental illness[2,16-18]. It's important to acknowledge that

economic considerations often influence antipsychotic prescribing patterns in low-income countries, where the lower cost of typical medications may lead to their continued dominance[19].

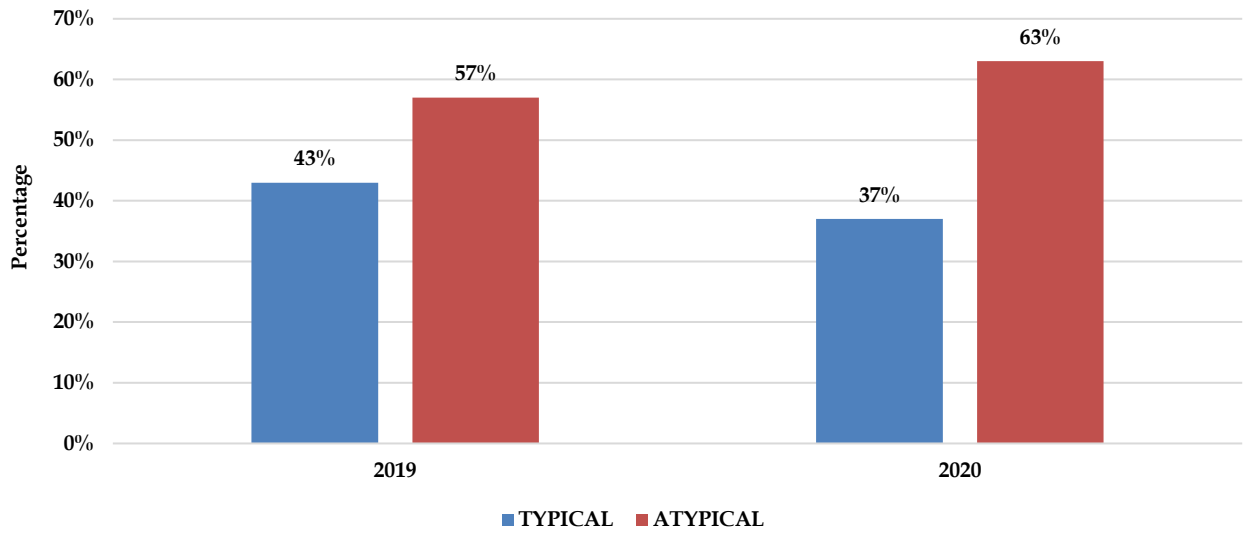


Figure 1. Use of typical and atypical antipsychotics in 2019 and 2020.

Figure 2 depicts the most frequently prescribed typical antipsychotics in 2019-2020. Trifluoperazine 5 mg emerged as the most commonly used medication (56.53%), followed by chlorpromazine 100 mg (21.95%) and haloperidol 5 mg (16.84%). **Figure 3** illustrates the most widely used atypical antipsychotics during the same period. Risperidone 2 mg stood out as the most prescribed medication in this category (33.23%), with clozapine 25 mg (24.51%) and risperidone 3 mg (22.07%) following closely behind.

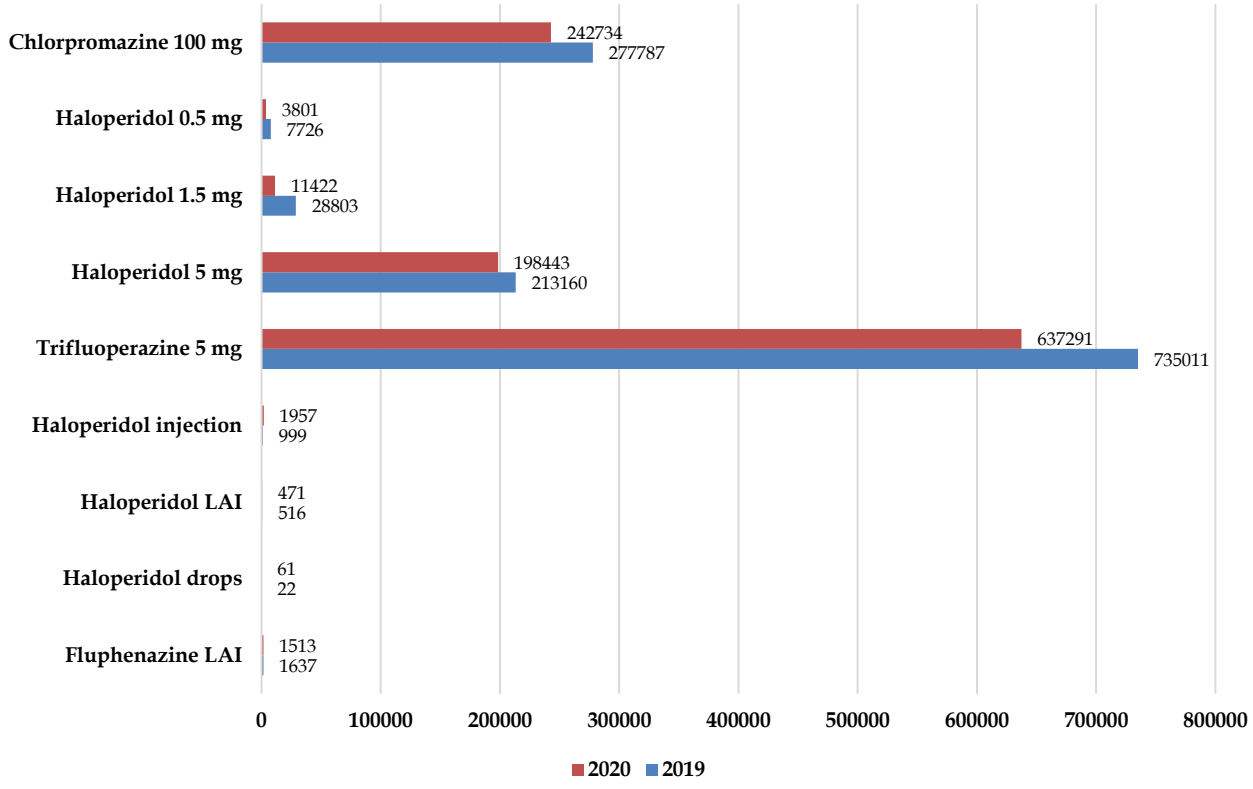


Figure 2. Number of typical antipsychotics use in 2019 and 2020.

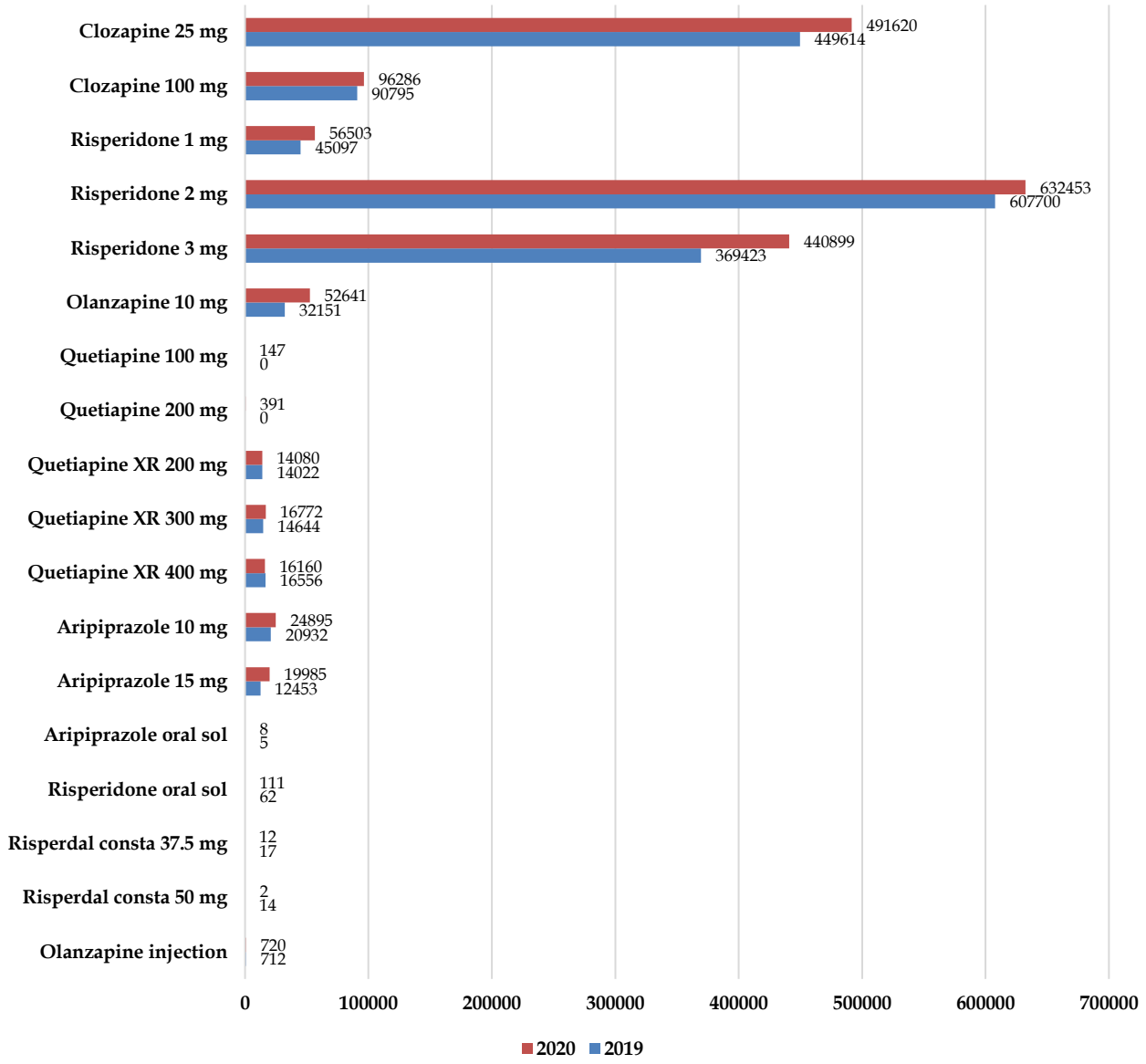


Figure 3. Number of atypical antipsychotics use in 2019 and 2020.

The National Mental Hospital, specializing in complex cases, may explain the observed higher use of clozapine, the second most commonly prescribed atypical antipsychotic. Clozapine is often reserved for patients with refractory schizophrenia or those who do not respond adequately to other atypical antipsychotics, aligning with established clinical guidelines for first-episode schizophrenia treatment, which recommend risperidone and aripiprazole as first-line options[20]. Trifluoperazine emerged as the most commonly prescribed typical antipsychotic in our study. This aligns with findings from a study in India[14]. However, these observations differ from reported practices in Palestine and Sudan, where chlorpromazine and haloperidol, respectively, were the preferred choices[15]. The cost-effectiveness of chlorpromazine and haloperidol might explain their wider use in low-income settings compared to middle- or high-income countries.

Risperidone, with a variable dosage of 2 mg, was the most commonly used atypical antipsychotic in this study. This finding is consistent with research conducted in Korea, Spain, Pakistan, and Canada, where risperidone was similarly favored[19,21-23]. Several factors likely contribute to risperidone's widespread use, including its affordability, availability in various dosage forms, and a generally lower risk of metabolic side effects compared to olanzapine and quetiapine[24]. However, our results diverge from those reported in Turkey, the USA, and India, where quetiapine and olanzapine, respectively, were prescribed more frequently[16,25].

Annual cost of antipsychotic

Our analysis of antipsychotic medication costs revealed distinct trends for atypical and typical medications over the two-year study period (**Figure 4**). The total annual cost of atypical antipsychotics exhibited an increasing trend, rising from IDR 3.2 billion in 2019 to IDR 3.8 billion in 2020. Conversely, the total annual cost of typical antipsychotics showed a decreasing trend, with costs dropping from IDR 475 million in 2019 to IDR 420 million in 2020. **Table I** provides a detailed breakdown of the total costs for each atypical and typical antipsychotic medication, respectively.

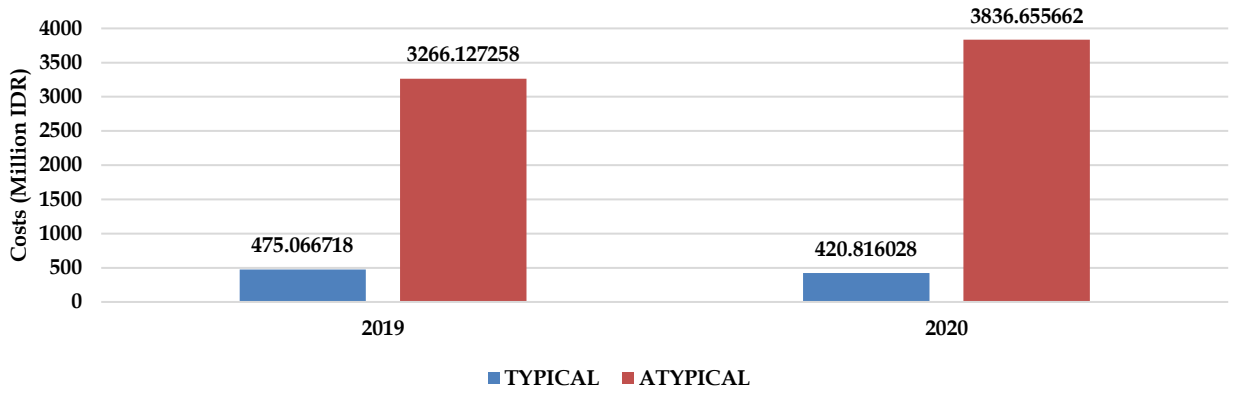


Figure 4. Total cost of typical and atypical antipsychotics in 2019 and 2020.

Table I. Total cost of typical and atypical antipsychotics in 2019 and 2020.

Name of drug	Strength of dose	Total cost of drug per year (IDR)	
		2019	2020
Haloperidol	0.5 mg	424,930	212,856
	1.5 mg	1,699,377	673,898
	5 mg	12,789,600	11,906,580
Haldol decanoate injection*	50 mg/mL	52,270,800	47,712,300
Lodomer injection	2 mg/mL	11,373,615	16,443,355
Lodomer drops	2 mg/mL	439,010	1,217,255
Trifluoperazine	5 mg	205,343,621	182,048,405
Stelazine	5 mg	17,731,424	2,345,207
Sikzonoate injection*	25 mg/mL	104,745,082	96,810,666
Chlorpromazine	100 mg	52,223,956	60,248,000
Cepezet	100 mg	-	431,145
Abilify	10 mg	482,147,688	573,431,430
	15 mg	345,675,600	559,871,136
	Oral solution	-	3,941,580
Arinia	15 mg	51,736,000	85,207,500
Clozapine	25 mg	460,079,699	501,580,240
	100 mg	291,872,472	310,748,880
Clorilex	25 mg	11,984,400	8,910,440
	100 mg	8,303,400	15,705,228
Sizoril	25 mg	306,295,640	106,272,738
	100 mg	-	2,727,296
Risperidone	1 mg	10,823,280	13,560,720
	2 mg	159,669,580	165,935,077
	3 mg	162,546,120	193,995,560
Rizodal	2 mg	313,072,500	76,773,150
Neripros	2 mg	21,956,040	7,462,917
Neripros oral solution	1 mg/mL	15,686,000	26,633,673
Risperdal consta	37.5 mg	18,849,600	10,452,332
	50 mg	19,771,500	1,924,923
Olanzapine	10 mg	151,355,925	248,653,125
Zyprexa injection	10 mg	100,666,832	101,797,920
Zyprexa zydis	10 mg	6,331,273	858,477
Serequel XR	200 mg	213,975,720	214,860,377
	300 mg	242,958,604	278,264,252
	400 mg	328,884,940	321,018,400
Quetvell	100 mg	-	1,474,758
	200 mg	-	4,593,530

Despite their higher cost compared to typical antipsychotics, several factors contribute to the widespread use of atypical antipsychotics in Indonesia. Firstly, atypical antipsychotics are more readily available in the country, with most being covered by National Health Insurance. Secondly, the Indonesian Psychiatric Society recommends atypical antipsychotics as the first-line treatment for schizophrenia. This preference stems from their potential advantages, including greater efficacy in managing both negative and positive symptoms, improved cognitive function in patients, and a higher quality of life. Additionally, atypical antipsychotics are associated with fewer EPS, a side effect that can significantly impact patient adherence to treatment regimens[26]. Poor adherence can lead to increased overall medication costs, creating a financial burden for patients and their families[27]. While atypical antipsychotics are typically eight to nine times more expensive than typical options, the long-term benefits they offer may justify the higher cost.

The cost of antipsychotic medication is influenced by both dosage and treatment duration. As shown in **Table I**, trifluoperazine was the most expensive typical antipsychotic per unit per year, followed by chlorpromazine and haloperidol. Among atypical antipsychotics, aripiprazole was the costliest per unit per year, followed by clozapine and quetiapine. The higher prices of aripiprazole and quetiapine can likely be attributed to the limited availability of generic versions of these medications. Risperidone, a more affordable atypical antipsychotic, is consequently used more widely in Indonesia[28].

Long-acting injection antipsychotics

Our study found that among the three currently available and nationally formulated LAIs in Indonesia (haloperidol-LAI, fluphenazine-LAI, and risperidone-LAI), fluphenazine-LAI was the most frequently used, followed by haloperidol-LAI and risperidone-LAI (**Table I**). This trend likely reflects the significantly higher cost of risperidone-LAI compared to the other two options[29]. In contrast to Indonesia's current focus on typical LAIs, high-income countries tend to favor atypical LAIs such as risperidone-LAI, paliperidone-LAI, and aripiprazole-LAI. These atypical LAIs offer several advantages, including the potential for shorter hospital stays, reduced need for daily medication administration, improved relapse prevention, and fewer hospital admissions[17,29-31]. However, it is important to acknowledge that both oral and atypical LAIs have potential drawbacks, including the risk of metabolic abnormalities like obesity, hypertension, hyperglycemia, and dyslipidemia[32].

CONCLUSION

This study highlights schizophrenia as the most frequently diagnosed mental disorder in tertiary referral hospitals within the context of national health coverage. While atypical antipsychotics, generally more expensive than typical medications, are widely distributed, the use of LAI antipsychotics remains dominated by conventional LAIs. This pattern warrants further investigation to determine if cost considerations or other factors are influencing the choice of LAI medications.

ACKNOWLEDGMENT

The authors would like to express their gratitude to the Director of Menur National Mental Hospital and the Head and staff of the Pharmacy Department for their invaluable assistance. This research was financially supported by the Indonesia Endowment Fund for Education (*Beasiswa Unggulan Dosen Indonesia*; *BUDI*) with reference number PRJ-5331/LPDP.3/2016.

AUTHORS' CONTRIBUTION

Conceptualization: Julaeha, Verra Yuliana, Josephine Paramita Ayuningtyas
Data curation: Julaeha
Formal analysis: Julaeha, Verra Yuliana, Josephine Paramita Ayuningtyas
Funding acquisition: Julaeha
Investigation: Julaeha

Methodology: Julaeha
Project administration: Julaeha, Verra Yuliana, Josephine Paramita Ayuningtyas
Resources: Verra Yuliana, Josephine Paramita Ayuningtyas
Software: -
Supervision: Verra Yuliana, Josephine Paramita Ayuningtyas
Validation: Julaeha
Visualization: Julaeha
Writing - original draft: Julaeha, Verra Yuliana, Josephine Paramita Ayuningtyas
Writing - review & editing: Julaeha

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Whiteford HA, Ferrari AJ, Degenhardt L, Feigin V, Vos T. The global burden of mental, neurological and substance use disorders: an analysis from the Global Burden of Disease Study 2010. PLoS One. 2015;10(2):e0116820. DOI: 10.1371/journal.pone.0116820; PMCID: PMC4320057; PMID: 25658103

2. Zhu B, Svanum HA, Faries DE, Correll CU, Kane JM. Cost of antipsychotic polypharmacy in the treatment of schizophrenia. BMC Psychiatry. 2008;8:9. DOI: 10.1186/1471-244X-8-19; PMCID: PMC2364616; PMID: 18394168

3. Sweileh WM, Odeh JB, Shraim NY, Zyoud SH, Sawalha AF, Al-Jabi SW. Evaluation of Defined Daily Dose, percentage of British National Formulary maximum and chlorpromazine equivalents in antipsychotic drug utilization. Saudi Pharm J. 2014;22(2):127–32. DOI: 10.1016/j.jsps.2013.03.003; PMCID: PMC3950502; PMID: 24648824

4. Patted UH, Hema NG, Nagaraj A. Antipsychotics in schizophrenia: a retrospective study of drug utilization pattern in outpatient department of psychiatry at a tertiary care hospital. Int J Basic Clin Pharmacol. 2017;7(1):167-72. DOI: 10.18203/2319-2003.ijbcp20175694

5. Fabrazzo M, Cipolla S, Camerlengo A, Perris F, Catapano F. Second-Generation Antipsychotics' Effectiveness and Tolerability: A Review of Real-World Studies in Patients with Schizophrenia and Related Disorders. J Clin Med. 2022;11(15):4530. DOI: 10.3390/jcm11154530; PMCID: PMC9369504; PMID: 35956145

6. Berger A, Edelsberg J, Sanders KN, Alvir JM, Mychaskiw MA, Oster G. Medication adherence and utilization in patients with schizophrenia or bipolar disorder receiving aripiprazole, quetiapine, or ziprasidone at hospital discharge: a retrospective cohort study. BMC Psychiatry. 2012;12:99. DOI: 10.1186/1471-244X-12-99; PMCID: PMC3480886; PMID: 22856540

7. Barnes TR, Paton C. Antipsychotic polypharmacy in schizophrenia: benefits and risks. CNS Drugs, 2011;25(5):383–99. DOI: 10.2165/11587810-000000000-00000; PMID: 21476610

8. Nappoe SA, Djasri H, Kurniawan MF. Chronic disease management programme (PROLANIS) in Indonesia: case study. Geneva: World Health Organization, Organisation for Economic Co-operation and Development; 2023.

9. Kadakia A, Catillon M, Fan Q, Williams GR, Marden JR, Anderson A, et al. The Economic Burden of Schizophrenia in the United States. J Clin Psychiatry. 2022;83(6):22m14458. DOI: 10.4088/jcp.22m14458; PMID: 36244006

10. Puspitasari IM, Sinuraya RK, Rahayu C, Witriani W, Zannah U, Hafifah A, et al. Medication Profile and Treatment Cost Estimation Among Outpatients with Schizophrenia, Bipolar Disorder, Depression, and Anxiety Disorders in Indonesia. Neuropsychiatr Dis Treat. 2020;16:815-28. DOI: 10.2147/NDT.S240058

11. Piparva KG, Parmar DM, Singh AP, Gajera MV, Trivedi HR. Drug utilization study of psychotropic drugs in outdoor patients in a teaching hospital. Indian J Psychol Med. 2011;33(1):54–8. DOI: 10.4103/0253-7176.85396; PMCID: PMC3195156; PMID: 22021954

12. Deshmukh S, Ismail T. Evaluation of Psychotropic Drugs Use Pattern Among Out Patients Attending Psychiatry Department at Government Medical College And Hospital, Nagpur: A Cross Sectional Study. Int J Pharm Bio Sci. 2012;3(3): 428-36.

13. Wold Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191-4. DOI: 10.1001/jama.2013.281053; PMID: 24141714

14. Thakkar KB, Jain MM, Billa G, Joshi A, Khobragade AA. A drug utilization study of psychotropic drugs prescribed in the psychiatry outpatient department of a tertiary care hospital. J Clin Diagn Res. 2013;7(12):2759-64. DOI: 10.7860/jcdr/2013/6760.3885; PMCID: PMC3919294; PMID: 24551631

15. Mohamed MMA, Yousef BA. Prescription patterns of antipsychotics in the management of first episode psychosis at three psychiatric hospitals in Khartoum, 2018: A descriptive cross-sectional study. J Family Med Prim Care. 2020;9(1):402–6. DOI: 10.4103/jfmpc.jfmpc_892_19; PMCID: PMC7014859; PMID: 32110626

16. Shaifali I, Karmakar R, Chandra S, Kumar S. Drug utilization audit of antipsychotics using WHO methodology: recommendations for rational prescribing. Int J Basic Clin Pharmacol. 2018;7(10):2021-7. DOI: 10.18203/2319-2003.ijbcp20183941

17. Cheung S, Hamuro Y, Mahlich J, Nakahara T, Sruamsiri R, Tsukazawa S. Drug Utilization of Japanese Patients Diagnosed with Schizophrenia: An Administrative Database Analysis. Clin Drug Investig. 2017;37(6):559–69. DOI: 10.1007/s40261-017-0517-0; PMCID: PMC5422449; PMID: 28361438

18. Lee L, Procyshyn RM, White RF, Woodward TS, Honer WG, & Barr AM. Antipsychotic prescribing patterns on admission to and at discharge from a tertiary care program for treatment-resistant psychosis. PLoS One. 2018;13(8):e0199758. DOI: 10.1371/journal.pone.0199758; PMCID: PMC6086406; PMID: 30096136

19. Roberto PN, Brandt N, Onukwugha E, Perfetto E, Powers C, Stuart B. The Impact of Coverage Restrictions on Antipsychotic Utilization Among Low-Income Medicare Part D Enrollees. Adm Policy Ment Health. 2017;44(6):943–54. DOI: 10.1007/s10488-017-0813-2; PMID: 28660370

20. Dipiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey LM. Pharmacotherapy: a pathophysiologic approach. 10th edition. New York: Mc Graw-Hill Medical Publishing Division; 2017.

21. Park SC, Lee MS, Kang SG, Lee SH. Patterns of antipsychotic prescription to patients with schizophrenia in Korea: results from the health insurance review & assessment service-national patient sample. J Korean Med Sci. 2014;29(5):719–28. DOI: 10.3346/jkms.2014.29.5.719; PMCID: PMC4024938; PMID: 24851031

22. Gaviria AM, Franco J G, Aguado V, Rico G, Labad J, de Pablo J, et al. A Non-Interventional Naturalistic Study of the Prescription Patterns of Antipsychotics in Patients with Schizophrenia from the Spanish Province of Tarragona. PLoS One. 2015;10(10):e0139403. DOI: 10.1371/journal.pone.0139403; PMCID: PMC4591292; PMID: 26427051

23. Mahmood S, Hussain S, Rehman TU, Barbui C, Kurdi AB, Godman B. Trends in the prescribing of antipsychotic medicines in Pakistan: implications for the future. Curr Med Res Opin. 2018;35(1):51–61. DOI: 10.1080/03007995.2018.1513834; PMID: 30122062

24. Julaeha J, Athiyah U, & Hermansyah A. The prescription patterns of second-generation antipsychotics in schizophrenia outpatient setting. J Basic Clin Physiol Pharmacol. 2019;30:(6):20190289. DOI: 10.1515/jbcpp-2019-0289; PMID: 31837257

25. Yazici E, Cilli AS, Yazici AB, Baysan H, Ince M, Bosgelmez S, et al. Antipsychotic Use Pattern in Schizophrenia Outpatients: Correlates of Polypharmacy. Clin Pract Epidemiol Ment Health. 2017;13:92–103. DOI: 10.2174/1745017901713010092; PMCID: PMC5633702; PMID: 29081826

26. Kim HY, Lee HW, Jung SH, Kang MH, Bae JN, Lee JS, et al. Prescription patterns for patients with schizophrenia in Korea: a focus on antipsychotic polypharmacy. Clin Psychopharmacol Neurosci. 2014;12(2):128–36. DOI: 10.9758/cpn.2014.12.2.128; PMCID: PMC4153859; PMID: 25191503

27. Julaeha J, Athiyah U, Yuliana V, Ayuningtyas JP, Hermansyah A. Revisiting the intractable barriers affecting medication adherence among outpatients with schizophrenia. Curr Trends Biotechnol Pharm. 2020;14(5):200-5. DOI: 10.5530/ctbp.2020.4s.24

28. Díaz-Castro L, Cabello-Ranger L, Arredondo A, de León EM, Pineda-Antúnez C. Cost-effectiveness of therapeutic interventions in schizophrenia. Eur J Psychiatry. 2017;31(1):11-6. DOI: 10.1016/j.ejpsy.2016.12.006

29. Fan SJ, Lu N, Chang HC, Tang CH, Huang KC. Health service utilization and medical costs among patients with schizophrenia receiving long-acting injectable risperidone versus oral risperidone: A nationwide retrospective matched cohort study in Taiwan. Int Clin Psychopharmacol. 2018;.33(4):204-12. DOI: 10.1097/YIC.0000000000000213; PMID: 29489495

30. Yan T, Greene M, Chang E, Hartry A, Touya M, Broder MS. All-cause hospitalization and associated costs in patients with schizophrenia or bipolar disorder initiating long-acting injectable antipsychotics. Cur Med Res Opin. 2018;34(1):41–7. DOI: 10.1080/03007995.2017.1395733; PMID: 29057674

31. Munday J, Greene M, Chang E, Hartry A, Yan T, Broder MS. Early initiation of long-acting injectable antipsychotic treatment is associated with lower hospitalization rates and healthcare costs in patients with schizophrenia: real-world evidence from US claims data. Cur Med Res Opin. 2019;35(7):1231–9. DOI: 10.1080/03007995.2019.1571295; PMID: 30649965

32. Julaeha J, Athiyah U, Ayuningtyas JP, Yuliana V, Hermansyah A. Metabolic Syndrome Risk Associated with Atypical Antipsychotic Medication: A Case Report. J Drug Deliv Ther. 2021;11(1):77-9. DOI: 10.22270/jddt.v11i1.4680