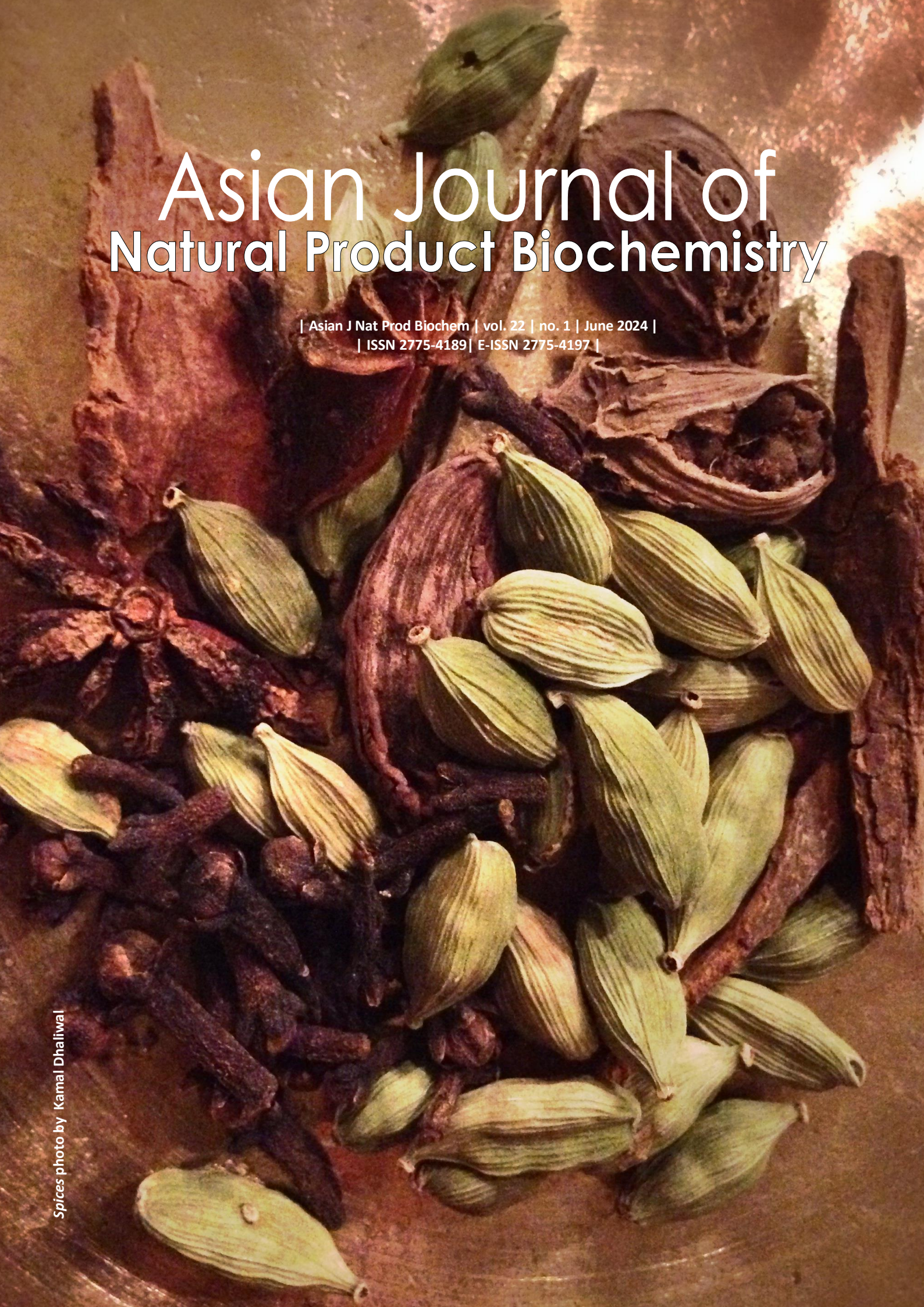
Asian Journal of Natural Product Biochemistry

| Asian J Nat Prod Biochem | vol. 22 | no. 1 | June 2024 |
| ISSN 2775-4189| E-ISSN 2775-4197 |



Spices photo by Kamal Dhaliwal

Asian Journal of Natural Product Biochemistry

| Asian J Nat Prod Biochem | vol. 22 | no. 1 | June 2024 |

Published semiannually **PRINTED IN INDONESIA**

ISSN: 2775-4189 E-ISSN: 2775-4197

9 772775 418434 9 772775 419431

Asian Journal of
Natural Product Biochemistry

| Asian J Nat Prod Biochem | vol. 22 | no. 1 | June 2024 |

ONLINE
http://smujo.id/jnpb

p-ISSN
2775-4189

e-ISSN
2775-4197

PUBLISHER
Smujo International

ASSOCIATION
Society for Indonesian Biodiversity

INSTITUTION
Universitas Sebelas Maret, Surakarta, Indonesia

OFFICE ADDRESS
Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia. Tel./fax. +62-271-663375, email: editors@smujo.id

PERIOD OF ISSUANCE
June, December

EDITOR-IN-CHIEF
Khalid Ahmad Khalid – National Research Center, Cairo, Egypt

EDITORIAL BOARD
Andri Frediansyah – Unit for Natural Product Technology, National Research and Innovation Agency, Indonesia
Asish Kumar Parida – CSIR-Central Salt & Marine Chemicals Research Institute, Bhavnagar, India
C.J. Sugiharjo – Universitas Gadjah Mada, Yogyakarta, Indonesia
Cedric B. Baker – Mercer University, Atlanta, USA
Chaerul – Research Center for Biology, National Research and Innovation Agency, Indonesia
Dayar Arbain – Universitas Andalas Padang, Indonesia
Enos Tangke Arung – Universitas Mulawarman, Samarinda, Indonesia
M. Ja'far Luthfi – UIN Sunan Kalijaga, Yogyakarta, Indonesia
Mallappa Kumara Swamy – Universiti Putra Malaysia, Malaysia
Supriyadi – Indonesian Spices and Medicinal Crops Research Institute, Bogor, Indonesia
Suranto – Universitas Sebelas Maret, Surakarta, Indonesia
Syamsul Arifin Achmad – Institut Teknologi Bandung, Indonesia
Taufik Hidayat – Institut Pertanian Bogor, Indonesia

List of reviewers: https://smujo.id/jnpb/reviewers

Society for Indonesia Biodiversity

Universitas Sebelas Maret Surakarta, Indonesia

Published by Smujo International for The Society for Indonesian Biodiversity and Sebelas Maret University, Surakarta, Indonesia

GUIDANCE FOR AUTHORS

Aims and Scope *Asian Journal of Natural Product Biochemistry* (**Asian J Nat Prod Biochem**) (*formerly Biofarmasi*) encourages submission of manuscripts dealing with all important contributions to natural product chemistry, include bioactivity compounds of land and sea and of plants, microbes and animals; activities of antimicrobial, antiviral, antiparasite, anti-cancer, etc.; ethnopharmacology, ethnomedicines and medical application; plant-derived drugs and pharmaceutical formulations and evaluation; as well as biochemistry, chemistry, biotechnology, physiology, cell structures, pharmacology, toxicology, and chemical ecology of natural products.

Article types The journal seeks for: (i) **Research papers**, (ii) **Reviews**, and (iii) **Short communications**. Original full-length research manuscripts are limited to 8,000 words (including tables and figures) or proportional to articles in this publication number (beyond that, it should be with notice). Review articles are also limited to 8,000 words, while Short communications should be less than 2,500 words, except for pre-study (can be more).

Submission: The journal only accepts online submissions through the open journal system **(https://smujo.id/jnpb/about/submissions)** or, for login problems, email the editors at unsjournals@gmail.com (or editors@smujo.id). Submitted manuscripts should be the original works of the author(s). Please ensure that the manuscript is submitted using the template, which can be found at (https://biodiversitas.mipa.uns.ac.id/D/template.doc). The manuscript must be accompanied by a cover letter containing the article title, the first name and last name of all the authors, and a paragraph describing the claimed novelty of the findings versus current knowledge. Please also provide a list of five potential reviewers in your cover letter. They should come from outside your institution and better from three different countries. Submission of a manuscript implies the submitted work has not been published (except as part of a thesis or report, or abstract) and is not being considered for publication elsewhere. When a group writes a manuscript, all authors should read and approve the final version of the submitted manuscript and its revision; and agree on the submission of manuscripts for this journal. All authors should have made substantial contributions to the concept and design of the research, acquisition of the data and its analysis, drafting the manuscript, and correcting the revision. All authors must be responsible for the work's quality, accuracy, and ethics.

Ethics Author(s) must be obedient to the law and/or ethics in treating the object of research and pay attention to the legality of material sources and intellectual property rights.

Copyright If the manuscript is accepted for publication, the author(s) still hold the copyright and retain publishing rights without restrictions. For the new invention, authors must manage its patent before publication.

Open Access The journal is committed to free-open access that does not charge readers or their institutions for access. Readers are entitled to read, download, copy, distribute, print, search, or link to the full texts of articles, as long as not for commercial purposes. The license type is CC-BY-NC-SA.

Acceptance Only articles written in US English are accepted for publication. Manuscripts will be reviewed by editors and invited reviewers (double-blind review) according to their disciplines. Authors will generally be notified of acceptance, rejection, or need for revision within 1 to 2 months of receipt. Manuscripts will be rejected if the content does not align with the journal scope, does not meet the standard quality, is in an inappropriate format, or contains complicated grammar, dishonesty (i.e., plagiarism, duplicate publications, fabrication of data, citations manipulation, etc.), or ignoring correspondence in three months. The primary criteria for publication are scientific quality and significance. **Uncorrected proofs** will be sent to the corresponding author by system or email as .doc or .docx files for checking and correcting typographical errors. The corrected proofs should be returned in 7 days to avoid publication delays. The accepted papers will be published online in chronological order at any time but printed at the end of each month.

A charge Authors are charged USD 320 (IDR 4,500,000). Additional charges may be billed for language improvement, USD 75-150 (IDR 1,000,000-2,000,000). **Reprints** The sample journal reprint is only available by special request. Additional copies may be purchased when ordering by email and sending back the uncorrected proofs.

Manuscript preparation Manuscript is typed on A4 (210x297 mm^2) paper size, in a single column, single space, 10-point (10 pt) Times New Roman font. The margin text is 3 cm from the top, 2 cm from the bottom, and 1.8 cm from the left and right. Smaller lettering sizes can be applied in presenting tables and figures (9 pt). Word processing program or additional software can be used; however, it must be PC compatible, use the template, and be Microsoft Word based (.doc or .rtf; not .docx). **Scientific names** of species (incl. subspecies, variety, etc.) should be written in italics, except in italicized sentences. Scientific names (genus, species, author) and cultivar or strain should be mentioned completely for the first time mentioning it in the body text, especially for taxonomic manuscripts. The genus name can be shortened after the first mention, except in early sentences, or where this may generate confusion; name of the author can be eliminated after the first mention. For example, *Rhizopus oryzae* L. UICC 524 can be written hereinafter as *R. oryzae* UICC 524. Using trivial names should be avoided. **Biochemical and chemical nomenclature** should follow the order of the IUPAC-IUB. For DNA sequences, it is better to use Courier New font. Standard chemical abbreviations can be applied for common and clear used, for example, completely written butilic hydroxyl toluene (BHT) to be BHT hereinafter. **Metric measurements** should use IS denominations, and other systems should use equivalent values with the denomination of IS mentioned first. A dot should not follow abbreviations like g, mg, mL, etc. Minus index (m^{-2}, L^{-1}, h^{-1}) suggested being used, except in things like "per-plant" or "per-plot." **Mathematical equations** can be written down in one column with text; in that case, they can be written separately. **Numbers** one to ten are written in words, except if it relates to measurement, while values above them are written in number, except in early sentences. The fraction should be expressed in decimal. In the text, it should be used "%" rather than "percent." Avoid expressing ideas with complicated sentences and verbiage/phrasing, and use efficient and effective sentences.

The title of the article should be written in compact, clear, and informative sentence, preferably not more than 20 words. Name of author(s) should be completely written, especially for the first and the last name. **Name and institution** address should also be completely written with street name and number (location), postal code, telephone number, facsimile number, and email address. We choose local names in Bahasa Indonesia for universities in Indonesia. The mention of "strata" program, should be avoided. Manuscript written by a group, author for correspondence along with address is required (marked with "♥"). **The title page** (first page) should include title of the article, full name(s), institution(s) and address(es) of the author(s); the corresponding authors detailed postage and e-mail addresses (P), and phone (O) and fax numbers (O).

Abstract A concise abstract is required (about 200 words). The abstract should be informative and state briefly the aim of the research, the principal results and major conclusions. An abstract is often presented separately from the article, thus it must be able to stand alone (completely self-explanatory). References should not be cited, but if essential, then cite the author(s) and year(s). Abbreviations should be avoided, but if essential, they must be defined at their first mention. **Keywords** are about five words, covering scientific and local name (if any), research themes, and special methods used; and sorted from A to Z. **Abbreviations** (if any): All important abbreviations must be defined at their first mention there. **Running title** is about five words.

Introduction is about 600 words, covering the aims of the research and provide an adequate background, avoiding a detailed literature survey or a summary of the results. **Materials and Methods** should emphasize on the procedures and data analysis. **Results and Discussion** should be written as a series of connecting sentences, however, for a manuscript with long discussion should be divided into subtitles. Thorough discussion represents the causal effect mainly explains why and how the results of the research were taken place, and do not only re-express the mentioned results in the form of sentences. **Concluding** sentence should be given at the end of the discussion. **Acknowledgements** are expressed in a brief; all sources of institutional, private and corporate financial support for the work must be fully acknowledged, and any potential conflicts of interest are noted.

Figures and Tables of a maximum of three pages should be clearly presented. The title of a picture is written down below the picture, while the title of a table is written above the table. Colored figures can only be accepted if the information in the manuscript can lose without those images; the chart is preferred to use black and white images. The author could consign any picture or photo for the front cover, although it does not print in the manuscript. All images property of others should be mentioned the source. Author is suggested referring to Wikipedia for international boundaries and Google Earth for satellite imagery. If not specifically mentioned, it is assumed to refer to these sources. **There is no appendix**, all data or data analysis is incorporated into Results and Discussions. For broad data, it can be displayed on the website as a supplement.

References Preferably 80% of it comes from scientific journals published in the last 10 years. In the text, give the author names followed by the year of publication and arrange from oldest to newest and from A to Z; in citing an article written by two authors, both of them should be mentioned; however, for three and more authors only the first author is mentioned followed by et al. For example, Saharjo and Nurhayati (2006) or (Boonkerd 2003a, b, c; Sugiyarto 2004; El-Bana and Nijs 2005; Balagadde et al. 2008; Webb et al. 2008). Extent citation should be avoided, as shown with the word "cit." Reference to unpublished data and personal communication should not appear in the list but should be cited in the text only (e.g., Rifai MA 2007, pers. com. (personal communication); Setyawan AD 2007, unpublished data). In the reference list, the references should be listed in alphabetical order. Names of journals should be abbreviated. Always use the standard abbreviation of a journal's name according to the **ISSN List of Title Word Abbreviations** (www.issn.org/2-22661-LTWA-online.php). Please include DOI links for journal papers. The following examples are for guidance.

Journal:

Saharjo BH, Nurhayati AD. 2006. Domination and composition structure change at hemic peat natural regeneration following burning; a case study in Pelalawan, Riau Province. Biodiversitas 7: 154-158. DOI: 10.13057/biodiv/d070213.

The usage of "et al." in long author lists will also be accepted:

Smith J, Jones M Jr, Houghton L et al. 1999. Future of health insurance. N Engl J Med 965: 325-329. DOI: 10.10007/s002149800025.

Book:

Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in the book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds.). Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta*. 50th annual symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.). Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

Information from the internet:

Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4: 187. DOI: 10.1038/msb.2008.24. www.molecularsystembiology.com.

THIS PAGE INTENTIONALLY LEFT BLANK

ASIAN J NAT PROD BIOCHEM
Volume 22, Number 1, June 2024
Pages: 1-7

P-ISSN: 2775-4189
E-ISSN: 2775-4197
DOI: 10.13057/biofar/f220101

Phytochemical and proximate compositions of frond extracts of *Nephrolepis biserrata*, *Phymatosorus scolopendria,* and *Microgramma mauritiana* in Rivers State University, Nigeria

ASIKIYE IBIYE, BLESSING O. GREEN, MERCY G. AJURU♥, LAURETTA C. CHIKERE
Department of Plant Science and Biotechnology, Faculty of Science, Rivers State University. Nkpolu-Oroworuko, Port Harcourt, Rivers State, Nigeria.
Tel.: +234-7036-834588, ♥email: mercy.ajuru@ust.edu.ng

Manuscript received: 16 December 2023. Revision accepted: 21 February 2024.

Abstract. *Ibiye A, Green BO, Ajuru MG, Chikere LC. 2024. Phytochemical and proximate compositions of frond extracts of* Nephrolepis biserrata, Phymatosorus scolopendria*, and* Microgramma mauritiana *in Rivers State University, Nigeria. Asian J Nat Prod Biochem 22: 1-7.* Pteridophytes have a great range of forms and are cosmopolitan in distribution. There are about 13,500 species of fern and allies distributed throughout the world. This research was carried out to investigate the phytochemical and proximate compositions of three species of ferns growing at Rivers State University, Nigeria. The three fern species were *Nephrolepis biserrata* (Sw.) Desv., *Phymatosorus scolopendria (*Burm.fil.) Pic.Serm., and *Microgramma mauritiana* (Willd.) Tardieu. The phytochemical and proximate composition analyses were done using standard procedures. Results from the phytochemical analysis showed that glycoside was the highest content in the three ferns, that is, *P. scolopendria* (17.24%), *N. biserrata* (14.47%), *M. mauritiana* (12.8%), followed by alkaloid in *M. mauritiana* (7.18%), *N. biserrata* (6.10%), *P. scolopendria* (4.42%). Phenol content in *M. mauritiana, P. scolopendria*, and *N. biserrata* were 4.09%, 2.17%, and 1.33%, respectively. Flavonoid content in *N. biserrata*, *P. scolopendria*, and *M. mauritiana* were 3.16%, 2.87%, and 2.43%, respectively. Saponin content in *P. scolopendria*, *M. mauritiana*, and *N. biserrata* were 1.65%, 1.35%, and 1.29%, respectively. Tannins content in *P. scolopendria*, *M. mauritiana,* and *N. biserrata* were 2.54%, 1.91%, and 0.98%, respectively. Results from proximate analysis showed that the highest moisture content was in *M. mauritiana* (53.39%), followed by *P. scolopendria* (50.78%) and *N. biserrata* (44.58%). The lowest content in the frond extracts was ash, with the values of *M. mauritiana* (0.84%), *N. biserrata* (0.83%), and *P. scolopendria* (0.73%). The results of the phytochemical and proximate evaluation of the species studied indicated that they are nutritionally and medicinally important, and their consumption can provide essential nutrients humans need.

Keywords: *Microgramma mauritiana, Nephrolepis biserrata, Phymatosorus scolopendria*, phytochemicals, proximate analysis

INTRODUCTION

Pteridophytes are non-flowering vascular cryptogamic plants. Pteridophytes comprise 400 genera and approximately 10,500 species, including living and fossil plants. Ferns are vascular plants with xylem and phloem bundles, which enable them to transport water from the root to other parts of the plant (xylem) and to translocate food to different plant parts (phloem). Most ferns are leptosporangiate, producing coiled fiddleheads that uncoil and develop into fronds. There are about 10,560 known living species of ferns, and they are generally grouped as Polypodiopsida, which comprises both the leptosporangiate (Polypodiidae) and eusporangiate ferns.

Nephrolepis biserrata (Sw.) Desv. plants are mainly used for ornamental purposes. In Sarawak, it is used to treat wounds, blisters, abscesses, and boils on the skin. In India, the rhizomes are used to treat respiratory diseases. It is also used to prevent miscarriage, promote fetal development, and treat stomach pain, bleeding, and wounds (Piggott 1996; Malan and Neuba 2011). Due to its high nutritional value, it serves as fodder for African dwarf goats (Christensen 1997). The tip of young shoots is used as a vegetable by the indigenous people of Malaysia.

Phymatosorus scolopendria (Burm.fil.) Pic.Serm. frond is pounded in Indo-China and used to treat boils and filariasis. Whole fronds are kept on beds to ward off bed bugs, while the young fronds are used to treat chronic diarrhea. In Polynesia, the fronds are ground and mixed with scrapings of *Atuna racemosa* Raf. to produce perfume. Also, the mashed fronds are wrapped with leaves of *Morinda citrifolia* L., then boiled and used as medicinal bandages. Frond juice is used in Fiji to treat stomachaches, swollen breasts, and boils (Snogan et al. 2007). They are also used to treat asthma, cough, and inflammatory diseases.

Microgramma mauritiana (Willd.) Tardieu is mainly found in moist scrubs, short forest patches, and climbing shrubs, and it produces fertile fronds in higher light intensity. Whole plants are used against pubic lice in humans and to prevent the reproduction of the lice.

Phytochemicals or plants' secondary metabolites are often restricted to a particular species within a phylogenetic group. Secondary metabolites are very important in treating diseases and in the ecological interaction of plants with other organisms. Proximate analyses of plant extract include moisture, ash, crude protein, crude lipid, crude fiber, and digestible carbohydrates. Proximate composition analysis is used to determine the nutritional constituents of

plants. It is essential because most medicinal plants are consumed due to their medicinal value; therefore, analyzing their nutrient content could provide added value to these plants (Pandey et al. 2006).

Ferns are plants widely distributed, including Nigeria, with emphasis on Rivers State. Generally, ferns have been classified into different taxonomic hierarchies, though there is very poor or little information on the relationships or differences between several ferns in their taxonomic classification. Most ferns are neglected, while some are endangered due to inadequate information, cultivation, and taxonomic studies. Researchers globally have carried out credible taxonomic works on several fern families. In Nigeria, the information characterizing these ferns is scanty, and the morphological approach seems to be predominant, especially those that are epiphytic and terrestrial. For example, two fern species of *Nephrolepis* have been investigated based on their morphological parameters. The use of biosystematic methods can improve the classification system. This work focused on providing systematic information using the phytochemical and proximate analysis.

MATERIALS AND METHODS

Collection, identification, and preparation of plant materials

The research work took place from July to November 2023. The different fern species were freshly collected from various trees in Rivers State University, Nkpolu-Oroworukwo, Rivers State, Nigeria. The plant samples were assigned accession numbers and identified at the Department of Plant Science and Biotechnology Herbarium, Rivers State University, Nigeria, by a Plant Taxonomist. The plant specimens were deposited in the Herbarium. Fresh fronds from each species were thoroughly washed with distilled water thrice and then dried in a shady place for five days. Dried plant samples were ground to powder using mortar and pestle.

Extraction of plant materials

About 100g of the powdered plant was carefully weighed and loaded into a soxhlet extractor. The powdered materials were extracted separately with redistilled methanol and petroleum spirit (60-80°C) using soxhlet extraction and cold maceration. The extracts were then concentrated in a vacuum using a rotary evaporator at 40°C. Dried extracts were used for further analysis.

Procedures

Proximate analysis

Determination of moisture content

The standard AOAC (2000) method was followed to deduce the Moisture contents. One gram of the sample was placed in a dry, empty, pre-weight (W1) clean petri dish (with lid). Samples were oven-dried at 105°C for 4-5 hours until a constant weight was obtained, and then the sample was placed in a desiccator for 30 minutes. After cooling the dish, the petri dish and sample were weighed as final weight (W2). The percentage of moisture was calculated as follows:

$$\%\text{Moisture} = \frac{\text{Wt. of residue } (W^2 - W^1)}{\text{Wt of Sample}} \times 100$$

Determination of ash

Ash content was determined according to the AOAC (2000) method. A clean crucible was heated in a muffle furnace for an hour at 660°C, then placed in a desiccator to cool. After cooling, it was weighed (W1). Ten g of dry sample was taken in the crucible. The sample was burned on the burner with the help a blowpipe. The crucible containing the sample was heated at 550°C for 6-8 hours in a muffle furnace. After cooling to room temperature, the crucible was weighed as (W2). Percent ash was calculated as follow:

$$\%\text{Ash} = \frac{\text{Wt. of Ash } (W^2 - W^1)}{\text{Wt of Sample}} \times 100$$

Determination of fat

A thimble with dry sample was prepared and its weight recorded as W1. Soxhlet extractor was used for the extraction, with diethyl ether as the solvent, the thimble inside the thimble holder was clipped, then 40 mL of diethyl ether was added into a round bottom flask to extract fat content. The round bottom flask was attached to the extractor with the ring clamped tightly. The temperature was adjusted to 40°C and the heater, main power and the condenser water were switched on. The extraction process was carried out for 16 hours at condensation rate of 2 to 3 drops per second. After the extraction, the ether was allowed to drain out of the thimble after about 30 minutes. The extract was transferred into a pre-weighed Beaker (W2) for further evaporation at room temperature. Room temperature drying was employed to avoid possible explosion from oven-induced drying of the ether solvent. The weight of Beaker and residue (W3) was recorded on completion of the drying process. Note: Excessive drying may oxidize the fat and give high results.

The remaining residue in thimble was used for fiber analysis and calculated as follows:

$$\%\text{Fat(Crude Lipid)} = \frac{\text{Wt. of Extract } (W^3 - W^2)}{\text{Wt of Sample } (W^1)} \times 100$$

Determination of crude fibre

Crude fiber was determined by acid and alkali digestion using fiber tec apparatus following AOAC (2000). The thimble residue was weighed (W1) and then digested with acid, followed by alkali. Then, 100 mL of 2.5% HCl was added to the sample into a beaker, heated with stirring for about 30 minutes, and drained into the beaker. The residue was redigested in 2.5% NaOH. The residue was transferred to a pre-weighted (W2) dried crucible to remove the moisture. The crucible was kept in the furnace until white and grey ash formed. The crucible was cooled in desiccators and weighed again. (W3) The loss in weight of

the dry residue upon ignition was taken as the amount of crude fiber. The crude fiber percentage was calculated as follows:

$$\%\text{Crude Fibre} = \frac{\text{Wt. of dry residue } (W^3 - W^2)}{\text{Wt of Sample } (W^1)} \times F \times 100$$

Where, F: Value of crude fat; W1: Weight of Sample; W2: Weight of dry crucible; W3: Weight of crucible after heating.

Determination of protein: Kjeldahl method

Stage 1

Digestion. 0.1 grams of the sample, 3 grams of the digestion catalyst, and 20 mL of concentrated sulphuric acid were placed in a 250 mL conical flask and then heated to digest until the mixture inside the conical flask turned black to sky blue. The digest was allowed to cool down to room temperature and then diluted with distilled water to 100 mL.

Stage 2

Distillation. The digested catalyst was measured (20 mL) and deposited into a distillation flask, which was inserted into the electrothermal heater. A Liebig condenser was connected to a receiver with the flask attached. The receiver contained 10 mL of 2% boric acid as an indicator. 40 mL of hydroxide solution was injected into the digest using a syringe attached to the single-arm steelhead until the digest became highly alkaline. Then, the mixture was boiled. Distilled ammonia gas was added to the receiver beaker through the condenser, which changed the color of boric acid from purple to green.

Stage 3

Titration. The distillate was titrated back to purple from green using a standard 0.1N hydrochloric acid solution. The quantity of hydrochloric acid used for the titration was the titer value and calculated as follows:

$$\%\text{Organic nitrogen} = \frac{\text{titer value x 1.4 x 100 x 100}}{\text{1000 x 20 x 0.1}}$$

The titer value is the HCL volume used to titrate the ammonium distillate. 1.4 is the quantity of Nitrogen equivalent to the volume of HCl used in the titration of 0.1N. 100 is the total volume of digest dilution, while the second 100 is the Percentage factor. 1000 is the conversion factor from gram to milligram, while the value 20 is the integral volume of digits analyzed and 0.1 is the weight of the sample in grams digested.

Screening of carbohydrate. Carbohydrates content was determined by subtracting the weights of protein, fats, crude fibers, ash, and moisture contents from 100.

TCH (%) = 100-%(CP+A+CF+M)

Phytochemical analysis

Qualitative analysis

Determination of Flavonoids

1 g of the extract was dissolved in 1% Aluminum chloride in methanol and then added with a few drops of concentrated HCL, magnesium, and potassium hydroxide solution. Orange to pink color change indicated the presence of flavonoids.

Determination of Alkaloids

1 g of the powdered sample was mixed with 5 mL of 1% HCL in a steam bath. Then, the mixture was filtered. After the filtration, 1 mL of the filtrate was exposed to a few drops of Dragendorff's reagent, which caused the sample to change to black, indicating the presence of alkaloids.

Determination of Saponin

2 g of the sample was boiled in 20 cm^3 of distilled water inside a water bath and then filtered. 5 cm^3 of the filtrate was mixed with 5 cm^3 distilled water and shaken thoroughly. The formation of a stable foam indicates the presence of saponin.

Determination of Phenol

2 mL of extract was added with 2 mL of ferric chloride ($FeCl_3$) solution in a test tube. A deep bluish-green solution indicated the presence of phenol.

Screening of Terpenoid

1 g of the powdered sample was stirred with 2 mL chloroform and 3 mL of conc. H_2SO4 to form a layer. A reddish-brown interface indicated the presence of terpenoids.

Screening of Tannins

10 mL of distilled water was mixed with 1 g of sample in a test tube, heated, and then filtered. A few drops of 5% ferric chloride were added to the filtrate. A black color indicated the presence of tannins (Banso and Adeyemo 2006).

Screening of Cyanogenic glycosides

3 mL of chloroform and 1 mL of 10% ammonium solution were added to 2 mL of the extract. The formation of a pink color indicated the presence of glycoside.

Quantitative phytochemical evaluation

Alkaloids analysis

Quantitative analysis for alkaloids followed the method by Harborne (1973). 5 g of the sample was placed in a 250 ml Beaker glass. Then, 200 mL of 10% acetic acid in ethanol was mixed with the sample and covered. The mixture was left for 4 hours and then filtered. After filtration, the extract was concentrated in a water bath until one-quarter of the original volume was obtained. Then, concentrated ammonium hydroxide was applied drop by drop until the residue was complete. It was allowed to stand for some time, after which the precipitate was collected and rinsed with dilute ammonium hydroxide before filtering. The residue was alkaloid, and it was dried and weighed. The alkaloid content was calculated as follows:

$$\text{Alkaloid}(\%) = \frac{\text{Filtered paper with residue} - \text{weight of dried filtered paper}}{\text{Weight of Sample}} \times 100$$

Determination of Flavonoids

Determination of quantitative flavonoids followed the method by Kumaran and Karunakaran (2006). The method is based on the formation of flavonoids-ammonium complex, which has an absorptivity maximum of 415 nm. 100 µL of the extracts in methanol (10 mg/mL) was mixed with 100 µL of 20% aluminum trichloride in methanol and a drop of acetic acid. The mixture was diluted with methanol to 5 mL. The absorption was observed at 415 nm and read after 40 min. The blank sample was prepared by adding a drop of acetic acid and added with methanol to 5 mL. Under the same conditions, the absorption of standard rutin solution (0.5 mg/mL) in methanol was measured. It was determined in triplicates.

$$\text{Flavonoid}(\%) = \frac{\text{Flask with residue} - \text{weight of empty flask}}{\text{Weight of Sample}} \times 100$$

Determination of Saponin

Obadoni and Ochuko's (2001) method was used to determine saponin quantitatively. 20 g plant powder was put into a conical flask and added with 100 ml of 20% aqueous ethanol. The samples were heated in a hot water bath (55°C) for 4 hours and stirred continuously. Then, it was filtered. The residue was re-extracted with another 200 mL of 20% ethanol. The combined extracts were reduced to 40 mL using a hot water bath at about 90°C. The concentrate was taken, placed into a 250 mL separator funnel, added with 20 mL of diethyl ether, and shaken vigorously. The ether layer was discarded while recovering the aqueous layer. This purification process was repeated, and 60 mL of n-butanol was added. The extracts were rinsed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. The samples were dried in the oven to a constant weight after evaporation. Saponin content was calculated as follows:

$$\text{Saponin}(\%) = \frac{\text{weight of conical flask with residue} - \text{weight of empty conical flask}}{\text{Weight of Sample}} \times 100$$

Determination of Phenol

1 g of the powdered sample was weighed and poured into a conical flask and added with 10 mL of ethanol. Then, the flask was sealed with aluminum foil. After shaking it vigorously, the mixture was left to stand for 30 min for proper extraction. The mixture was centrifuged to obtain a clear supernatant to determine the total phenolic content quantitatively.

$$\text{Phenol content mg/kg (TAE)} = \frac{\text{Conc. obtained in } mg/l \text{ x volume of sample x DF}}{\text{Sample weight}}$$

DF: Dilution factor, if not diluted, then DF = 1

Determination of Terpenoid

0.4 g of the sample was placed into a conical flask, added with 9 mL ethanol, and macerated for 24 hours. After maceration, the mixture was filtered using a funnel, filter paper, and a conical flask. 20 mL of petroleum ether was employed for the extraction with a separating funnel, then 10 mL of distilled water was added to the extract. The plant sample was then added to a pre-weighed moisture can and dried in the oven for 1 hour at 150°C for complete dryness. Calculation:

Terpenoid = Can + Solvent - Can weight

$$\text{Terpenoid}(\%) = \frac{\text{Total Terpenoid}}{\text{Sample weight}} \times 100$$

Determination of Tannins

500 mg of the sample was placed into a 50 mL plastic bottle, added with 50 mL of distilled water, and shaken for an hour in a mechanical shaker. It was then filtered into a 50 mL volumetric flask and added to the mark with distilled water. Afterward, 5 mL of filtrate was pipetted into a test tube and mixed with 2 mL of 0.1M $FeCL_3$ in 0.1N, HCL, and 0.008M potassium ferrocyanide. The absorbance was measured at 120nm in 10 minutes and calculated as follows:

$$\text{Tannin (mg/kg)} = \frac{\text{The gradient of tannin x absorbance of sample x 100}}{2}$$

Determination of Cyanogenic glycosides

Five grams of the sample was placed into a clean distillation flask, added with 20 mL of distilled water, and allowed to stand overnight for proper hydrolysis. The sample was distilled using 200 mL of 0.5g NaOH pellets/ 1 L distilled water (AOAC 2000). The distillate was titrated with 0.02 N Silver Nitrate, 5% Potassium iodide, and 1 mL of 6 N Ammonia hydroxide solution to permanent turbidity, which indicated the endpoint.

Cyanogenic glycoside in the sample was calculated as follows: 1 mL 0.02N $AgNO_3$ = 1.08 mg HCN.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) software version 25 was used to analyze data, and the results were expressed as the mean ± Standard Error (SE).

RESULTS AND DISCUSSION

Proximate analysis

The result of the proximate analysis of the studied ferns is presented in Table 1. The highest moisture content was in *M. mauritiana* (53.39±0.16), followed by *P. scolopendria* (50.78±0.13) and *N. biserrata* (44.58±0.25). Protein content in *N. biserrata* was (35.56±0.68), followed by *M. mauritiana* (31.20±0.05) and *P. scolopendria* (28.12±0.09). Fibre content in *P. scolopendria* is (11.25±0.06), *N. biserrata* (8.07±0.07), *M. mauritiana* (6.23±0.13). Carbohydrate content in *N. biserrata* (8.29±0.48), *P. scolopendria* (7.83±0.12), *M. mauritiana* (6.59±0.26). Crude Lipid in *N. biserrata* was (2.65±0.08), *M. mauritiana* (1.76±0.06), *P. scolopendria* (1.34±0.03), and ash content in *M. mauritiana* was (0.84±0.03), *N. biserrata* (0.83±0.03), *P. scolopendria* (0.73±0.04).

Phytochemical composition

Qualitative phytochemical analysis of three ferns species

The result of the qualitative phytochemical analysis is presented in Table 2. Glycosides were the highest (++) in all three fern species, followed by Saponin (+), Flavonoid (+), Alkaloids (+), Tannins (+), and Phenols (+).

Quantitative phytochemical analysis of three ferns species studied

The result of the quantitative phytochemical analysis is presented in Table 3. Glycoside content was the highest in the three ferns, i.e., *P. scolopendria* (17.24±0.13), *N. biserrata* (14.47±0.26), *M. mauritiana* (12.8±0.18), followed by Alkaloid in *M. mauritiana* (7.18±0.18), *N. biserrata* (6.10±0.20), *P. scolopendria* (4.42±0.16), the next was Phenol in *M. mauritiana* (4.09±0.08), *P. scolopendria* (2.17±0.04), *N. biserrata* (1.33±0.77), next to Flavonoid in *N. biserrata* (3.16±0.19), *P. scolopendria* (2.87±0.04), *M. mauritiana* (2.43±0.02), Saponin in *P. scolopendria* (1.65±0.03), *M. mauritiana* (1.35±0.04), *N. biserrata* (1.29±0.08) and Tannins in *P. scolopendria* (2.54±0.09), *M. mauritiana* (1.91±0.02), *N. biserrata* (0.98±0.11).

Table 1. Proximate compositions in the fronds of the studied ferns

Parameter (%)	*Nephrolepis biserrata*	*Phymatosorus scolopendria*	*Microgramma mauritiana*
Moisture Conten	44.58±0.25	50.78±0.13	53.39±0.16
Carbohydrate	8.29±0.48	7.83±0.12	6.59±0.26
Crude lipid	2.65±0.08	1.34±0.03	1.76±0.06
Proteins	35.56±0.68	28.12±0.09	31.20±0.05
Fibre	8.07±0.07	11.25±0.06	6.23±013
Ash	0.83±0.03	0.73±0.04	0.84±0.03

Note: Mean (±) SD, n=3

Table 2. Qualitative phytochemical constituents of the ferns

Phytochemical	*Nephrolepis biserrata*	*Phymatosorus scolopendria*	*Microgramma mauritiana*
Saponin	+	+	+
Flavonoid	+	+	+
Alkaloids	+	+	+
Tannins	+	+	+
Phenols	+	+	+
Glycosides	++	++	++

Note: +: Present; ++: Highly present

Table 3. Quantitative phytochemical constituents of the ferns

Phytochemical (%)	*Nephrolepis biserrata*	*Phymatosorus scolopendria*	*Microgramma mauritiana*
Saponin	1.29±0.08	1.65±0.03	1.35±0.04
Flavonoid	3.16±0.19	2.87±0.04	2.43±0.02
Alkaloids	6.10±0.20	4.42±0.16	7.18±0.18
Tannins	0.98±0.11	2.54±0.09	1.91±0.02
Phenols	1.33±0.77	2.17±0.04	4.09±0.08
Glycosides	14.47±0.26	17.24±0.13	12.18±0.18

Note: Mean (±) SD, n=3

Discussion

The proximate analysis of the three species showed that the fern has a high moisture content. The high moisture content of these fern species results in the high activity of water-soluble enzymes and co-enzymes needed for metabolic activities. It helps their leaves stay in a dry environment for an extended period.

The protein content of three fern species ranged from 28-35%. Plants with more than 12% protein are considered good sources of protein. Therefore, the fern species in this study are a good protein source and can be used as supplements in protein-deficient diets. It is comparable to the daily protein requirement of 23-65 g (Chaney 2006). Proteins in the body are used to produce hormones, enzymes, and blood plasma. Proteins increase immunity and can help cell division and growth. Proteins are also needed to replace dead tissues, energy supply, and amino acid source (Igile et al. 2013).

Fibre content in three ferns ranged from 6.23%-8.07% in the fern species. According to Bouttwell (1998), dietary fiber could slow down the rate of glucose absorption into the bloodstream, thereby reducing the risk of hyperglycemia. They also reduce plasma cholesterol levels and prevent colon cancer and cardiovascular diseases. The fiber in plants aids digestion, softens stool, and prevents constipation.

Carbohydrate content in the three fern species ranged from 6.59%-8.29%. The presence of carbohydrates in ferns shows they could be a good energy source. Carbohydrates are by-products of photosynthetic processes. It is used as the primary source of energy. Carbohydrates are hydrolyzed in the body to produce glucose, which can be utilized immediately or stored as glycogen in the muscles and liver (Raven et al. 2005).

Crude Lipid in *N. biserrata* was 2.65±0.08%, 1.76±0.06% in *M. mauritiana*, and 1.34±0.03% in *P. scolopendria*. The results showed that ferns in this study have a low content of crude lipids and can be easily incorporated into a weight-reducing diet. Dietary fats are important because of their high energy value and the soluble vitamins and essential fatty acids in natural foods' fat. Dutta (2003) reported that fats and oil help regulate blood pressure and play a role in synthesizing and repairing vital cell parts.

Ash content in the three ferns in this study was low. Ash content is an indicator of the total mineral content of a sample. Minerals can be used to establish and maintain the acid-alkaline balance of the blood system (Vunchi et al. 2011).

The results of the phytochemical analysis in three fern species are presented in Table 3. Glycoside was the highest compound in the three ferns, i.e., *P. scolopendria* was 17.24±0.13%, 14.47±0.26% in *N. biserrata*, and 12.8±0.18% in *M. mauritiana*. Glycosides are natural toxins found in plants (Walker et al. 2000) and, if consumed in excess, can cause chronic and acute health problems (Singh and Upadhyay 2012; Khoja et al. 2022; Murthy et al. 2023).

Alkaloid content in *M. mauritiana* was 7.18±0.18%, *N. biserrata* was 6.10±0.20%, and in *P. scolopendria* was 4.42±0.16%. Alkaloids are naturally occurring organic compounds found in plants with various pharmacological

activities, including anti-malaria, anti-cancer, and anti-asthma (Kittakoop et al. 2014). Other alkaloids possess psychotropic and stimulant activities, while some can be toxic. A high alkaloid content may be used as an antimalarial, anti-cancerous, and anti-asthmatic (Bandyopadhyay and Dey 2022).

Phenol content in *M. mauritiana* was 4.09±0.08%, in *P. scolopendria* was 2.17±0.04%, and in *N. biserrata* was 1.33±0.77%, Phenolic compounds are natural, large, and complex substances in plants. Phenolic compounds possess defense functions. They display several properties that are quite beneficial to man, including antioxidant properties to protect plants against free radical-mediated diseases. They also perform a defensive role against pathogens, parasites, and predators and provide colors to plants (Walton et al. 2003; Priti et al. 2021).

Flavonoid content in *N. biserrata* was 3.16±0.19%, in *P. scolopendria* was 2.87±0.04%, and in *M. mauritiana* was 2.43±0.02%. The presence of flavonoids in the fronds of these ferns makes them possible to be used in food, medicine, and cosmetics because flavonoids have anti-oxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties in addition to their ability to modulate key cellular enzyme functions. Flavonoids in plants are responsible for the color and aroma of flowers and fruits to attract pollinators (Griesbach 2005; Antonisamy et al. 2023).

Saponin in *P. scolopendria* was 1.65±0.03%, in *M. mauritiana* was 1.35±0.04%, and in *N. biserrata* was 1.29±0.08%. Saponin in the fern species showed that it can be used as a surfactant with the potential ability to interact with cell membranes (Lorent et al. 2014). Saponins can be utilized as adjuvants in vaccine development, soap making, medicine, fire extinguishers, dietary supplements, producing steroids and carbonated beverages, anti-feedants, and protecting plants against microbes and fungi (Sun et al. 2009).

Tannin in *P. scolopendria* was 2.54±0.09%, in *M. mauritiana* was 1.91±0.02%, and in *N. biserrata* was 0.98±0.11%. Tannins play a role in protecting against predators and help regulate plant growth (Ferrell 2006). McGee (2004) showed that the astringency from tannin can cause a dry and pucker feeling in the mouth following consumption.

In conclusion, the results of the proximate evaluation of *P. scolopendria, M. mauritiana,* and *N. biserrata* support the utilization of the fronds as food supplements. It also showed that they have good nutritional value. They also possess antioxidant activity, which may have potential in the pharmaceutical industry.

ACKNOWLEDGEMENTS

The authors acknowledge the Laboratory Technologists in the Department of Plant Science and Biotechnology, Rivers State University, Nigeria for assisting in the laboratory procedures. We also declare that there were no conflicts of interest.

REFERENCES

Antonisamy JMA, Narayanan J, Saleride JC, Sivaraman A. 2023. Phytochemistry of Indian Pteridophytes: A review. In: Marimuthu J, Fernandez H, Kumar A, Thangaiah S (eds). Ferns. Springer Nature, Singapore.

AOAC [Association of Official Analytical Chemists]. 2000. Official Methods of Analysis. 17th Edition. Association of Official Analytical Chemists, Washington DC, USA.

Bandyopadhyay A, Dey A. 2022. Medicinal Pteridophytes: Ethnopharmacological, phytochemical and clinical attributes. Beni-suef Univers J Basic App Sci 11: 113. DOI: 10.1186/543088-022-00283-3.

Banso A, Adeyemi S. 2006. Phytochemical screening and antimicrobial assessment of *Abutilon mauritianum*, *Bacopa monnifera* and *Datura stramonium*. Biokemistri 18 (1): 44-52. DOI: 10.4314/biokem.v18i1.56390.

Bouttwell RK. 1998. An overview of the role of nutrition in Carcinogenesis. In: Harris CC, Cerutti PA (eds). Nutrition Growth and Cancer. Alan R. Liss, London.

Chaney SG. 2006. Principles of nutrient 1: Macronutrients. In: Devlin TM (eds). Textbook of Biochemistry, with Clinical Correlation. John Wiley and Sons, New York

Christensen J. 1997. Medical Ethnobotany, Phytochemistry and Bioactivity of the Ferns of Moorea, French and Polynesia. Nichole Baltrushes Publishers, Chicago.

Dutta AC. 2003. Botany for Degree Students. 6th Ed. Oxford University Press, New Delhi, India.

Griesbach R. 2005. Biochemistry and genetics of flower color. Plant Breed Rev 25: 89-114. DOI: 10.1002/9780470650301.ch4.

Harborne JB. 1973. Phytochemical Methods. Chapman and Hall, London.

Igile GO, Iwara IA, Mgbeye BI, Uboh FE, Ebong PE. 2013. Phytochemical, proximate and nutrient composition of *Vernonia calvaona* Hook (Asteraceae): A Green-leafy vegetable in Nigeria. J Food Res 2 (6): 111-122. DOI: 10.5539/jfr.v2n6p1.

Khoja AA, Haq SM, Majeed M, Hassan M, Waheed M, Yagoob U, Bussmann RW, Alataway A, Dewidar AZ, Al-Yafrsi M, Elansary HO, Yessoufou K, Zaman W. 2022. Ecological and traditional knowledge of Pteridophytes in Western Himalayas. Diversity 14 (8): 628. DOI: 10.3390/d14080628.

Kittakoop P, Mahido C, Ruchirawat S. 2014. Alkaloids as important scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis, and smoking cessation. Curr Top Med Chem 14 (2): 239-252. DOI: 10.2174/1568026613666131216105049.

Kumaran A, Karunakaran RJ. 2006. Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus.* Food Chem 97 (1): 109-114. DOI: 10.1016/j.foodchem.2005.03.032.

Lorent JH, Quetin-Lecleberq J, Mingeot-Leclercq MP. 2014. The Amphiphilic nature of saponins and their effects on artificial and biological membranes and potential consequences for red blood and cancer cells. Org Biomol Chem 12 (44): 8803-8822. DOI: 10.1039/C4OB01652A.

Malan DF, Neuba DFR. 2011. Traditional practices and medicinal plants use during pregnancy by Anyi-Ndenye women (Eastern Côte d'Ivoire). Afr J Reprod Health 15: 85-93.

Mcgee H. 2004. On Food and Cooking: The Science and Lore of the RT Kitchen. Scribner, New York.

Murthy HN, Yadav GG, Bhat MA. 2023. Bioactive compounds of Pteridophytes. In: Murthy HN (eds). Bioactive Compounds in Bryophytes and Pteridophytes Reference Series in Phytochemistry. Springer, Cham. DOI: 10.1007/978-3-031-23243-5.

Obadoni BO, Ochuko PO. 2001. Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. Glob J Pure Appl Sci 8 (2): 203-208. DOI: 10.4314/gjpas. v8i2.16033.

Piggott AG. 1996. Ferns of Malaysia in Color. Tropical Press, Kuala Lumpur.

Priti G, Pratiba K, Prabha S, Prem L. 2021. Ethnomedicinal uses of Pteridophytes for treating various human ailments in India. In: Singh LJ, Ranjan V (eds). New Vistas in Indian Flora. Messrs Bishen Singh Mahendra Pal Singh, India.

Raven PH, Evert RF, Eichhorn SE. 2005. Biology of Plants (7th edition). W.H.Freeman, New York.

Singh BP, Upadhyay R. 2012. Ethnobotanical importance of Pteridophytes used by the tribe of Pachmarhi, Central India. J Med Plants Res 6 (11): 14-18. DOI: 10.5897/JMPR11.367.

Snogan E, Vahirua-Lechat I, Ho R, Bertho G, Girault JP, Ortiga S. 2007. Ecdysteroids from the medicinal fern *Microsorum scolopendria* (Burm. f.). Phytol Anal 18 (5): 441-450. DOI: 10.1002/pca.1000. PMID: 17624884.

Sun HX, Xie Y, Ye J, Yi P. 2009. Advances in saponin-based adjuvants. Vaccine 27 (12): 1787-1796. DOI: 10.1016/j.vaccine.2009.01.091.

Vunchi MA, Umar MA, King AA, Liman GJ, Aigbe CO. 2011. Proximate, vitamins and mineral composition of *Vitex doniana* (black plum) fruit pulp. Nig J Bas Appl Sci 19 (1): 97-101. DOI: 10.4314/njbas. v19i1.69352.

Walker E, Pacold M, Perisic O. 2000. Structural determinants of Phosphoinositide 3-Kinase inhibition by Wortmannin, LY294002, Quercetin, Myricetin, Staurosporine. Molec Cell 6 (4): 909-919. DOI: 10.1016/S1097-2765(05)00089-4.

Walton NJ, Mayer MJ, Narbad A. 2003. Molecules of interest: Vanillin. Phytochemistry 63 (5): 505-515. DOI: 10.1016/S0031-9422(03)00149-3.

ASIAN J NAT PROD BIOCHEM
Volume 22, Number 1, June 2024
Pages: 8-18

P-ISSN: 2775-4189
E-ISSN: 2775-4197
DOI: 10.13057/biofar/f220102

Medicinal plants used in the management of cancer and other diseases in Swat District, Pakistan

SHUJAT ALI[1,♥], MEHMOODA MUNAZIR[2]

[1]Independent Researcher. Swat, Khyber Pakhtunkhwa 19120, Pakistan. ♥email: alishujat119@gmail.com
[2]Department of Botany, Government College, Women University. Kutchehry Road, Sialkot 51040, Pakistan

Manuscript received: 10 December 2023. Revision accepted: 27 February 2024.

Abstract. *Ali S, Munazir M. 2024. Medicinal plants used in the management of cancer and other diseases in Swat District, Pakistan. Asian J Nat Prod Biochem 22: 8-18.* The provision of healthcare in impoverished nations is significantly influenced by traditional medicine. It has been revealed that many cancer patients use traditional medicine, either as a complementary therapy or as a primary treatment. Among noncontagious infections, cancer is one of the main causes of morbidity and mortality worldwide. This study aimed to determine the plants that people in the Swat regions, Pakistan used to treat cancer through traditional medicine. Interviewing consenting individuals about the ethno-medicinal plants they use to treat cancer was done using a structured questionnaire. Also, an assessment of the literature published on the mentioned plants was done. Questions about plants used to cure cancer, parts of plants used, the form of cancer cured, therapeutic applications of the plants, and the preparation and administration of the plant parts were posed to the practitioners and the locals. About 12 plants in all, comprising five herbs, three climbers, three trees, and one shrub, were used to treat cancer. About 50% of plants contain flavonoids, compounds with various anticancer properties. Fruits accounted for 41% of all the parts used in the documented species, with leaves coming in second at 25%, bark at 17%, roots at 17%, and the entire plant at 8%. However, the highest RFC was shown by *Vitis vinifera* L. (0.56), the lowest was shown by *Viola biflora* L. (0.03), while the highest FL was shown by *Hedera nepalensis* K.Koch (83), and the lowest was shown by *V. biflora* (30). According to the study, the gathered plants were used to treat a variety of malignancies: general tumors were treated by 75% of the plants, breast cancer was treated by 17%, and lung cancer was treated by 8%. The reported uses of the medicinal plants from prior ethno-pharmacological studies conducted in Swat regions align well with the traditional uses of the plants mentioned in this study. Therefore, if sufficiently investigated, the Swat district's indigenous herbs used to treat cancer may play key roles in searching for and creating anticancer medications.

Keywords: Bioactive compounds, cancer, local people, medicinal plants, Swat

INTRODUCTION

The term "traditional medicine" describes methods of maintaining and regaining health that predate the development of contemporary medicine (Domfeh 2007). According to WHO (2003), a third of people on the planet do not regularly have access to basic modern medicine. An estimated half of the population in some regions of Africa, Asia, and Latin America is thought to lack access to basic healthcare, primarily due to inadequate government funding. This explains the stark disparities in healthcare quality in underdeveloped nations. People in developing nations suffer greatly from the high drain of infectious syndromes (HIV, AIDS, Malaria, TB, Pneumonia and Diarrhea) also the rising menace of non-infectious ailments (NCDS) like diabetes, cancer, hypertension, and ischemic heart disease, among several others (Payyappallimana 2010). Human health is enhanced using ethnomedicinal plants, which support the primary healthcare needs of the local population (Kambizi and Afolayan 2001). Traditional medicine is used for primary healthcare by around (80%) of individuals in remote areas of emerging nations (Bodeker et al. 2005). Several cancer patients generally use customary remedies as key treatment and complementary medication (Cassileth and Deng 2004; Verhoef et al. 2005).

Many plant-based anticancer medicines are used in clinical practice, including taxol, vincristine, vinblastine, etoposide, irinotecan and topotecan. Worldwide research is still being done on ethnomedicine to treat cancer, and the National Cancer Institute USA is a major participant in medicinal plant investigation for cancer treatment. The NCI gathered around thirty-five thousand plant models from twenty different nations, and roughly 114,000 excerpts have been tested for anticancer bustle (Manju et al. 2017). According to projections, there will be 11.5 million cancer deaths by 2030, up from seven million in 2002 (Mathers and Loncar 2006). Deterioration of quality of life is linked to cancer, not only for the patients but also for spouse caregivers. The quality of life for spouse caregivers is significantly influenced by the diagnosis, period of hospitalization, intensity, and duration of care (Chen et al. 2004). Among several serious side effects, treatment for the condition with some medications has been linked to a decline in the worth of life, as well as in the stimulation of exhaustion and roughly marginal neuropathy.

Many commonly used anticancer medications can induce Chemotherapy-Induced Peripheral Neuropathy (CIPN), which compensates for equally large and small afferent sensory neurons (Mantyh 2006). Other medications have been shown to suppress bone marrow, which leaves patients more vulnerable to infections and

other illnesses. Therefore, it is crucial to design medications that provide the best possible treatment for an illness without lowering a patient's quality of life. In an attempt to reduce side effects, many patients often combine traditional medicine with their conventional therapy or, in some cases, forego it entirely. In many developing nations, a sizable section of the populace gets their healthcare from traditional healers and the usage of medicinal herbs; these health treatments are supposed to be inexpensive, easily accessible, and effectively treated (Konno 2004). Particularly in wealthy nations, many traditional remedies are now more widely accessible commercially. Strict manufacturing requirements are applied to producing these medications in certain nations (WHO 2002).

Traditional medicine knowledge has been primarily transmitted verbally between generations without extensive documentation. The absence of documentation may have resulted in the loss of some knowledge. Most communities' dependence on ethnomedicinal plants for basic healthcare has assisted in sustaining the body of knowledge on medicinal plants (Maroyi 2011). Preserving medicinal plants and protecting indigenous knowledge systems (IKS) in traditional medicine may be aided by documenting the IKS. Globally, especially for indigenous people, it depends on acknowledging IKS for cultural and economic emancipation. Therefore, developing successful adaptation solutions that may be affordable, inclusive, and sustainable might result from including native information in relevant strategies, like those about healthiness or the environment (Robinson and Herbert 2001).

Although traditional medicine is becoming more and more popular in the Swat district, little is known about the indigenous expertise in using medicinal plants to treat cancer. This specific ethnobotanical survey was conducted in an attempt to close the gap. Documentation helps conserve medicinal plants by ensuring that knowledge of traditional medicine is maintained. It also simplifies the process for further research projects that can concentrate on verifying the detected plants' activity in contrast to the claims.

MATERIALS AND METHODS

Study area

Swat is known as "Paradise on Earth" for its outstanding natural appearance, spreading across its domain of 5,337 km^2. Swat is famous for its abundant greenish-beautiful scenery, including snow-covered mountains, broad water springs and streams, colorful meadows, clear environmental conditions, and pleasing and welcoming people (Ali 2023). Until 1969, Swat persisted as an independent and sovereign state known as "The Yusafzai State of Swat." The State of Swat voluntarily merged with Pakistan on 14 October 1969. This union occurred 22 years later, in 1947, when Pakistan gained independence from British rule. Swat is bordered by Chitral to the Northwest, Dir to the West, Malakand to the South, and Buner to the Southeast. Also, to the east is Shangla, to the Northeast is Kohistan, and to the North is Gilgit-Baltistan (Figure 1) (Ali et al. 2023). Swat is known for its impressive mountains at the foot of the Hindu-Kush range. The Swat River Valley, which endeavors through the district's southern boundaries, has an elevation of about 600 meters and emerges rapidly to the north. Various peaks have altitudes ranging from 4,500 meters to over 6,000 meters above sea level. Its climate is quite intermediate and changeable but often highly hot. It is an area where you can see all four seasons in one day, but it is a 20 to 30-minute drive one way, and the weather is often completely different. During the spring, high temperatures in March to May range from about 7°C to 13°C. Summers in Swat, such as June to August, are usually the warmest months, with average highs of around 15°C to 17°C. Swat's Autumn (or fall) temperatures can range from about 8 °C to 14 °C from September to November. December to February are usually the coldest months in Swat, with average high temperatures around 5°C.

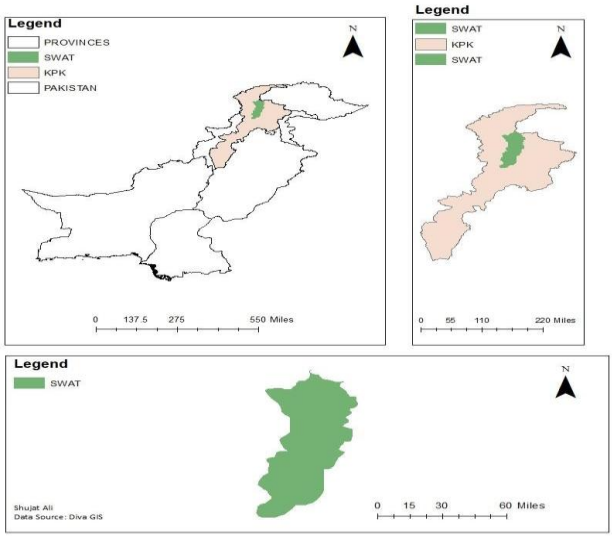


Figure 1. Study area

Data collection

This study was conducted to gather information about plants used to fight or prevent cancer. The data were collected using questionnaire-type forms. The data were collected over almost one year (December 2021 to December 2022). We interviewed well-informed respondents based on their knowledge of ethno-medicinal plants, and all respondents' consent was obtained before the interviews. About 300, including 216 male and 84 female respondents, were chosen based on specific criteria, such as who prescribes treatment recipes, is involved in selling, is a plant collector, is an elderly member, or is a young, educated individual. We also included some professionals for interviews, such as physicians, pathologists, nurses, medical technologists, radiologic technicians, and other health- and hospital-related individuals. Generally, the professions were investigated to collect data about the signs and symptoms, clinical treatment, and benefits and side effects of clinical treatments like chemotherapy, radiotherapy, and other related treatments. We also used different literature databases, including PubMed, Scopus, Online Science Web, Google Scholar, and previously published literature.

Data analysis

The collected data were analyzed statistically via Microsoft Excel (2016) and cross-checked with old published literature. Additionally, the quantitative indices, including Fidelity Level (FL) and Relative Frequency of Citation (RFC), were used to assess the obtained data statistically.

Fidelity Level (FL)

Multiple plant species are certainly utilized in the treatment of a single ailment within a certain category. The formula established was carefully considered while determining the Fidelity Level (FL) index.

$$FL = Np/N \times 100$$

Where N is the total number of informants who cited the species for any ailments, and Np is the number of informants who mentioned using the plant for a particular disease. A high FL score indicates that the study area's informants use the plant species more frequently to treat a certain illness category.

Relative Frequency of Citation (RFC)

RFC is the most utilized plant taxon by the native people. It was determined by using the formula:

$$RFC = FC/N$$

Where ($0 < RFC < 1$), FC is the 'Number of informants citing a useful species' and N is the 'total number of informants' in the survey.

RESULTS AND DISCUSSION

Information

A total of 40 field trips were to collect data on medicinal plants. The entire period of the fieldwork was about one year, from December 2021 to December 2022. A total of 300 individuals were interviewed. Almost all the participants were natives of the area. The greatest number of people were aged between 60 to 80 years (50%), 40 to 60 years (33%), and less than 30 years (17%), as shown in Table 1. Approximately 200 people were illiterate, 30 were metric passers, 20 held bachelor's or master's degrees, and 50 were chosen from professional fields. Among these 50, 10 were selected from the laboratory (a pathologist or microbiologist), 20 were nurses by profession, 10 were medical technologists, and 10 were radiologic technicians, as shown in Table 1. Most of the individuals were Pashto speakers. Most informants were male (72%) rather than female (28%). This could be because the male interviewer made them feel at ease and allowed them to speak freely.

Using plants as a complementary and alternative medicine for cancer

Plants and their product-based treatments play a vital role in cancer prevention. Nowadays, scientists have been trying to make drugs or introduce possible ways to treat cancer, but it is unlikely they have not successfully introduced a proper form of medication for cancer except chemo and radiotherapy. Therefore, people across the world still depend on plant-based products for cancer treatments. As a result, the current study involves examining the plants to document their importance in cancer prevention. The current study documented about 12 plants that were used to treat various diseases. In addition to being effective against other diseases, these plants are also effective against cancer in the study area, as shown in Tables 2 and 3. Current research indicates that about 12 plants belong to 9 families. As shown in Table 3, the documented 12 plant species have been described to be used in treating human cancer, most of them being 42% herbs, 25% vines, 8% shrubs, and belonging to different plant families, including 25% trees, as shown in Figure 2. For the treatment of cancer, the highest RFC was shown by *Vitis vinifera* L. (0.56), and the lowest was shown by *Viola biflora* L. (0.03), while the highest FL was shown by *Hedera nepalensis* K.Koch (83), and the lowest was shown by *V. biflora* (30), as shown in Table 3.

The most common compounds were flavonoids, found in 50% of plants and have various anticancer effects because they regulate the activity of enzymes that scavenge Reactive Oxygen Species (ROS), contribute to cell cycle perturbations, and persuade and suppress apoptosis and autophagy cancer cell growth and invasion. Along with cancer, the documented 12 plant species custom to cure a range of other ailments, including laxatives, expectorants, heart problems, stomach problems, as a plaster, carbuncles, diuretic effects, aphrodisiac tonics, rheumatism, skin inflammation, fever, purgatives, respiratory diseases, dyspepsia, brain disorders, asthma, jaundice, piles, joint aches, diabetes, blood purification, and epilepsy.

Plant parts used against cancers

Locals used several parts of the plant, like leaves, roots, bark, seeds, fruits, and occasionally the whole plant, to create medicines for careful control. The fruits and leaves were the most commonly used parts. In 12 plant species, fruits (41%) were extracted, followed by leaves (25%), bark (17%), roots (17%), the whole plant (8%), gum (8%), kernel (8%), needle (8%), and twigs (8%) as shown in Figure 3 and Table 3. It was also noted that the reported plants were used to treat cancer and many other diseases. However, we noticed that a single plant can treat more than one disease. During the interviews with local people, these plants were also brought from an adjacent area when they were not available there.

Table 1. Demographic characteristics

Demographic Characteristics	Number	Percentage
Age		
30 or below	50	17%
40 to 60 years	100	33%
60 to 80 years	150	50%
Sex		
Male	215	72%
Female	85	28%
Education status		
Educated	100	33%
Uneducated	200	67%
Professions		
Farmer and others	200	67%
Pathologist or microbiologist	10	3%
Nurses	20	7%
Medical technologists	10	3%
Radiologic technicians	10	3%
Govt school teachers	15	5%
Private school teachers	5	2%
No proper profession (jobless)	30	10%

Types of cancer treated with plants

During the field study, we documented that several forms of cancer have been cured with medicinal plants such as blood, lung, colon, rectum, prostate, skin, breast, uterus, thyroid, and lymphatic system. Chemotherapy, precision medicine, radiation therapy, surgery, stem cell transplantation, hormone therapy, immunotherapy, and targeted therapies have all been used to treat cancer. However, the present study revealed that they were used to treat various cancers, such as generalized cancer. As shown in Table 2, 75% of plants treated general cancers, 17% treated breast cancer, and 8% treated lung cancer, as shown in Figure 4. The study showed that all the collected plants were used to treat all types of cancer.

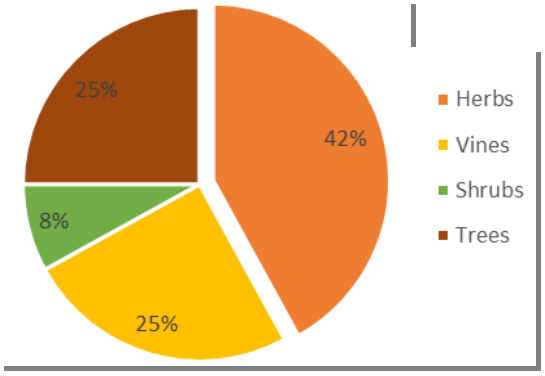


Figure 2. Plants forms

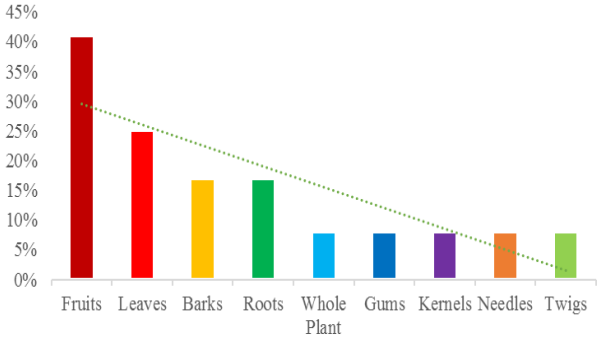


Figure 3. The plant parts used

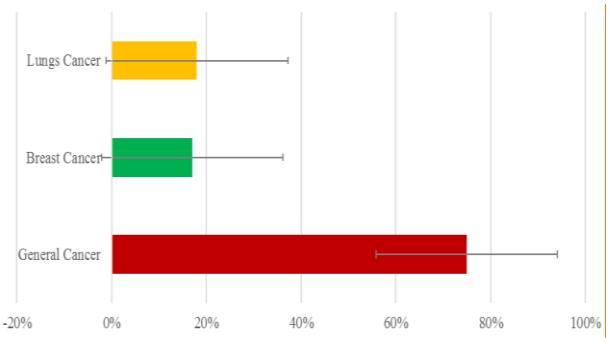


Figure 4. Cancer type treated with plants

Secondary metabolites and their anticancer effects

Secondary metabolites serve crucial supporting roles in plant development and evolution, even though they are not necessary for it. These include promoting herbivore defense, preventing the growth of rival plants, thwarting the growth of bacterial and fungal diseases, and facilitating pollination. They include many antioxidant and anti-inflammatory substances (Adebayo et al. 2015). The chelation of redox-active metal ions, which results in lipid peroxidation and free radical outflow, is a component of antioxidant action. These items aid in the management of cancer as well. Plant secondary metabolites are categorized into alkaloids, terpenoids, polyphenols, and flavonoids based on their structural makeup (Singh et al. 2016). Vinblastine, vincristine, and camptothecin are examples of

known anticancer alkaloids; lycopene and gamma-tocopherol are examples of terpenoids. Resveratrol, curcumin, etoposide, and epigallocatechin gallate (EGCG) are examples of polyphenols. Camphorol, genistein, and apigenin are examples of flavonoids. These bioactive substances are necessary for the development, proliferation, microtubule formation, and cell death of cancers. They can act alone or in concert with other substances to produce anti-tumor effects through classical metabolic and signaling pathways (Kojima-Yuasa et al. 2015). Numerous botanical and herbal infusions have been studied for their antiproliferative qualities over time; many show ontogeny and effectiveness in slowing the spread of cancer.

Table 3 summarizes some of the plants that have been studied and their active ingredients with good cancer-preventing and limiting effects. A detailed study of the implicit chemical processes behind the actions of these compounds and the actions of several other new compounds can shed light on the biological underpinnings of their anticancer properties. As a result of this goal, it is important to recognize changes in cancer physiology that differ from normally proliferating cells to transfer the side effects of anticancer drugs to normal cells.

Table 2. Plants used in the treatment of cancers

Plants	Extract from	Anticancer Potential
Carthamus oxyacantha M.Bieb.	Seeds and flowers	According to an anti-oxidation investigation, a 40µg extract from this plant indicated more than 40% inhibition of cancers (Souri et al. 2004).
Convolvulus arvensis L.	Whole plant	Its alcoholic infusions have essential anticancer effects against cell lines. Oral administration of proteins and polysaccharides suppressed tumor growth dose-dependent (Meng et al. 2002).
Hedera nepalensis K. Koch	Leaves and barks	It has two major compounds that are prudent for anticancer activities: hederagenin 3-O-L-arabinopyranoside and pulsatilla saponin A (Li et al. 2015).
Malus pumila L.	Fruits	Apple has bioactive substances that contribute to the prevention of cardiovascular disease, diabetes, inflammation, and cancer (Han et al. 2020).
Prunus armeniaca L.	Kernel and gums	The plant has bioactive compounds, which can activate different anticancer processes and signaling pathways, such as tumor suppressor proteins that reduce the proliferation of tumor cells (Kitic et al. 2022).
Rosa moschata J. Herm.	Fruits and flowers	It is also used as a food that may reduce the incidence of cancer and as a means of stopping or reversing cancer growth (Mathews 1994).
Solanum nigrum L.	Leaves and fruits	The plant's crude infusion has demonstrated anti-tumor effects in a variety of cancer types, including colorectal, endometrial, cervical, and breast malignancies in humans (Li et al. 2008; Liu et al. 2021).
Taxus wallichiana Zucc.	Barks, needles, twigs, and roots	The plant has recently attracted attention because of the discovery that taxol, a powerful anticancer medication, is mostly present in its leaves and bark. Numerous other biological functions are also present in it (Juyal et al. 2014).
Viburnum grandiflorum Wall. ex DC.	Fruits and barks	The plant extract activated apoptosis through a caspase-dependent route, thereby inhibiting the viability of lung cancer cells. As a result, its extract effectively inhibits the growth of lung cancer cells (Liu et al. 2021).
Viola biflora L.	Flowers	The plant has anticancer properties (Hamayun et al. 2007). The present study recommended this plant for further phytochemical studies.
Vitis vinifera L.	Fruits	Its extract showed cytotoxic against cancer cells. Infusion isolated from seeds and stems investigated anti-tumor action in human breast cancer cell lines (Kaur et al. 2009).
Withania somnifera (L.) Dunal	Leaves and roots	The plant has main constituents like withanolide A and withaferin A; withaferin A is mostly found in the plant leaves and produces fast apoptosis in cancer cells (Malik et al. 2007).

Table 3. Plants used for the treatment of different diseases in the area

Family	Plants	Local Name	Habitat	Extract From	Medicinal Uses	RFC	FL
Araliaceae	*Hedera nepalensis* K. Koch	*Zela Bota*	Climber	Leaves and bark	Used as cancer, also used as fodder and ornamental.	0.2	83
Asteraceae	*Carthamus oxyacantha* M.Bieb.	*Kareeza*	Herb	Seed and flower	Used as fuel and food. Seed oil is used to control cancer, urination, and stomach problems.	0.4	58
Caprifoliaceae	*Viburnum grandiflorum* Wall. ex DC.	-	Shrub	Fruit and Bark	Fruit is used to cure cancer and stomach problems.	0.23	71
Convuluaceae	*Convolvulus arvensis* L.	*Perwathai*	Herb	Whole plant	Used for cancer, diabetes, and blood purification.	0.31	42
Rosaceae	*Malus pumila* L	*Manra*	Tree	Fruits	It is an economical fruit, laxative, and expectorant, used in jams and jellies and for heart diseases. Its tough wood is used to make agricultural tools and fuel wood. Leaves are used as fodder.	0.5	40
Rosaceae	*Prunus armeniaca* L.	*Khobanay*	Tree	Kernel and gum	Used as fresh or dried fruits and seeds. In addition to being utilized as fuel wood and a honeybee species, it is a purgative for cancer. Foliage is made from leaves.	0.43	60
Rosaceae	*Rosa moschata* J. Herm.	*Qaroch*	Climber	Fruits and flowers	Used for cancer and curing stomach disorders	0.1	67
Solanaceae	*Solanum nigrum* L.	*Kachmacho*	Herb	Leaves and fruit	The leaves are used to treat skin inflammation, while the fruits are used to treat fever.	0.13	38
Solanaceae	*Withania somnifera* (L.) Dunal	*Kotilal*	Herb	Leaves and root	Used as a plaster, it is also used for cancer, ulcers, and carbuncles. Fruit has a diuretic effect. Root is an aphrodisiac tonic, diuretic, narcotic, and rheumatism treatment.	0.51	58
Taxaceae	*Taxus wallichiana* Zucc.	-	Tree	Bark, needles, twigs, and roots	Its leaves are used for cancer and respiratory diseases. It is also used in dyspepsia and brain disorders; the leaves and fruits are sedative and aseptic; it is utilized in constructing roofs; and due to its strength against dense snowfall, its wood is placed on graves.	0.33	40
Violaceae	*Viola biflora* L.	*Banfsha*	Herb	Flower	It is used as a diaphoretic, antipyretic, cancer preventive, and febrifuge, as well as for epilepsy and nervous diseases.	0.03	30
Vitaceae	*Vitis vinifera* L.	*Kowar*	Climber	Fruits	It is used to cure piles, joint aches, cancer, fever, asthma, jaundice, vomiting, and stomach issues. It is also used as a laxative, purgative, diuretic, and aphrodisiac.	0.56	59

Compounds found in different parts of plants can help treat diverse forms of cancer, and all compounds collected from plants belong to different classes. For example, flavonoids are plant-derived polyphenol secondary metabolites. Sterols are assigned to lipids (fat in the broadest sense). There are 11 major classes of saponins (dammarane, tirucallane, lupine, hopane, oleanane, taraxasterane, ursan, cycloartane, lanostane, cucurbitan, and steroid). Squalene is an acyclic triterpene, while achilleol A, lanostane, dammarane, and euphane are monocyclic, pouoside A is a bicyclic triterpene, and oleane group, ursan group, lupine group, and hopane group are pentacyclic triterpenes. The two primary classes of carotenoids are carotenes and xanthophylls. Antioxidant qualities are present in both forms of carotenoids.

Furthermore, taxol is a member of the plant alkaloids class of chemotherapeutic medicines. Because each amygdalin molecule contains a nitrile class that beta-glucosidase might release as a deadly cyanide anion, amygdalin is categorized as a cyanogenic glycoside. Mannose is a member of the hexose class of chemical compounds, where monosaccharides are molecules with six carbons as the sugar component. Luteolin is a flavone, a type of flavonoid, and kaempferol belongs to the flavonol group of organic compounds. Sesquiterpenes are a group of terpenes belonging to three isoprene units. Sesquiterpenes, like monoterpenes, can be cyclic or have several specific combinations of rings. Vibsin-type diterpenoids are thought to be common in natural products.

Why plant-based drugs are best for the treatment of cancer instead of other clinical drugs

During a field survey, some professionals and cancer patients said that about 85% of cancer patients faced various side effects when treated with chemotherapy and radiotherapy, such as impotence, fatigue, nausea, hair loss, and vomiting. Sore mouth and numbness are also common side effects, and less common are diarrhea, abdominal cramps, and memory loss, which are reported as erogenous zone side effects. A similar report was made by Aslam et al. (2014) from Pakistan. Current research, therefore, indicates that chemotherapy aims to be as efficient as possible with manageable side effects. The morbidity of chemotherapy drugs can pose significant difficulties in treating cancer with symptomatic or conventional drugs. Several therapies have been developed to cure cancer, many of which use plant-derived products, and those plants have great potential for new drugs and are endowed with anticancer chemo-protective properties. Remarkable improvement has been made in warning and guiding malignancy (cancer) development, but there is still considerable validity and room for enhancement. Some unexpected side effects may occur during chemotherapy. Natural remedies can reduce harmful side effects, such as using herbal products to cure malignancies (cancer), and recent investigations have found about a dozen botanical products. Nevertheless, a myriad of products prepared from the documented 12 plants have been found to have very promising anticancer properties but need to be evaluated for further applications in human cancer treatments. It is necessary to determine the efficacy of these botanical products.

Knowledge and awareness of cancer among residents of Swat

During the fieldwork for the present study, we asked the people about cancer, and their responses were very surprising; they did not know about cancer. Cancer is referred to as "*Sakha*" (bad) or "*Khatrnaka Bemari*" (dangerous diseases) by the locals. Thus, the current survey results show extremely low cancer awareness or consciousness among Swat residents. According to our general observations, about 95% of illiterate people did not know about cancer. As a result, this study found that few residents knew much about cancer, while most knew almost nothing about the risk factors and early symptoms of the disease. In this regard, several efforts must be made to increase people's awareness of cancer and its prevention and treatment.

Discussion

Medicinal plants, including in many countries, contain frequent alternative medicines for treating cancers worldwide (Tascilar et al. 2006). About 3,000 plants are reported as anticancer sources worldwide (Graham and Quinn 2000). Worldwide, the frequency of use of products derived from plants for cancer ranges from 10 to 40%, but now it is reaching 50% in Asian patients. Throughout the Middle East and Europe, herbal medicine has been used for a long time (Cassileth and Deng 2004; Molassiotis et al. 2006). A recent WHO (2004) report reveals that many developing countries believe traditional herbs are legitimate anticancer drugs. About 5-15% of these herbs are being studied to find bioactive anticancer compounds (Ahmad et al. 2016).

The study shows that the root of *V. biflora* is used for stomach disorders and jaundice, expectorants, epilepsy, nervous disorders, antispasmodics, diaphoretic purposes, colds, flu, and laxatives, as shown in Table 3; similar applications are found for *V. biflora* in the study area described by Hamayun (2007). The plant contains compounds like aurantiamide acetate, solalyratin B, esculetin, scopoletin, lupeol, 2S-hydroxypheophytin, vomifoliol, dibutyl phthalate, (-)-dihydrovomifoliol, grasshopper ketone, crassifol, and -sitosterol (Cong et al. 2016). The *Convolvulus arvensis* L. is used in medicine to cure purgatives and skin disorders. Similar uses for the plant were also suggested by Akhtar et al. (2013) and Sher et al. (2003); it is used as an anticancer agent on different cell lines. Saponins, steroids, flavonoids, alkaloids, proteins, and lipids are the major compounds found in this plant (Kaur and Kalia 2012). However, alcoholic infusions have essential anticancer effects against IMR-32 and Colo-205 cell lines (Kaur and Kalia 2012). Particularly, oral administration of proteins and polysaccharides suppressed cancer progression in a dose-dependent; around 70% of tumor development was suppressed at a broad, indefinite dose (200 mg) daily. Over 70% of tumor growth is suppressed by subcutaneous or intraperitoneal administration at 50 mg daily (Meng et al. 2002).

The *Carthamus oxyacantha* M.Bieb. controls urination and cures stomach problems and cancer, as shown in Table 3; similar uses for the plant are also documented by Khan et al. (2015). Its flowers and seeds contain glycosides, serotonin, flavonoids and sterols (Souri et al. 2004). Among the chemical components, it also contains oils such as oleic and linoleic derivatives; the pyrrolizidine alkaloids were also isolated and defined from the plant. Its fruit contains 20-25% protein. HPLC was used to isolate two glycosides, 2-O-methyl glucopyranosyl carthamo side and beta-D-fructo furanosyl carthamo side, as well as compounds 3', 4', 5, and 7-tetrahydroxy flavanone (Ahmad et al. 2010). Oxidative stress is linked to several degenerative illnesses, such as cancer. Therefore, the trend of looking for antioxidants from natural sources is growing daily. In an antioxidant study, a 40 µg extract of *C. oxyacantha* showed over 40% inhibition (Souri et al. 2004). studied the antifungal activity of crude extracts (methanol, ethanol, and aqueous) of this *C. oxyacantha* against fungal strains (*Bipolaris sorkiniana* Shoemaker, *Fusarium oxysporum* Schltdl., *Rhizoctonia solani* J.G.Kühn, *Phytophthora drechsleri* Tucker) and found that the extracts were effective against these strains found to exhibit a broad spectrum of activity. A dichloromethane extract of *C. oxyacantha* at a 25 µg/ml concentration exhibited 27.5% neuroprotection and inhibited 44.5% of ROS (Abdolmaleki et al. 2011).

The *H. nepalensis* is used for diabetes, scabies, boils, cancer, heart disease, and diabetes, as shown in Table 3; a similar result was also found by Ahmad et al. (2014). It has inositol, carotenes, and cardiac glycosides (hederagain) (Kanwal et al. 2011). Cytotoxic can affect HeLa and HeLa cancer cell lines. N-hexane and ethyl acetate from the examined plants appear to offer good potential in cancer chemoprevention, according to the evaluation's results. The n-hexane fractions containing lupeol and ethyl acetate have a reduced IC50 (0.20 ± 1.9 µM) when assessed by NFκB. The three cancer cell lines' ontogeny was reduced by roughly 60% by the crude extract and its fractions, and their IC50 values for lupeol ranged from 2.32 to 10.2 µM. Plant leaves are a rich source of lupeol, as demonstrated by the HPLC-DAD-based measurement of lupeol in diverse plant tissues (0.196 mg/100 mg dry weight).

The *V. vinifera* is used to cure skin malignancy (cancer), heart ailment, and antimicrobial activity, as shown in Table 3; similar uses are also noted by Ahmad et al. (2014). The grape infusion did, however, demonstrate cytotoxicity against PC-3, A-549, and MCF-7 cancer cells. In human breast cancer cell lines (MCF-7 and MDA-MB-23), colon (HT29), kidney (786-0 and Caki-1), thyroid (K1), hepatocellular carcinoma, oral squamous cell carcinoma, and normal human fibroblasts, infusions derived from seeds and stems were employed to investigate anti-tumor activity in cell lines (Kaur et al. 2009). Resveratrol, a chemo-protective compound found in grape skins, stimulates autophagy and has anticancer properties. In gastric cancer cells that were activated with TNF-α and whose ICAM-1 mRNA levels were suppressed by methanol infusion, the result was cell death and the control of inflammation (Kaliora et al. 2008). However, increasing evidence from human medical institution trials has shown that consumption of grape juice promotes many health problems and may have anticancer effects. Therefore, there is a great deal of promise for using grape skin and seed infusions to prevent cancer, and further research in this promising area is needed (Zhou and Raffoul 2012).

Rosa moschata J. Herm. is widely used to cure stomach disorders, as shown in Table 3; a similar result was found by Ali et al. (2011). Moreover, it contains important fatty acids, which is uncommon for a fruit. It has also been researched as a diet that can stop or reverse the growth of cancer and lower the incidence of cancer (Matthews 1994). It is an extremely rich source of minerals and vitamins, particularly flavonoids, bioactive compounds, and vitamins A, C, and E.

Taxus wallichiana Zucc. is used as a hypnotic and antispasmodic. The leaves are used in bronchitis, whooping cough, and asthma. It is also used in indigestion and epilepsy; the leaves and fruits are sedatives and antiseptics, as shown in Table 3; a similar result was also found by Ilyas et al. (2013). Its bark and leaves have garnered attention recently since it was discovered that they are the primary source of taxol, a highly effective anticancer medication. Numerous other biological functions are also present in it (Juyal et al. 2014).

Prunus armeniaca L., locally known as *Khobani*, belongs to the Rosaceae family. It is a laxative, and the gum extracted from the stem is anticancer, as shown in Table 3; similar uses of the plants mentioned by Akhtar et al. (2013) from the area. The gum extracted from the stem of *P. armeniaca* was used to treat cancer (Iqbal and Hamayun 2004; Akhtar et al. 2013). Various parts of the apricot plant are used worldwide for their anticancer properties, either as a primary remedy in traditional medicine or as complementary or alternative medicine (Kitic et al. 2022). Bioactive compounds may mediate anticancer properties, activating various anticancer processes and signaling pathways, such as tumor suppressor proteins that reduce tumor cell proliferation (Kitic et al. 2022). It strongly and concentration-dependently reduced cell growth during the incubation period (P 0.05). In both types of cancer cells, the expression levels of the Bax and c-FLIP genes were consistently higher in the untreated group as compared to the control group ($P < 0.001$). According to Mahmoudi et al. (2019), it had a very significant time-dependent mode (P 0.001) of inhibiting the expression of the Bax and c-FLIP genes in cancer cells.

Solanum nigrum L. is an effective treatment for digestive disorders, chronic skin disease, hepatitis, inflammation, and liver problems, as shown in Table 3; similar uses for the plants mentioned by Akhtar et al. (2013), Ali et al. (2011), and Ilyas et al. (2013) from the study area. Plant infusions have shown anti-tumor effects on various cancers, including human melanoma, colorectal, endometrial, and cervical breast cancer (Wang et al. 2010; Liu et al. 2021). Aqueous plant infusions are essential components of a number of traditional Chinese medicine recipes that demonstrate anti-tumor effects in human HCC cells and are used to cure cancer in Hep3B and HepJ5 cells

carefully. These recipes help the cells integrate AE-SN-enhanced cytotoxicity induced by doxorubicin and cisplatin by accumulating microtubule-associated protein-1-light chain-3 A/1B II (LC-3 A/B II), which in turn causes both cells to undergo autophagy and apoptotic cell death (Wang et al. 2010).

Withania somnifera (L.) Dunal is used in Swat to treat aphrodisiacs, as a poultice for swellings, ulcers, and carbuncles, as a diuretic, a narcotic, and to treat rheumatism, as shown in Table 3; similar uses for the plants also mentioned by Akhtar et al. (2013). It has sitoindosine, anferine, isopellertierine, withanolides, and withaferins. The plant's infusion has a number of biological effects (Winters 2006). Because of its anti-stress, anti-aging, anti-peroxidative, anti-inflammatory, antioxidant, anti-tumor, cardio-tonic, and immunomodulatory qualities, it is utilized in a variety of preparations (Malik et al. 2007). Among the plant's primary components are withanolide A and withaferin A, the latter of which is mostly present in the leaves and causes cancer cells to undergo rapid apoptosis. This plant preparation's cell signaling pathways largely rely on the wide range of withferin (Malik et al. 2007). Preparation of *W. somnifera* induced cell cytotoxicity in a number of human cancer cell lines. Additionally, by upregulating the expression of IL-2 and IFN-gamma, its preparation alters the T cell population in tumor-bearing mice (Malik et al. 2007).

Viburnum grandiflorum Wall. ex DC. belongs to the Lotus family. It has been used to cure gastrointestinal complications in the study area, as shown in Table 3; similar uses for the plant were also mentioned by Akhtar et al. (2013) and Ali et al. (2011). Plants are pretreated with UVB-exposed cells, and inflammatory and apoptotic signaling cascades are profoundly regulated. Its VG can act against UVB-induced photodamage (Liu et al. 2021). Viability of H1650, HCC827, and H1299 cells by VGE happened in a way that was dependent on both concentration and time. At 48 and 72 hours, the VGE treatment markedly elevated the apoptotic rate of H1650 ($P < 0.05$) and H1299 ($P < 0.02$) cells. The number of cells in the sub-G1 phase was dramatically increased when 10 µM VGE was applied to H1650 and H1299 cells. In H1650 and HCC827 cells, VGE treatment resulted in the cleavage of procaspase-8/-9 and -3 after 72 hours. His VGE treatment decreased the expression of the Mcl-1 protein in HCC827 and H1650 cells. VGE treatment significantly decreased p-Akt levels in H1650 and HCC827 cells. On H1650 and HCC827 cells, however, the viability-inhibitory effects of VGE were neutralized by transfection with the caspase-9 dN plasmid. When VGE was applied to H1650 and HCC827 cells, cytosolic cytochrome C levels rose. (Han et al. 2020).

Malus pumila L., also called apple, belongs to the Rosaceae family. It is used as a purgative, a source of iron, an expectorant, in jams and jellies, and is good for the heart, as shown in Table 3; similar study uses of the plant also collected from the study area by Iqbal and Hamayun (2004). Fruit-derived biologically active substances are attracting attention as regulators for various diseases because they have fewer side effects than chemical drugs. Apples, one of the most popular fruits, are high in bioactive components and a rich source of nutritious elements. Pentacyclic triterpenes, phytosterols, polyphenols, and polysaccharides (pectins) are the main structural classes of apple components. Trace minerals and vitamins complete the apple fruit's nutritional profile. These physiologically active ingredients found in apples and their skins have benefits for human health, including the prevention of cancer, diabetes, inflammation, and cardiovascular disease. Numerous scientific studies have demonstrated that it might enhance health (Patocka et al. 2020).

In conclusion, people in the area used different plants to treat diseases, including cancer. However, this investigation showed that the same plant was used to cure different ailments in some cases, while in others, different plants were used to cure different ailments. Before, people were not well aware of the diseases, but currently, cancer is the most spread disease with a high ratio in the area. Now, the locals face this extremely uncured disease, of which the most common are breast, colon, blood, and lung cancers. As a result, the current study is being carried out to create a primary report on cancer in the area and to assess the outcome and significance of plants in this dangerous disease. Hence, this study reported that 12 plants are superior for cancer treatment. Studying these plants could serve as a template or conductor for malignancy medicine innovation and development. The increasing trend of malignancy (cancers) in Swat is alarming. This preliminary study will be important in cancer prevention, treatment, and future planning in Swat.

As other nations have done, the Pakistani government, through the Ministry of Health, is obligated to incorporate medicinal plants into the healthcare system so that folks can freely take advantage of the opportunities it presents and freely discuss with physicians the various herbal medicines without fear of retaliation. To facilitate long-term preservation and increase access, further research has to support documenting the existing plant practices.

ACKNOWLEDGEMENTS

We appreciate the locals' contributions to the study, punctual interviews, and hospitality. The author said that they have no competing interests.

REFERENCES

Abdolmaleki M, Bahraminejad S, Abbasi S. 2011. Antifungal activity of some plant crude extracts on four phytopathogenic fungi. J Med Plant 10 (38): 148-155.

Adebayo SA, Dzoyem JP, Shai LJ, Eloff JN. 2015. The anti-inflammatory and antioxidant activity of 25 plant species used traditionally to treat pain in southern African. BMC Complement Altern Med 15 (1): 1-10. DOI: 10.1186/s12906-015-0669-5.

Ahmad M, Sultana S, Fazl-i-Hadi S, Ben Hadda T, Rashid S, Zafar M, Mir AK, Khan MPZ, Yaseen G. 2014. An ethnobotanical study of medicinal plants in high mountainous region of Chail valley (District Swat-Pakistan). J Ethnobiol Ethnomed 10 (1): 1-18. DOI: 10.1186/1746-4269-10-36.

Ahmad P, Jaleel CA, Salem MA, Nabi G, Sharma S. 2010. Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic

stress. Crit Rev Biotechnol 30 (3): 161-175. DOI: 10.3109/07388550903524243.

Ahmad S, Ullah F, Sadiq A, Ayaz M, Imran M, Ali I, Zeb A, Ullah F, Shah MR. 2016. Chemical composition, antioxidant and anticholinesterase potentials of essential oil of *Rumex hastatus* D. Don collected from the North West of Pakistan. BMC Complement Altern Med 16 (1): 1-11. DOI: 10.1186/s12906-016-0998-z.

Akhtar N, Rashid A, Murad W, Bergmeier E. 2013. Diversity and use of ethno-medicinal plants in the region of Swat, North Pakistan. J Ethnobiol Ethnomed 9 (1): 1-14. DOI: 10.1186/1746-4269-9-25.

Ali H, Sannai J, Sher H, Rashid A. 2011. Ethnobotanical profile of some plant resources in Malam Jabba Valley of Swat, Pakistan. J Med Plant Res 5 (18): 4676-4687.

Ali S. 2023. A study on different plant species of the Rosaceae family and their ethnobotanical uses among the local communities at Swat District, Pakistan. Ethnobot Res Appl 25: 1-16. DOI: 10.32859/era.25.13.1-16.

Ali S, Sayed AS, Saeed RF, Iqbal J, Ijaz S, Munazir M, 2023. Ethnomedicinal plant use value in Lower Swat, Pakistan. Ethnobot Res Appl 16 (25): 1-22. DOI: 10.32859/era.25.23.1-22.

Aslam MS, Naveed S, Ahmed A, Abbas Z, Gull I, Athar MA. 2014. Side effects of chemotherapy in cancer patients and evaluation of patients' opinion about starvation based differential chemotherapy. J Cancer Ther 5 (5): 817-822. DOI: 10.4236/jct.2014.58089.

Bodeker G, Ong CK, Grundy C, Burford G, Shein K. 2005. WHO global atlas of traditional. Complement Altern Med 1: xiii-xiv.

Cassileth BR, Deng G. 2004. Complementary and alternative therapies for cancer. Oncol Lett 9(1): 80-89. DOI: 10.1634/theoncologist.9-1-80.

Cassileth BR, Schraub S, Robinson E, Vickers A. 2001. Alternative medicine use worldwide: The international union against cancer survey. Cancer 91 (7): 1390-1393. DOI: 10.1002/1097-0142(20010401)91:7<1390::AID-CNCR1143>3.0.CO;2-C.

Chen M-L, Chu L, Chen H-C. 2004. Impact of cancer patients' quality of life on that of spouse caregivers. Support Care Cancer 12 (7): 469-475. DOI: 10.1007/s00520-004-0636-z.

Cong WL, Chen YT, Zhao WB, Zhang Z, Wang Q. 2016. Chemical constituents of ethyl acetate extract from *Viola biflora*. Zhong Yao Cai 39 (5): 1041-1044. [Chinese]

Domfeh KA. 2007. Indigenous knowledge systems and the need for policy and institutional reforms. Tribes Tribals 1: 41-52.

Graham JG, Quinn ML. 2000. Fabricant DS and Farnsworth N. Plants used against cancer–an extension of the work of Jonathan Hartwell. J Ethnopharm 73 (3): 347-377. DOI: 10.1016/S0378-8741(00)00341-X.

Hamayun M. 2007. Traditional uses of some medicinal plants of Swat Valley, Pakistan. Indian J Tradit Knowl 6 (4): 636-641.

Han B, Wu J, Huang L. 2020. Induction of apoptosis in lung cancer cells by *Viburnum grandiflorum* via mitochondrial pathway. Med Sci Monit Intl Med J Exp Clin Res 2020 (26): e920265-1. DOI: 0.12659/MSM.920265.

Ilyas M, Qureshi R, Shinwari ZK, Arshad M, Mirza SN. 2013. Some ethnoecological aspects of the plants of Qalagai Hills, Kabal Valley, Swat, Pakistan. Intl J Agric Biol 15 (5): 801-810.

Iqbal I, Hamayun M. 2004. Studies on the traditional uses of plants of Malam Jabba Valley, District Swat, Pakistan. Ethnobot leafl 15 (1): 15.

Juyal D, Thawani V, Thaledi S, Joshi M. 2014. Ethnomedical properties of *Taxus wallichiana* zucc. (Himalayan yew). J Tradit Complement Med 4 (3): 159-161. DOI: 10.4103/2225-4110.136544.

Kaliora AC, Kountouri AM, Karathanos VT, Koumbi L, Papadopoulos NG, Andrikopoulos NK. 2008. Effect of Greek raisins (*Vitis vinifera* L.) from different origins on gastric cancer cell growth. Nutr Cancer 60 (6): 792-799. DOI: 10.1080/01635580802295776.

Kambizi L, Afolayan AJ. 2001. An ethnobotanical study of plants used for the treatment of sexually transmitted diseases (*Njovhera*) in Guruve District, Zimbabwe. J Ethnopharm 77 (1): 5-9. DOI: 10.1016/s0378-8741(01)00251-3.

Kanwal S, Ullah N, Haq IU, Afzal I, Mirza B. 2011. Antioxidant, anti-tumor activities and phytochemical investigation of *Hedera nepalensis* K. Koch, an important medicinal plant from Pakistan. Pak J Bot 43 (8): 85-89.

Kaur M, Agarwal C, Agarwal R. 2009. Anticancer and cancer chemopreventive potential of grape seed extract and other grape-based products. J Nutr 139 (9): 1806S-1812S. DOI: 10.3945/jn.109.106864.

Kaur M, Kalia A. 2012. *Convolvulus arvensis*: A useful weed. Intl J Pharma Pharm Sci 4 (1): 38-40.

Khan AA, Ali F, Ihsan M, Hayat K, Nabi G. 2015. Ethnobotanical study of the medicinal plants of Tehsil Charbagh, District Swat, Khyber Pakhtunkhwa, Pakistan. Am Eurasian J Agric Environ Sci 15: 1464-1474. DOI: 10.5829/idosi.aejaes.2015.15.7.94235.

Kitic D, Miladinovic B, Randjelovic M, Szopa A, Sharifi-Rad J, Calina D, Seidel V. 2022. Anticancer potential and other pharmacological properties of *Prunus armeniaca* L.: An updated overview. Plants 11 (14): 1885. DOI: 10.3390/plants11141885.

Kojima-Yuasa A, Huang X, Matsui-Yuasa I. 2015. Synergistic anticancer activities of natural substances in human hepatocellular carcinoma. Diseases 3 (4): 260-281. DOI: 10.3390/diseases3040260.

Konno B. 2004. Integration of Traditional Medicine with Modern Medicine. EHNRI, Addis Ababa. DOI: 10.4314/gjs.v60i1.4.

Li J, Li Q, Feng T, Li K. 2008. Aqueous extract of *Solanum nigrum* inhibit growth of cervical carcinoma (U14) via modulating immune response of tumor bearing mice and inducing apoptosis of tumor cells. Fitoterapia 79 (7-8): 548-556. DOI: 10.1016/j.fitote.2008.06.010.

Li T, Pan H, Feng Y, Li H, Zhao Y. 2015. Bioactivity-guided isolation of anticancer constituents from *Hedera nepalensis* K. Koch. S Afr J Bot 100: 87-93. DOI: 10.1016/j.sajb.2015.05.011.

Liu H, Zhang H, Dang M, Lin Y, Yan H. 2021. Protective effect of *Viburnum grandiflorum* against ultraviolet-B radiation-induced cellular and molecular changes in human epidermal keratinocytes. Pharmacogn Mag 16 (05): 200. DOI: 10.4103/pm.pm_397_19.

Mahmoudi E, Abolfathi M, Hassanzadeh N, Milasi YE, Dehghani-Samani M, Khaledi M, Kerdarian H, Najafipour M, Arshi A. 2019. *Prunus armeniaca* effects on expression of genes related to apoptosis in human breast cancer cells. Transl Med Commun 4 (1): 1-6. DOI: 10.1186/s41231-019-0036-5.

Malik F, Singh J, Khajuria A, Suri KA, Satti NK, Singh S, Kaul MK, Kumar A, Bhatia A, Qazi GN. 2007. A standardized root extract of *Withania somnifera* and its major constituent withanolide-A elicit humoral and cell-mediated immune responses by up regulation of Th1-dominant polarization in BALB/c mice. Life Sci J 80 (16): 1525-38. DOI: 10.1016/j.lfs.2007.01.029.

Manju K, Jat RK, Anju G. 2017. A review on medicinal plants used as a source of anticancer agents. Intl J Drug Res Technol 2 (2): 6.

Mantyh PW. 2006. Cancer pain and its impact on diagnosis, survival and quality of life. Nat Rev Neurosci 7 (10): 797-809. DOI: 10.1038/nrn1914.

Maroyi A. 2011. An ethnobotanical survey of medicinal plants used by the people in Nhema communal area, Zimbabwe. J Ethnopharm 136 (2): 347-354. DOI: 10.1016/j.jep.2011.05.003.

Mathers CD, Loncar D. 2006. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 3 (11): e442. DOI: 10.1371/journal.pmed.0030442.

Matthews V. 1994. New Plantsman: Royal Horticultural Society. RHS, Cornell.

Meng XL, Riordan NH, Casciari JJ, Zhu Y, Zhong J, González MJ, Miranda-Massari JR, Riordan HD. 2002. Effects of a high molecular mass *Convolvulus arvensis* extract on tumor growth and angiogenesis. P R Health Sci J 21 (4): 323-328.

Molassiotis A, Scott JA, Kearney N, Pud D, Magri M, Selvekerova S, Bruyns I, Fernadez-Ortega P, Panteli V, Margulies A, Gudmundsdottir G, Milovics L, Ozden G, Platin N, Patiraki E. 2006. Complementary and alternative medicine use in breast cancer patients in Europe. Support Care Cancer 14 (3): 260-267. DOI: 10.1007/s00520-005-0883-7.

Patocka J, Bhardwaj K, Klimova B, Nepovimova E, Wu Q, Landi M, Kuca K, Valis M, Wu W. 2020. *Malus domestica*: A review on nutritional features, chemical composition, traditional and medicinal value. Plants 9 (11): 1408. DOI: 10.3390/plants9111408.

Payyappallimana U. 2010. Role of traditional medicine in primary health care: An overview of perspectives and challenging. Yokohama J Soc Sci 14 (6): 58-77.

Robinson JB, Herbert D. 2001. Integrating climate change and sustainable development. Intl J Glob Environ Issues 1 (2): 130-149. DOI: 10.1504/IJGENVI.2001.000974.

Sher H, Midrarullah G, Coll PGJ. 2003. Medicinal plants of Udigram, District Swat, Pakistan. Pak J For 53: 65-74.

Singh A, Gupta R, Srivastava M, Gupta MM, Pandey R. 2016. Microbial secondary metabolites ameliorate growth, in planta contents and

lignification in *Withania somnifera* (L.) Dunal. Physiol Mol Biol Plant 22 (2): 253-260. DOI: 10.1007/s12298-016-0359-x.

Souri E, Amin GH, Farsam H, Andaji S. 2004. The antioxidant activity of some commonly used vegetables in Iranian diet. Fitoterapia 75 (6): 585-588. DOI: 10.1016/j.fitote.2004.04.007.

Tascilar M, de Jong FA, Verweij J, Mathijssen RH. 2006. Complementary and alternative medicine during cancer treatment: Beyond innocence. J Oncol 11 (7): 732-741. DOI: 10.1634/theoncologist.11-7-732.

Verhoef MJ, Balneaves LG, Boon HS, Vroegindewey A. 2005. Reasons for and characteristics associated with complementary and alternative medicine use among adult cancer patients: A systematic review. Integr Cancer Ther 4 (4): 274-286. DOI: 10.1177/1534735405282361.

Wang HC, Chung PJ, Wu CH, Lan KP, Yang MY, Wang CJ. 2010. *Solanum nigrum L*. polyphenolic extract inhibits hepatocarcinoma cell growth by inducing G2/M phase arrest and apoptosis. J Sci Food Agric 91: 178-185. DOI: 10.1002/jsfa.4170.

WHO. 2003. Factsheet 134. Traditional medicine: Report by The Secretariat. WHO, Geneva.

WHO. 2002. International Agency for Research on Cancer (IARC) Monographs Volume 82; Some Traditional Herbal Medicines. WHO, Geneva.

Winters M. 2006. Ancient medicine, modern use: *Withania somnifera* and its potential role in integrative oncology. Altern Med Rev 11 (4): 269-277.

Zhou K, Raffoul JJ. 2012. Potential anticancer properties of grape antioxidants. J Oncol 2012: 803294. DOI: 10.1155/2012/803294.

ASIAN J NAT PROD BIOCHEM
Volume 22, Number 1, June 2024
Pages: 19-26

P-ISSN: 2775-4189
E-ISSN: 2775-4197
DOI: 10.13057/biofar/f220103

Hesperidin and quercetin modulate carbon tetrachloride (CCl_4) induced hepatotoxicity in male rats

GRACE IME EKPO[1], OFEM EFFIOM ETENG[2,♥], VICTOR SAMPSON EKAM[1], MARYJANE UKAMAKA ONYEMAIZU[3], NDODO OFONIME[1], OBINAJU EFFIONG BLESSING[1], ONOFIOK LUKE UDUAK[4], ANITIE EYO ROBERT[5], SAVIOUR UFOT[2], UBANA EYONG[2]

[1]Department of Medical Biochemistry, University of Uyo. P.M.B 1017, Uyo, Akwa Ibom State, Nigeria
[2]Department of Biochemistry, Federal University of Agriculture. P.M.B 2240, Abeokuta, Ogun State, Nigeria
Tel.: 234-803-337439, ♥email: ofemeffiom@gmail.com
[3]Department of Biochemistry, University of Calabar. PMB 1115 Etagbor, Cross River State, Calabar, Nigeria
[4]Department of Chemical Engineering, University of Surrey. Guildford, United Kingdom
[5]Depatrment of Medical Biochemistry, Alex Ekwueme Federal University. Ndufu-Alike Ikwo, Abakaliki, Ebonyi State, Nigeria

Manuscript received: 12 December 2023. Revision accepted: 19 March 2024.

Abstract. *Ekpo GI, Eteng OE, Ekam VS, Onyemaizu MU, Ofonime N, Blessing OE, Uduak OL, Robert AE, Ufot S, Eyong U. 2024. Hesperidin and quercetin modulate carbon tetrachloride (CCl_4) induced hepatotoxicity in male rats. Asian J Nat Prod Biochem 22: 19-26.* Quercetin and hesperidin are bioactive chemicals that have shown considerable promise in both conventional and herbal medicine due to their significant impact on treating various human diseases. Oxidative stress is linked to the etiology of many liver disorders. Quercetin-containing foods and fruits include kales, onions, berries, apples, cherries, and other citrus fruits like grapefruit, oranges, lemons, and mandarins. The purpose of this study is to examine the potential therapeutic benefits of quercetin and hesperidin on oxidative stress-induced hepatotoxicity using male rats treated with carbon tetrachloride (CCl_4). A total of (30) male rats weighing 160-180 g were divided into 5 groups (n = 6). For twenty-one days, rats were gavaged with quercetin and hesperidin (2.4 and 2.4 mg/kg each) after being exposed to CCl_4 (0.5 mg/kg). Samples were taken to evaluate several biochemical markers. Nitric oxide (NO), Hydrogen peroxide (H_2O_2), and Malonialdehyde (MDA) levels were assayed for oxidative damage. The enzymes alanine transferase (ALT) and alkaline phosphatase (ALP) activity were estimated to represent liver function. Glutathione peroxidase (GPx), Catalase (CAT), and Glutathione (GSH) levels were evaluated for antioxidants, and histopathology was also assessed. CCl_4 increased significantly ($P < 0.05$) the concentration of MDA, NO, H_2O_2, SOD, CAT, GPX, GSH, and also, the activities of ALT, ALP, and AST in serum. Following treatment with quercetin and hesperidin, significantly ($P < 0.05$) reduced MDA, NO, H_20_2, ALT, AST, and ALP activities and modified SOD, CAT, GPX, GSH, in rats substantially ($P < 0.05$). Hepatotoxicity caused by carbon tetrachloride (CCl_4) was protected by quercetin and hesperidin treatment. This study concludes that by recovering from ROS-mediated oxidative stress, the injection of quercetin and hesperidin helps to offset the damage that CCl_4-induced hepato-renal ailments generate. Hence, quercetin and hesperidin supplements may be recommended as an adjunctive natural therapy because they can scavenge free radicals and prevent hepato-renal damage.

Keywords: Antioxidant, carbon tetrachloride (CCl_4), hepatotoxicity, oxidative damage

INTRODUCTION

The condition known as hepato-renal syndrome (or toxicity) is linked to the development of kidney failure as well as severe liver damage when exposed to carbon tetrachloride (CCl_4) by multiple routes such as ingestion, inhalation, and cutaneous absorption (Khan et al. 2012). This disorder is frequently observed in people who have severe liver failure. Symptoms include weight gain, jaundice, confusion, delirium, nausea, vomiting, dementia, reduced urine output, dark urine, swollen abdomen, and decreased antioxidant-mediated body defense (Tabeshpour et al. 2020). The severity of this condition is thought to be caused by decreased urine production, which leads to the buildup of nitrogenous waste materials. These materials then help to produce reactive oxygen species, which interact with macromolecules to cause organ damage (Low et al. 2015). Enhancing the body's antioxidant capacity, which comes from natural sources like fruits and vegetables, has also been demonstrated to help lessen the effects of hepatorenal syndrome (Douglas et al. 2020). Although it is well known that nutrition can prevent a wide range of diseases, including infectious ones, it seems that modern medicine, especially when treating infectious diseases, has overlooked or undervalued this aspect of health care (Aune et al. 2018)

Over the years, research has evaluated the role and possible mechanisms by which most chemical agents induce tissue damage, especially in the liver and kidney, which are directly responsible for the metabolism and excretion of chemicals following exposure (El-Boshy et al. 2017). Among the compounds mentioned is carbon tetrachloride (CCl_4), one of the principal xenobiotics that have been demonstrated to produce both acute and chronic tissue damage with a well-established hepatotoxicity (Xu et al. 2010). Douglas et al. (2020) state that quercetin is a polyphenolic flavonoid component widely found in red wine, tea, onions, berries, apples, broccoli, cherries, and

red grapes. According to Douglas et al. (2020), quercetin's high solubility, bioavailability, and capacity to form complexes or combine to generate certain unique preparations utilized for human health care contribute to its medicinal value. According to Tabeshpour et al. (2020), hesperidin (HSP), a pharmacologically active subclass of flavonoids (flavonoid aglycone), is widely distributed in citrus species, including blood orange, orange, lemon, and lime. According to Tabeshpour et al. (2020), it is a disaccharide derivative that has hesperidin substituted by a 6-O-(alpha-L-rhamnopyranosyl)-beta-D-glucopyraosyl moiety at position 7 by a glycosidic linkage with a diphenol structure with the molecular formula C28H34O15. According to the paper by Zanwar et al. (2018), it has been shown that flavanone exhibits various of pharmacological activities such as anti-inflammatory antioxidant, analgesic, anticarcinogenic, antiviral, and anti-coagulant. It is utilized in herbal formulations. Recent studies on the protective effects of hesperidin have shown that, as a major dietary polyphenol, its ability to guard against organ damage is well known (de Aja et al. 2020). It is used as a supplemental agent in complementary therapy protocols with success because of its biological and pharmacological properties, which are effective in lowering lipid levels, scavenging free radicals, and acting as powerful antioxidant, anti-inflammatory, anti-carcinogenic, and anti-hypertensive agents (Sedky et al. 2017). Hesperidin's antioxidant properties protect the testicles from cadmium poisoning and regulate the hepatic synthesis of cholesterol by blocking the action of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, as per a study by Saleh et al. (2022). The vital roles that bioactive chemicals from plants play in combating various human diseases have led to their widespread usage in conventional and botanical medicine. Calas, onions, berries, apples, red grapes for quercetin, and a variety of citrus fruits, including oranges, lemons, mandarins, and grapefruit for hesperidin, are excellent sources of them (Low et al. 2015). They have higher antioxidant activity due to their high solubility and bioavailability. Examples of this activity include their effect on glutathione (GSH), enzymatic activity, signal transduction pathways, and the elimination of Reactive Oxygen Species (ROS) brought on by chemical and environmental toxicological factors, such as the CCl_4 used in this study. Therefore, the study aimed to clarify how hesperidin and quercetin can work in concert to lessen the harmful effects of CCl_4.

MATERIALS AND METHODS

Chemicals and reagents

Pfizer International (NY, USA) provided the CCl_4 that was used. Randox Laboratories Limited (Admore, Crumlin, Co-Antrim, UK) produced the alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (-GT), and total protein kits. Cayman Chemical (Ann Arbour, Michigan, USA) supplied the Griess reagent kit, Potassium persulfate ($K_2S_2O_8$), dipotassium hydrogen phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), potassium chloride (KCl), sodium nitroprusside ($C_5FeN_6Na_2O$), hydrogen peroxide (H_2O_2), butylated hydroxytoluene, 2-deoxy-D-ribose, 2-thiobarbituric acid (TBA), and Aldrich Sigma Chemical Company (St. Louis, MO, USA) provided Ellman's reagent. Randox Laboratories Limited (Crumlin, UK) biochemical test kits were used instead of Lab Kit Biochemical Kits (Barcelona) to estimate urea, uric acid, and creatinine. All other chemicals and reagents, except those noted otherwise, were of the analytical grade and were bought from British Drug Houses in Poole, UK.

Animal model

Male albino test subjects In the Animal House of the Department of Biochemistry at the University of Calabar in Calabar, Nigeria, Wistar rats were produced artificially. The weight of the rats varied between 150 and 200 grams. They were housed in a plastic hanging cage with standard 12-hour light/12-hour dark cycles in a rat housing that was appropriately ventilated and maintained at 25°C. Fresh water and standard pellet meal were provided to the rats without restriction. The animals were allowed to acclimate for two weeks before the start of the tests. The National Academy of Science's (NAS) "Guide for the Care and Use of Laboratory Animals" by National Research Council (2010), provided guidelines that all of the animals were treated with care. The institution has accepted the researcher's experiment under number 17/042144175.

Experimental protocol

Therefore, according to their treatments, 30 rats were randomly divided into five (n = 6) groups. Before testing, hesperidine and quercetin were added to the vehicle, sweetened condensed milk diluted 1:6 in water. For 14 days consecutively, the animals consecutively were given oral aliquots containing doses of hesperidin and quercetin (2.4, 2.4, and 1.4 mg/kg bw) every day between 8.00 and 9.00 AM. These different doses of quercetin and hesperidin have been demonstrated to shield rats against oxidative stress, inflammation, and liver damage (Seema et al. 2021). The vehicle was given to the rats that were CCl_4 alone and the normal control, as indicated in Table 1. Except for normal control group I, which just received sterile injection water, all of the groups (II - 6) were subsequently given an intraperitoneal (i.p.) injection of CCl_4 (0.5 mg/kg) formulated in sterile injection water.

Preparation of serum

At the end of the experiment, precisely 24 hours after the CCl_4 administration, the animals were slaughtered under light ether anesthesia, meaning the CCl_4 would have fully metabolized by then. After piercing the heart, blood samples were taken using basic centrifuge tubes. Serum was created by centrifuging it at 3000 g for 10 minutes using an MSE bench centrifuge. The clear supernatant was used to estimate serum enzymes.

Table 1. Treatment protocols

Groups (n = 6)	Treatment
I	Control (administered vehicle)
II	CCl_4 (0.5 mg/kg b.wt., respectively)
III	Hesperidin (2.4 mg/kg b.wt.)
IV	Quercetin (2.4 mg/kg b.wt.)
V	Hesperidin (1.2 mg/kg b.wt.)

Preparation of tissue

The livers and kidneys of the sacrificed rats were quickly removed. A portion was dried using filter paper, weighed, and rinsed with ice-cold 1.15 percent KCl. To create 10% homogenates, the liver and kidney were chopped with scissors in 9 vol of ice-cold potassium phosphate buffer (0.1 M, pH 7.4). Next, a Teflon pestle homogenizer was used to homogenize the kidney and liver. (Thomas Scientific, Swedesboro, NJ, US). An aliquot of the homogenate was centrifuged at 12,000 g at 4°C for 15 minutes in a TGW16 Micro Centrifuge (Tingtai, China). This process produced the Post-Mitochondrial Fractions (PMF). We kept the supernatant at 20°C until we needed it for biochemical testing.

Biochemical assays

The manufacturer's instructions for the kit are available here. Total bilirubin, total protein, urea, uric acid, and creatinine were measured using spectrophotometry at 546 nm, while the interaction between cupric ions and protein peptide bonds in an alkaline medium to form a colored complex was observed.

Determination of liver integrity biomarkers

Measurement of ALT (alanine transaminase) activity in the blood and liver. ALT catalyzes the transamination of L-alanine to pyruvate, which subsequently combines with 2,4-dinitrophenylhydrazine (DNPH) to produce a brown-colored complex, pyruvate2,4-dinitrophenylhydrazone (PDNPHO) in alkaline medium (L-alanine + α-oxoglutarate → L-glutamate + pyruvate; + DNPH → PDNPHO), the intensity of the brown colored complex, PDNPHO at 340 nm is proportional to the activity of ALT, as described by Reitman and Frankel (1957). Units/mg protein was used to express the ALT-specific activity. The generation of reactive species – H_2O_2 and nitric oxide (NO) – was estimated according to the methods of Junglee et al. (2014). Oxidative degradation of lipids and proteins was assessed as malondialdehyde (MDA), and MDA was determined according to the method of Buege and Aust (1978) as thiobarbituric acid reactive substances (TBARS).

Determination of serum and hepatic aspartate transaminase (AST) activity.

A combination of malate and NAD+ is produced by the combination of AST-catalyzed transamination of aspartate to oxaloacetate and L-glutamate + oxaloacetate; + NADH malate + NAD+, according to Reitman and Frankel (1957). The AST-specific activity was expressed in units/mg of protein.

Determination of serum and hepatic alkaline phosphatase (ALP) activity

ALP catalyzes the hydrolysis of p-nitrophenyl phosphate to pnitrophenol, a yellow chromogen, at a wavelength of 405 nm, according to Akamo et al. (2015) technique. The activity of ALP, quantified in units per milligram of protein, is directly correlated with the intensity of yellow chromogen.

Determination of antioxidants

The liver homogenate's 12,000 g post-mitochondrial fraction was tested for antioxidant content.

Determination of reduced glutathione (GSH) concentration

The GSH concentration was determined spectrophotometrically at 412 nm by tracking the rate of production of the chromophoric product 2-nitro-5-thiobenzoate (TNB) after Ellman's reagent DTNB [5,5-dithiobis(2-nitrobenzoic acid)] was reduced. The method of Jollow et al. (1974) indicates that the intensity of the yellow-colored complex formed is directly proportional to the amount of -SH groups. by the reduced glutathione's free sulphydryl group (2GSH + DTNB TNB + GSSG). The GSH data were expressed as GSH/mg protein using a GSH molar extinction value () of 9.6 0.017 mM 1 cm 1.

Determination of superoxide dismutase (SOD) activity

SOD activity was measured spectrophotometrically at 420 nm by measuring the suppression of autoxidation of pyrogallol, a superoxide-reacting indicator molecule (SRIM) that rivals SOD for its interaction with superoxide in an alkaline media. Applying the method (pyrogallol/SOD + O2 • + 2H+ H2O2 + O2) described by Marklund and Marklund (1974). The units/mg protein or pyrogallol auto-inhibition/min/mg protein was calculated using the pyrogallol molar extinction coefficient () of 8.0 105 M 1 cm 1 to express the specific activity of SOD.

Determination of catalase (CAT) activity

The method of Hadwan and Abed (2016) involved measuring the rate of hydrogen peroxide ($2H_2O_2.2H_2O$ + O_2) oxidation at 374 nm was used to quantify catalase activity spectrophotometrically. The specific activity of catalase was expressed as Units/mg protein or mmol H_2O_2 degraded/min/mg protein using the H2O2 molar extinction value () of 43.6 M 1 cm 1.

Determination of glutathione peroxidase (GPx) activity

Using GSH as a co-factor (H_2O_2 + 2GSH →$2H_2O$ + GSSG), the remaining GSH content during the breakdown of hydrogen peroxide was measured Spectrophotometrically at 420 nm to quantify the GPx activity, following the methodology of Mohandas et al. (1984). Using a GSH molar extinction coefficient (Σ) of 9.6 0.017 mM− 1 cm− 1, GPx-specific activity was reported as Units/mg protein or nmol of residual GSH/min/mg protein.

Histopathological examinations

Dr. Kris Uko, a consultant pathologist at the University of Calabar Teaching Hospital in Nigeria, conducted the

histological analysis of the liver and kidney samples. Small pieces of liver tissue were gathered in 10% neutral buffered formalin for the proper fixing. These tissues were processed and embedded in paraffin wax. Sections of 5 mm thickness that had been cut, mounted, and stained were stained with hematoxylin and eosin. After that, the slices were examined under a light microscope.

Statistical analysis

Data were expressed as the mean ± Standard Deviation (SD) of five replicates in each group. Analysis of Variance (ANOVA) was conducted to test for the level of homogeneity among the groups. Where heterogeneity occurred, the groups were separated using the Duncan Multiple Range Test (DMRT). A p-value of less than 0.05 was considered statistically significant. All the statistics were carried out by SPSS (Statistical Package for Social Sciences) software for Windows version 20 (SPSS Inc., Chicago, Illinois, USA). Graphs were plotted using Graph Pad Prism 8 Software (Graph Pad Software Inc., San Diego, USA).

RESULTS AND DISCUSSION

Quercetin and hesperidin pre-treatment preserved hepatic integrity

It was found that quercetin and hesperidin may reduce CCl_4-mediated liver injury by assessing the activities of ALT, AST, and ALP in the serum (Figure 2). Therapy with CCl_4 markedly ($p < 0.05$) raised the activities of serum ALT (Figure 2), AST (Figure 2.B), and ALT. The findings of comparing each serum's ALP, AST, and ALT levels to the corresponding negative control (normal) group are displayed in Figures 2.C, 2.D, and 2.E, respectively.

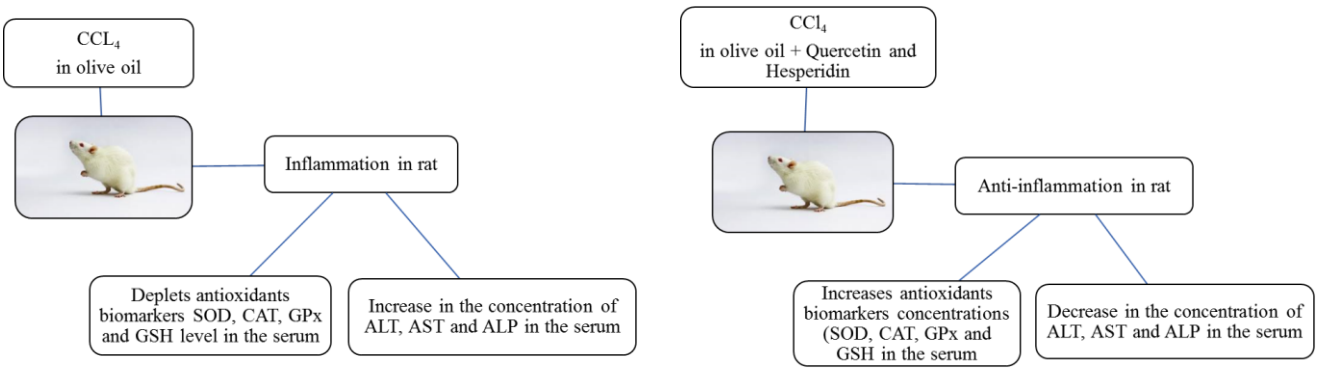
In contrast to the corresponding negative control (normal) group, the function and activity of the hepatic liver enzymes were considerably ($p < 0.05$) decreased following the administration of CCl_4. When quercetin and hesperidin were administered to the CCl_4-intoxicated group at doses of 2.4, 2.4, and 1.4 mg/kg, they significantly ($p < 0.05$) reduced the altered activities of ALT, AST, and ALP that were released into serum as a result of CCl_4-mediated liver injury. By increasing their hepatic activities compared to the CCl_4 alone treated groups, the various doses of quercetin and hesperidin also significantly ($p < 0.05$) increased the hepatic functional activities of ALT, AST, and ALP. In the two compartments, quercetin and hesperidin exhibited a concentration-dependent protective effect against hepatotoxicity generated by CCl_4.

Quercetin and hesperidin pre-treatment prevents the alterations of the oxidative stress markers induced by CCl_4 in rat organs

The effects of a single dose of 0.5 mg/kg CCl_4 were measured on the levels of reduced glutathione and the liver's activities of glutathione-S-transferase, superoxide dismutase, catalase, and glutathione reductase after pre-treatment with 2.4, 2.4, and 1.4 mg/kg quercetin and hesperidin for 21 days in a row (Figure 1). After CCl_4 injection, intracellular GSH levels were considerably ($p < 0.05$) lower than in the healthy control group (Figure 3.A). Conversely, CCl_4-induced decreases in the activities of glutathione reductase (Figure 3), glutathione peroxidase (Figure 3), and catalase (Figure 3) were seen in comparison to the healthy control group. Glutathione-S transferase and superoxide dismutase activities were significantly ($p < 0.05$) increased by CCl_4-intoxication compared to the healthy control group. The oral administration of the rats with quercetin and hesperidin to varying degrees significantly ($p < 0.05$) reverses the CCl_4-mediated changes in the oxidative stress markers.

Histopathological changes in the liver

Even though there were no reported deaths in any of the groups during this experiment, some of the liver tissues from group A (0.4% DMSO) showed a conserved architecture with a noticeable modification observed in the liver and kidney tissues of the CCl_4 exposed groups (Figure 4). After pre-treatment with Q and H, the liver and kidney tissues are clearly repaired, suggesting that the histopathological study indicates a little increase in nucleated cells.



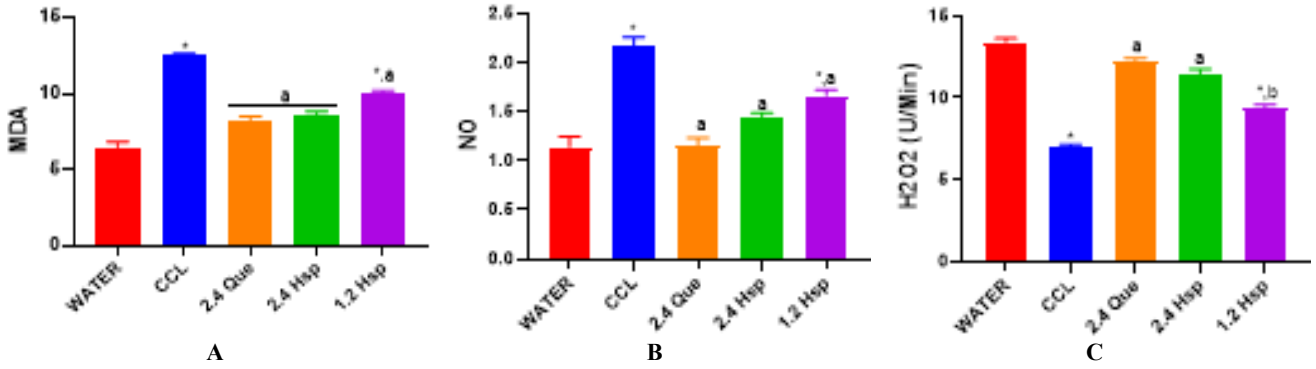


Figure 1. Effects of quercetin and hesperidin pretreatment on CCl_4 mediated increased in oxidative stress markers in rats on kidney parameters. A. MDA level, B. Nitric oxide, C. Hydrogen peroxide. Bars represent mean ± SEM (=5). Bars with different letters are significantly different at P <0.05

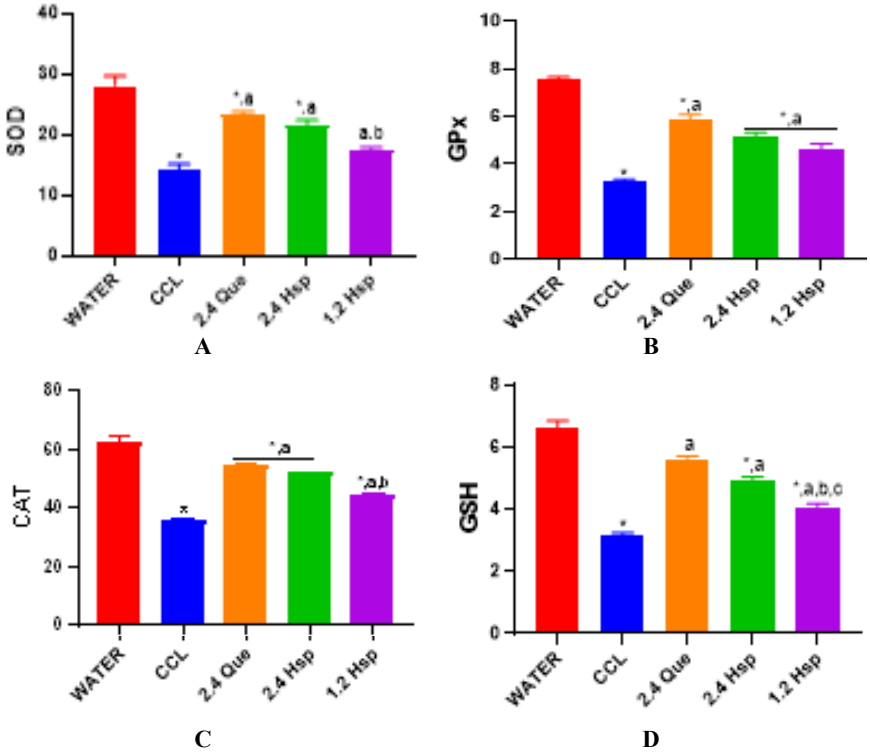


Figure 2. Effects of quercetin and hesperidin pretreatment on CCl_4 mediated increase in antioxidant markers in rats on kidney parameters. A. SOD, B. CAT, C. GPx, D. GSH. Bars represent mean ±SEM (=5). Bars with different letters are significantly different at P<0.05

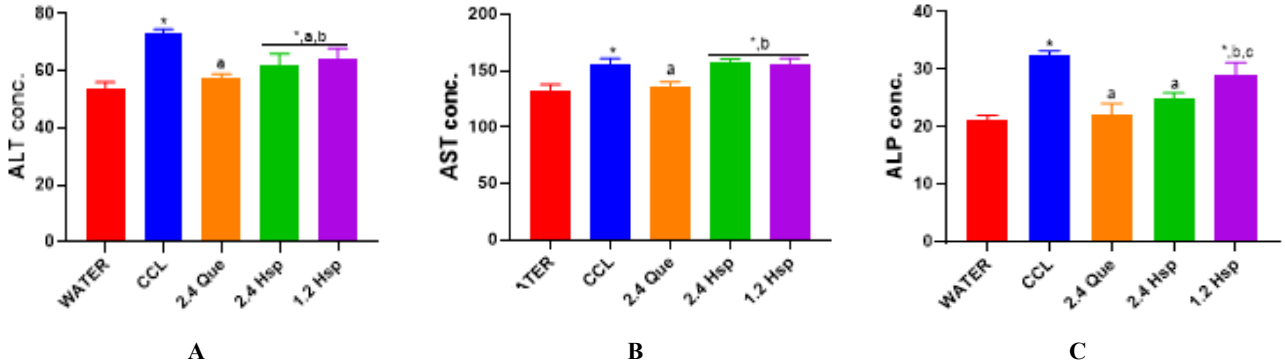


Figure 3. Effects of quercetin and hesperidin pretreatment on CCl_4 mediated increase in liver enzymes markers in rats on kidney parameters. A. AST, B. ALT, C. ALP. Bars represent mean ±SEM (=5). Bars with different letters are significantly different at P<0.05

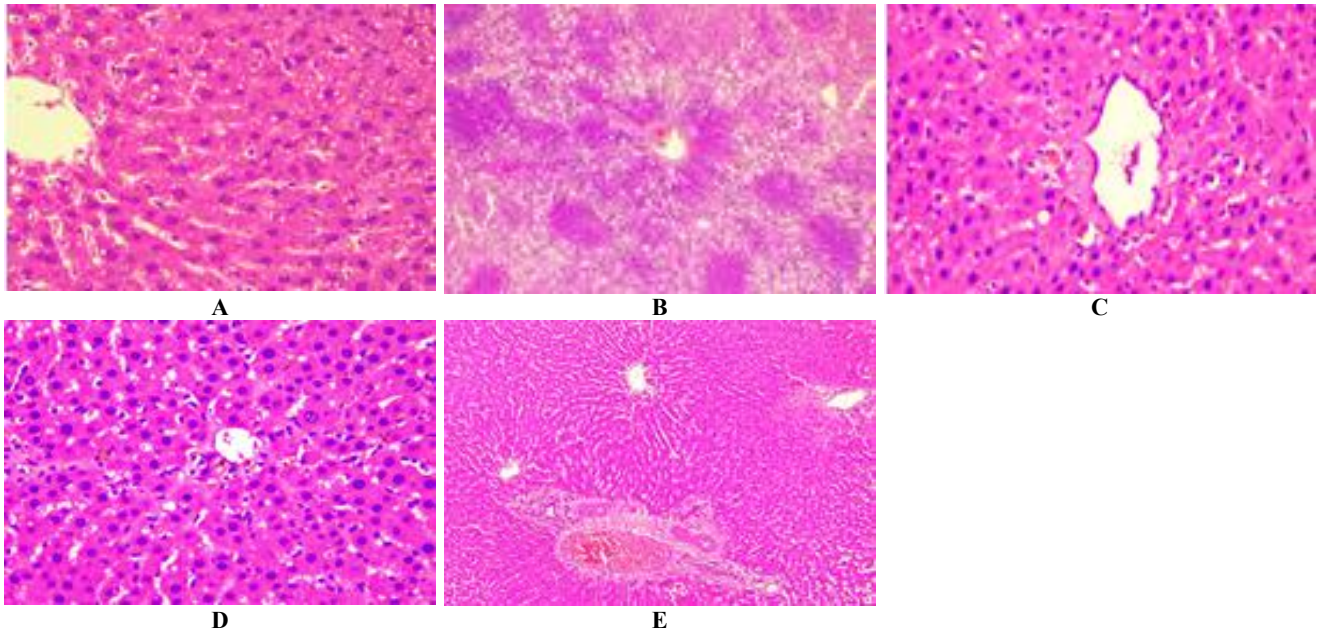


Figure 4. Liver histology of control and CCL_4-exposed rats treated with quercetin and hesperidin. Group A: 0.4% DMSO, Group B: CCL_4; 0.5 mg/kg, Group C: Quercetin (2.4 mg/kg), Group D: Hesperidin (2.4 mg/kg), Group E: Hesperidin 1.4 mg/kg). Photomicrograph of the liver section showing arrays of hepatocytes (black arrows) severe diffused fatty degeneration (#) with both a mixed microvesicular (mi), macrovesicular (ma) steatosis and hepatocellular ballooning degeneration (asterisk). Also seen was a congested blood vessel (V) necrotic liver cell (Red arrow). Haematoxylin and Eosin (H&E) Stain. X100 and x400 magnification on the CCL4 mediated group upon treatment with quercetin and hesperidin changes showed hepatocytes (black arrows), Central Vein (CV) and average-sized sinusoidal spaces (Thin Blue arrow). No pathological lesion was seen. H&E stain. x400 magnification

Discussion

Reactive Oxygen Species (ROS) produced by a biological system are not balanced with the system's capacity to detoxify the reactive intermediates or repair the damage that results in the impacted tissues quickly (El-Boshy et al. 2017). This imbalance is known as oxidative stress. According to a study by Medline (2022), this led to the accumulation of oxidative stress indicators and a notable decrease in antioxidant indicators.

The current study's findings demonstrated that the injection of CCl_4 resulted in an imbalance in the level of oxidative stress indicators tested, as seen by differences between the CCl_4 and the water groups. According to El-Boshy et al. (2017), peroxides and free radicals, which harm all parts of the cell, including proteins, lipids, and DNA, are produced when there is a disruption in the normal redox state of cells. According to the results of this investigation, CCl_4 administration was able to cause damage to cellular components, increase the generation of reactive oxygen species, and entirely reduce their clearance from the system. These reported damages may help to explain them. However, other results showed that serum CCl_4 poisoning led to a considerable decrease in antioxidant levels, including SOD, CAT, GPx, and GSH. On the other hand, CCl_4 injection and liver and kidney homogenates cause an increment in AST, ALT, ALP, SOD, and CAT activities. Therefore, a high level of ALT may indicate liver damage, which could be brought on by toxic chemicals or certain drugs that restrict blood supply to the liver. While ALP levels are reported to be a sign of liver disease or specific bone disorders, AST is directly linked to heart issues that are associated with damage to the kidneys or liver (Medline 2022). The current study's results corroborate the earlier report since exposure to CCl_4, which has a well-documented history of hepatorenal toxicity, led to a rise in the levels of these enzymes. All live cells include the enzyme superoxide dismutase (SOD), which aids in the breakdown of potentially hazardous oxygen molecules within the cells and guards against cellular damage. Because it plays a part in detoxifying harmful produced O_2 in the system, its concentration has been found to increase in excessive accumulation of reactive oxygen species. According to (Biju et al. 2014), one of the host defense mechanisms facilitating a quick rise in oxygen uptake may be the protective and adaptive mechanisms against oxidative stress that are developing in the tissue, which could explain the increase in SOD. This report is consistent with the findings in liver and kidney homogenates from the current study, which indicated that the system produced more SOD and its activity to protect itself and promote O_2 clearance from the system as a result of the damage caused in the tissues by CCl_4; this resulted in low substrate in the serum and reduced SOD activity. Similar decreases in serum levels of catalase (CAT) were also observed; they have been linked in earlier studies to CAT gene mutations, and the decreases encourage the accumulation of hazardous levels in specific cells Medline (2022). CAT is responsible for maintaining an optimal amount of the molecule in the cell, which is linked to crucial cellular signaling pathways, by using hydrogen

peroxide, a non-radical ROS, as its substrate and neutralizing it by breakdown (El-Boshy et al. 2017). However, the rise in CAT activity observed in the tissues in this study may result from the body's attempt to correct the disruption in the antioxidant levels and reduce the negative effects of reactive species and their byproducts. CAT is essential for reducing oxidative stress to a great degree since they break down cellular hydrogen peroxide to create oxygen and water. The present study's observation of elevated CAT activity indicates the deleterious consequences of administering CCl_4.

The present study indicate that serum levels of glutathione peroxidase (GPx), a family of peroxide-active enzymes whose primary biological function is to protect the body from oxidative damage by reducing lipid hydroperoxides to their corresponding alcohols and free hydrogen peroxide to water, were decreased. This study suggests that CCl4 toxicity caused a reduction in GPx concentration, which may indicate disease condition, but higher concentrations of GPx were recorded in the tissues. El-Boshy et al. (2017) also reported a similar reduction and stated that the measured reduction may contribute to vitiligo and most other disease conditions, including diabetes. As stated in the study by Dibal et al. (2018), reducing oxidative stress, which can exacerbate symptoms of various chronic illnesses, including autoimmune disease, is one of the potential health benefits of boosting GPx concentration. The rise in this study could be attributed to a systematic process used by the system to counteract the tissue-damaging effects of CCl_4. Glutathione (GSH), which is known as the body's master antioxidant because of its function in cell protection, is another significant antioxidant that is of interest in the current investigation. The report claims that it immediately quenches radical centers on DNA and other biomolecules, as well as other free radicals with oxygen centers and reactive hydroxyl centers. This enzyme's decrease has been linked to long-term exposure to pollutants, alcohol, chemicals, cadmium, and other medical conditions. This could account for the serum result that showed a decrease, indicating that the administration of CCl4 induced a medical state, including liver illness. Glutathione plays a role in detoxifying endogenous and xenobiotic substances and the excretion of oxidative molecules. This could explain the rise in tissue homogenate seen in the current study. Nonetheless, dietary antioxidants support the antioxidant activity of cells (El-Boshy et al. 2017). By scavenging active oxygen and free radicals and neutralizing lipid peroxides, antioxidants and anti-inflammatory drugs play a crucial role in the fight against CCl_4 intoxication. This raises antioxidant levels, restores the structural integrity of injured organs, and improves ameliorative effects. This was observed in both the liver and kidney but not in the serum for SOD, CAT, GPx, and GSH; it was also shown in a dose-dependent manner in the hesperidin groups. The groups treated with 2.4 mg/kg quercetin showed substantial results. According to a study by Dibal et al. (2018), elevated levels of AST, ALP, and ALT in the liver indicate a dysfunctional liver condition with an unbalanced level of oxidative stress markers. nevertheless, most research indicates that this outcome could result from a disordered lipid profile in the liver. Furthermore, the results should support the findings of Dibal et al. (2018), which showed that quercetin has the potential to significantly reduce ALT, AST, and ALP activity in hepatorenal injury; similarly, Apaydin et al. (2018) reported hesperidin's ability to ameliorate chemically induced hepatorenal toxicity. Treatment with plant extracts should also be able to restore these effects, particularly in the groups treated with 2.4 mg/kg quercetin.

In conclusion, this current study concludes that by recovering from ROS-mediated oxidative stress, the injection of quercetin and hesperidin helps to offset the damage that CCl_4-induced hepato-renal ailments generate. The quercetin and hesperidin supplements may be recommended as an adjunctive natural therapy because they can scavenge free radicals and prevent hepato-renal damage.

ACKNOWLEDGEMENTS

We gratefully recognise the contributions made by the study team and the entire laboratory staff of the Department of Biochemistry, University of Calabar, Nigeria.

REFERENCES

Akamo JA, Ugbaja RN, Amah GH, Fabuluje I, Edaferiemu JO, Lepzem NG, Oyekale KO. 2015. The relationship between indices of hepatocellular injury and anthropometric measurements in some Babcock University Students, Ilisan, Ogun State, Nigeria. Braz J Biol Sci 2 (3): 23-37.

Apaydin FG, Kaltalioğlu K, Balabanli B, Coskun Cevher Ş. 2018. Morin and hesperidin ameliorate cisplatin-induced hepatotoxicity and nephrotoxicity in rats: A histopathological study. Gazi Univ J Sci 31 (2): 399-406.

Aune D, Keum N, Giovannucci E, Fadnes LT, Boffeth P, Greenword DC. 2018. Dietary intake and bllod c oncentration of antioxidants and the risk of cardiovascular disease, total cancer and all cause mortality: a systematic review and dose-response meta-analysis of prospective studies. Am J Clin Nutr 108 (5): 1069-1091. DOI: 10.1136/bmj.i2716.

Biju SD, Garg S, Mahony S, Wijayathilaka N, Senevirathne G, Meegaskumbura M. 2014 DNA barcoding, phylogeny and systematics of Golden-backed frogs (Hylarana, Ranidae) of the Western Ghats-Sri Lanka biodiversity hotspot, with the description of seven new species. Contrib Zool 83 (4): 269-335. DOI: 10.1163/18759866-08304004.

Buege JA, Aust SD. 1978. Microsomal lipid peroxidation. Method Enzymol 30: 302-310. DOI: 10.1016/S0076-6879(78)52032-6.

de Aja JS, Dost A F M, Kim C F. 2020. Alveolar progenitor cells and the origin of lung cancer. Key *Symposium.* J Intern Med 289: 629-635, DOI: 10.1111/joim.13201.

Dibal NI, Garba SH, Jacks TW. 2018. Onion peel quercetin attenuates ethanol-induced liver injury inmice by preventing oxidative stress and streatosis. Biomed Res Ther 9 (6): 5102-5112. DOI: 10.15419/bmrat.v9i6.745.

Douglas AS, Pere G, Patrick SK. 2020. Hepatorenal syndrome: Pathophysiology, diagnosis, and management BMJ 370: m2687. DOI: 10.1136/bmj.m2687.

El-Boshy ME, Abdelhamidb F, Richab E, Ashshia A, Gaitha M, Qustya N. 2017. Attenuation of CCl_4 Induced oxidative stress, immunosuppressive, hepatorenaldamage by fucoidan in rats. J Clin Toxicol 30 (1): 13-24. DOI: 10.1177/0394632017694898.

Hadwan MH, Abed HN. 2016. Data supporting the spectrophotometric method for the estimation of catalase activity. Data Brief 6: 194-199. DOI: 10.1016/j. dib.2015.12.012.

Jollow DJ, Mitchell JR, Zampaglione NA, Gillette JR. 1974. Bromobenzeneinduced liver necrosis. Protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology 11 (3): 151-169. DOI: 10.1159/000136485.

Junglee S, Urban L, Sallanon H, Lopez-Lauri F. 2014. Optimized assay for hydrogen peroxide determination in plant tissue using potassium iodide. Am J Anal Chem 5 (11): 730. DOI: 10.4236/AJAC.2014.511081.

Khan R, Khan M, Sahreen S. 2012. CCl_4-induced hepatotoxicity: Protective effect of rutin on p53, CYP_2E_1 and the antioxidative status in rat. Complement Altern Med 12: 178-184. DOI: 10.1186/1472-6882-12-178.

Low G, Alexander GJM, Lomas DJ. 2015. Hepatorenal syndrome: Aetiology, DIAGNOSIS, AND TREATMENT. Gastroenterol Res Pract 2015: 207012. DOI: 10.1155/2015/207012.

Marklund S, Marklund G. 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 47 (3): 469-474. DOI: 10.1111/j.1432-1033.1974.tb03714.x.

Mohandas J, Marshall JJ, Duggin GG, Horvath JS, Tiller DJ. 1984. Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney: Possible implications in analgesic nephropathy. Biochem Pharmacol 33 (11): 1801-1807. DOI: 10.1016/0006-2952(84)90353-8.

National Research Council. 2010. Guide for the Care and Use of Laboratory Animals. National Academies Press, Nigeria.

Reitman S, Frankel S. 1957. A colorimetric method for the Determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28 (1): 56-63. DOI: 10.1093/ajcp/28.1.56.

Saleh N, Allam T, Korany R, Abdelfattah MS, Omran MA, Eldaim MAA, Hassan AM, El-Borai NB. 2022. Protective and therapeutic efficacy of hesperidin versus cisplatin against ehrlich ascites carcinoma-induced renal damage in mice. Pharmaceuticals 15: 294. DOI: 10.3390/ph15030294.

Sedky A, Mahboub F, Elsawy H, Eid R. 2017. Protective potential of quercetin on Cd-induced hepatorenal damage. Pol J Environ Stud 26 (5): 21972205. DOI: 10.15244/pjoes/68954.

Seema S, Rohilla KK, Kalyani VC, Babbar P. 2021. Prevalence and contributing factors for adolescent obesity in present era: Cross-sectional Study. J Family Med Prim Care 10: 1890-1894. DOI: 10.4103/jfmpc.jfmpc_1524_20.

Tabeshpour J, Hosseinzadeh H, Hashemzaei M, Karimi G. 2020. A review of the hepatoprotective effects of hesperidin, a flavanonglycoside in citrus fruits, against natural and chemical toxicities. DARU J Pharm Sci 28: 305-317. DOI: 10.1007/s40199-020-00344-x.

Xu C, Bailly-Maitre B, Reed JC. 2010. Endoplasmic reticulum stress: Cell life and death decisions. J Clin Invest 15 (10): 26564. DOI: 10.1172/JCI26373.

Zanwar P, Heyn PC, McGrew G, Raji M. 2018. SIG proceedings paper in word Format. In: Proceedings of ACM Human-Habitat for Health conference, Boulder, CO USA, October 2018 (H3'18): 9. DOI: 10.1145/3279963.3279971.

ASIAN J NAT PROD BIOCHEM
Volume 22, Number 1, June 2024
Pages: 27-34

P-ISSN: 2775-4189
E-ISSN: 2775-4197
DOI: 10.13057/biofar/f220104

Ethnomedicine, improved traditional medicine from *Cocos nucifera* water and evaluation of antibacterial activity on four bacterial strains in Center, Cameroon

GEORGES MAXIME LAMY LAMY[1,♥], JULIENNE LOUISE NGO LIKENG[2,3], ROSETTE CHRISTELLE NDJIB[1], SANDRINE EMILIE NGO NDOUNGA[2], L'AFRICAINE BLANCHE ELOMO[4], SALIOH M. MBUH[1], LEILA SANDRA NNANGA[1], MAHAMA[5], JACQUES BRUNO NGOTTA BIYON[6], KARL HARMSEN[7], EMMANUEL NNANGA NGA[1,8], ALEMBERT TIABOU TCHINDA[1]

[1]Institute of Medical Research and Medicinal Plants Studies (IMPM). PO Box 13033 Yaoundé, Cameroon. Tel.: +237-2222-1001, ♥email: geomaxlamy@gmail.com
[2]High Institute of Sciences and Techniques Applied to Health (ISSTAS). PO Box 33422 Yaoundé, Cameroon
[3]School of Health Sciences of Catholic University of Central Africa. P.O Box 11628, Yaoundé, Cameroon
[4]Paramedical Training Center. PO Box 25602 Yaoundé, Cameroon
[5]Local Co-Author, Local Counterpart for Informants who Participated by Name
[6]Departement of Plant Biology, Faculty of Sciences, University of Douala. PO Box 24157, Douala, Cameroon
[7]Marderhoek 41, NL-8223 XC Lelystad, Netherlands
[8]Department of Galenic Pharmacy and Pharmaceutical Legislation, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I. PO Box 812 Yaoundé, Cameroon

Manuscript received: 2 April 2024. Revision accepted: 30 May 2024.

Abstract. *Lamy GML, Likeng JLN, Ndjib RC, Ndounga SEN, Elomo LB, Mbuh SM, Nnanga LS, Mahama, Biyon JBN, Harmsen K, Nga EN, Tchinda AT. 2024. Ethnomedicine, improved traditional medicine from* Cocos nucifera *water and evaluation of antibacterial activity on four bacterial strains in Center, Cameroon. Asian J Nat Prod Biochem 22: 27-34.* In 2017, the WHO published its first list of "priority pathogens" resistant to antibiotics to combat growing antimicrobial resistance globally. These include bacteria that are multi-resistant to several antibiotics (*Acinetobacter*, *Escherichia*, etc.) and others (*Salmonella*, *Shigella*, etc.). Currently, research is focused on new antibiotics and medicinal plants are among the favored natural resources. Worldwide, *Cocos nucifera* water (Arecaceae) or coconut water, is traditionally reported to fight bacterial diseases. Unfortunately, information is lacking on its antibacterial potential in Center, Cameroon. The aim is to determine the traditional antibacterial uses of this water in Central, Cameroon. Then, transform this water into Improved Traditional Medicine (ITM). Finally, evaluate the antibacterial activity of ITM on 4 bacterial strains following the respective Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of 0.015 and 0.25 µg/mL. The methods were ethnomedicinal surveys, the pre-formulation protocol of medicinal syrup, and sensitivity tests in liquid and solid media. Therefore, 76 informants belonging to several ethnolinguistic groups participated. Women (55.27%) exceed men (44.73%) traditionally used coconut water more to treat digestive disorders (stomachache and constipation). An antibacterial ITM named Coco Water Cure (CWC) was manufactured. The more sensitive *Salmonella enteritidis* and *Shigella dysenteriae* bacteria with CWC had an MIC of 0.25 µL/mL, respectively. In conclusion, coconut water containing actives compounds such as lauric acid is eligible among the natural antibiotic resources from Center, Cameroon.

Keywords: Antibiotic resistance, coconut water, MIC, pathogens, traditional medicine

INTRODUCTION

The number of deaths due to antibiotic-resistant infections worldwide is worrying. To this end, the WHO published in 2017 a list of "priority pathogens" resistant to antibiotics. This led to and promoted new antibiotics research and development (Asokan et al. 2019), with 12 families of bacteria most threatening to human health; these including *Acinetobacter baumannii*, *Escherichia coli*, *Salmonella enteritidis* and *Shigella dysenteriae*. Certain antimicrobials, called conventional antibiotics, face resistance from many pathogenic microorganisms. This microbial resistance can be intrinsic or acquired because microbes can observe resistance during a spontaneous mutation or when new genes are transferred from another species. Several factors linked to antimicrobial resistance are, in general, microbial characteristics, selection pressure, and social and technological changes, without ignoring the abusive and uncontrolled use of antibiotics. This phenomenon, which can be described as universal, is the leading cause of infant mortality and morbidity in the world, killing around 50, 000 people per day (O'neill 2016); microbial agents are responsible for 70% of these deaths. Among antimicrobial agents, antibiotics or even antibacterial have been the subject of numerous studies (Gianluigi et al. 2015; Privalsky et al. 2021; David and Wessel 2022). Moreover, bacteria cause several diseases in humans such as cholera, diarrhea; among them responsible for these diseases are Campylobacteriosis, Salmonellosis, Shigellosis, Listeriosis, etc. These bacteria are increasingly resistant to conventional antibiotics (Maertens de Noordhout et al. 2017; Agnieszka and Katarzyna 2018). An antimicrobial evaluation obeys certain technical criteria such as the determination of the Minimum Inhibitory

Concentration (MIC), the Minimum Bactericidal Concentration (MBC), and the Susceptibility Test. Therefore, to compensate for the resistance of bacteria to antibiotics, which are high costs of modern drugs for predominantly poor populations, many hopes remain placed in the therapeutic effect of medicinal plants and the consideration of traditional medicine in Public Health systems in sub-Saharan Africa. Medicinal plants offer a varied and infinite range of secondary metabolites, making it possible to find new molecules with unprecedented antibacterial properties. Therefore, it is crucial to research new antibiotics targeting the priority pathogens on the published WHO list, and natural resources seem to be an avenue to explore in this search for new antibiotics. This is the case for medicinal plants, which are increasingly in demand; *Cocos nucifera* (Arecaceae) is among these medicinal plants. The water or juice of *C. nucifera* commonly called "coconut water," has multiple uses worldwide. More than five decades have passed since this water's use in traditional medicine was reported (Kheraro 1975). In Benin, coconut water is traditionally used against several ailments, such as colic, gastroenteritis, and abdominal pain (Dougnon et al. 2017). In Nigeria, there are numerous uses of *C. nucifera* water in ethnomedicine (Amujoyegbe et al. 2016). In Ivory Coast, the study of the biochemical parameters of coconut water reveals that it can be consumed (Konan et al. 2016) and that it contains soluble sugars (Assa et al. 2007). In Guadeloupe, coconut water is also used in traditional medicine (Jiounandan 2019). Unfortunately, in Central region of Cameroon, there is a lack of information on the traditional antibacterial medicinal knowledge of *C. nucifera* water. However, in the Center, Cameroon, such information has recently been the subject of transformation of traditional preparations into easily accessible Improved Traditional Medicine (ITM) syrups (Lamy et al. 2023). The aim is to determine the traditional antibacterial uses of this water in Central, Cameroon. Then, transform this water into Improved Traditional Medicine (ITM). Finally, evaluate the antibacterial activity of ITM on 4 bacterial strains following the respective Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of 0.015 and 0.25 µg/mL.

MATERIALS AND METHODS

Study area

The study took place in the Center region, Cameroon, precisely in two subdivisions (Lékié and Mfoundi) belonging to the agro-ecological zone of humid forests with bimodal rainfall (zone V) favorable to the cultivation of *C. nucifera* (IRAD 2008). Botanical samples of coconut palms (coconut, inflorescences, stipules, etc.) were collected in the study area. Then, they were brought to the National Herbarium of Cameroon (NHC). NGANSOP Eric (Botanist and Systematician) identified said samples, as *C. nucifera* (Arecaceae) compared with the specimen from herbarium collection No. 67488 HNC.

Ethnomedicine

The ethno-medicine part was carried out using an ethno-medicinal survey sheet. All informants voluntarily gave their informed consent by name or anonymously. In accordance with the recommendations of the Nagoya Protocol on access and benefit sharing from local knowledge, informants who agreed to participate by name were cited as co-authors (Lamy et al. 2020). The questions focused on the informant's identity, socio-economic life, nomenclature, and therapy (illness to treat, part used, method of preparation, consumption by age, duration of treatment, quantity used).

Improved traditional medicine

Pre-formulation of syrup based on coconut water

Pre-formulation consists of preparing 15 vials of 125 g of coconut water extract. Excipients will be associated with the active ingredients.

Generally, the preparation of the syrup is done in two stages (preparation of the simple syrup and incorporation of the medicinal and aromatic principles). In addition, the European Pharmacopoeia only recommends the dosages for 100 g of simple syrup, in particular, preservative 0.4 g and flavoring 0.03 g (Dénou et al. 2021). Because the recommended dosages (100 g, 0.4 g and 0.03 g) were insufficient for the quantity of final syrup, i.e. 1,875 g; thus, a multiplication factor named K was used to adapt the values of each ingredient to the final quantity.

Yield at first pre-formulation trial

The quantity of simple syrup obtained is (100-(0.4+0.03)) = 99.57 g. For the 15 bottles of 125 mL to be packaged, we will have 15x125 g = 1,875 g (the exact quantity of syrup to prepare). For a 10% loss compensation, we have, 1/10x1875 = 185.5 g. That is a total to be prepared of 1,875+187.5 = 2,062.5 g. The multiplication factor (K), which justifies the individual quantity of simple syrup, flavoring and preservative to be obtain in a quantity of final syrup, is:

100 % — 2,062.5 g

1% — 20,625 g, So that K = 20,625

Preparation of sugar syrup or simple syrup

The ingredients used to prepare the simple syrup were sugar (sucrose) and distilled water (Table 1). The method of preparation was decoction. The preparation was done hot, at 70°C, with purified water. The incorporation of 650 g of sugar (sucrose) was done by dissolution, followed by filtration (Dénou et al. 2021).

Table 1. Ingredients, their quantities (100 g and 3,500 g), and functions necessary to prepare a simple syrup

Ingredients	Quantity for 100 g or 1 L	Quantity for 3,500 g or 3.5 L	Functions (Roles)
Sugar (sucrose)	650 g	2275 g	Taste, viscosity
Distilled water	175 g	612.5 g	Solvent, excipient

Second step

Incorporation of medicinal and aromatic principles

The active ingredient was coconut water, collected through immature eyes coconuts using new syringes. The flavoring used was vanilla and the antimicrobial preservative sodium benzoate.

Examination of organoleptic characters

The sense organs (eyes, tongues, and nose) recorded some organoleptic parameters (color, flavor, smell, texture, etc.) of the syrup obtained from coconut water to evaluate the organoleptic characteristics.

Packaging and labeling

It will be a question of producing 15 bottles with a capacity of 125 mL each, labeled with white packaging.

Evaluation of antibacterial activity

Sensitivity test in liquid media

Mueller Hinton Broth (MHB) media were prepared to perform the test in liquid media. The inoculum consisted of solutions (Mueller Hinton Broth), and the microorganisms consisted of bacterial strains (*S. enteritidis*, *E. coli*; *S. dysenteriae*, and *A. baumannii*) and 3 extracts (oil from coconut kernels or HA, Coco water cure or CWC and COVID Med) with different transplanting strains methods (reactivating the microbial strains). This subculturing is carried out in a microplate using the micro-dilution method. It is carried out in triplicate (3 times).

Determination of inhibition parameters (MIC, MBC)

The microdilution technique determined the extracts' inhibition parameters in a liquid medium following the CLSI protocol (2011). This is a reference method, which consists of distributing decreasing concentrations of an antimicrobial substance in the wells of a plate under the same volume, then adding, under the same volume, a culture of bacteria in the exponential growth phase. After incubation for 24 to 48 hours, microbial activity can be visible to the naked eye or by color change inside the cup. The reference antibiotic used was Ciprofloxacin.

Determination of the Minimum Inhibitory Concentration (MIC)

The stock solutions of the two syrups and the almond oil used were prepared at a concentration of 100 mg/mL, and the stock solution of Ciprofloxacin concentrated at 20 mg/mL. The bacterial inoculum was prepared to obtain a turbidity corresponding to the 0.5 Mc Farland standard (1.5 $x10^8$ Cells/mL) and 250x 10^{-3} µg/mL.

Three extracts (oil from coconut almonds, medicated syrup from coconut water and Covid Med syrup) were triplicate for a single isolate. In each well of a 96-well microplate, 100 µL of MHB culture medium was introduced. Subsequently, 100 µL of a stock solution of the 3 extracts or Ciprofloxacin® was introduced into the first 3 wells of column 1 (lines A, B, and C). In columns 1 to 12, successive dilutions following a geometric progression of reasons 2 were carried out (from wells A, B, and C) up to the 11th well, which should vary the concentration range of 500 µL/mL to 0.48 µL/mL for the 3 extracts and from 500 µg/mL to 0.48 µg/mL for Ciprofloxacin. Finally, 100 µL of bacterial inoculum was introduced into each well, thus varying the concentrations from $250x10^{-3}$ µL/mL to 0.24 µL/mL for the extracts and from $250x10^{-3}$ µg/mL to 0.24 µg/mL for Ciprofloxacin. All tests were carried out in triplicate.

The fourth line of the microplate was used as a negative control for the activity of our 3 extracts and MHB. The wells of column 12 were used as positive controls for bacterial growth (MHB+ inoculum). The microplate was sealed with its lid and covered with film paper, then incubated at 37°C for 18 to 24 hours.

After incubation, bacterial growth was demonstrated by adding 20 µL of Blue Alamar at a concentration of 0.4 mg/Ml in two (2) of the three (3) wells of the test lines; the wells of the third line will used for determining the MBC. The MIC was defined as the lowest concentration of our extracts and Ciprofloxacin, for which no bacterial growth was visible to the naked eye (CLSI 2011).

Determination of Minimum Bactericidal Concentration (MBC)

A volume of 50 µL from the wells of the third line, whose concentrations of extracts and Ciprofloxacin® are greater than or equal to the MIC, was transferred into a microplate containing 150 µL of sterile culture broth. The plate was incubated under optimal conditions. After incubation, 20 µL of Blue Alamar was added to the wells at concentration of 0.4 mg/mL and left to act for 30 minutes. The smallest dilution of the extracts where no color change was observed corresponds to its CMB.

Data processing and analysis

The data were processed with Word and Excel 2013 software. Data analysis was done using STATGRAPHICS Plus 5.0 software.

RESULTS AND DISCUSSION

Ethnomedicine

Uses of coconut water in traditional medicine

Regardless of the subdivisions, constipation and stomachaches have the greatest incidence (Figure 1). These results reflect that coconut water is mainly used in the study area to treat digestive disorders (stomachaches and constipation). The pharmacological activities of coconut water could explain these results. Indeed, previous studies have demonstrated that coconut water has antibacterial and antifungal activities, as in Benin (Dougnon et al. 2017).

Characteristics of informants

Moreover, 76 informants participated in the study, i.e. 35 people in the Lékié Department and 41 in Mfoundi (Table 2). Regardless of the department, women were in the majority, with a total of 42 or 55.27%, than men, with 34 or 44.73%. These results reflect that women in the study area have more knowledge than men about the coconut water's antibacterial uses in traditional medicine. These results could be explained by the fact that, in rural areas, women being closer to the sick use coconut water for

primary health care as practiced from generation to generation. Using coconut water as an antibacterial in traditional medicine for primary health care has already reported in Ivory Coast (Assa et al. 2007).

Improve traditional medicine

Pre-formulation of syrup based on coconut water

The results show that the pre-formulation of coconut water as medicated syrup is real (Table 3). These results could be explained by the meticulous follow-up of the observed syrup preparation protocol, which the European Pharmacopoeia recommends. According to Ouedraogo et al. (2021), herbal medicines can be packaged in liquid forms, including syrup, thanks to the introduction of modern technology in the commercial production of herbal products.

Packaging and labeling

Figure 2 shows that the final syrup was packaged in bottles with a label filled in as follows: name of the syrup (Coco Water Cure); active ingredient (coconut water); simple syrup (99.5%); preservative (Sodium Benzoate); aroma (0.03%); place of manufacture (Galenics and Pharmaceutical Legislation Laboratory); date of manufacture (2022.09.16); expiration date (2026.09.15) and bundle number (001). These results are consistent with drug packaging and label presentation (Begert 2015). From the manufacturing and expiration dates on the label, we see that coconut water can be stored for 4 years. This long-term conservation was made possible thanks to the preservative. However, the shelf life of coconut water is generally one year. Recently, the use of sodium benzoate as a preservative has made it possible to preserve a traditional preparation based on medicinal plants in the long term (Lamy et al. 2023).

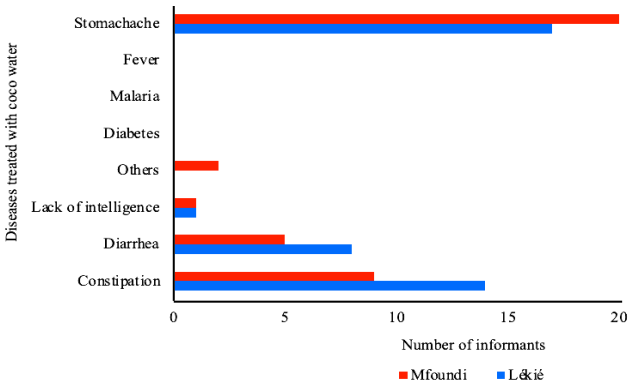


Figure 1. Diseases treated by coconut water depend on the number of people

Table 2. Socio-economic characteristics of coconut water informants in the Center region, Cameroon

Characteristics	Lékié	Mfoundi
Gender		
Women	20	22
Men	15	19
Ages groups (years)		
15-35	15	16
36-55	12	13
55-75	8	12
Religions		
Christians	34	37
Muslims	2	4
Others	0	0
Matrimonial status		
Single	15	17
Divorcee	3	10
Married	12	8
Windwer/ Window	5	6
Educational levels		
Illiterate	0	4
Primary	8	6
Secondary	12	20
Superior	15	11
Number of individuals per family		
1-5	6	8
6-10	25	22
11-20	4	7
20	0	4
Income per day (FCFA)		
655-1300	5	0
1300-2000	13	11
2000-5000	12	19
Others	5	11
Ethnolinguistics groups		
English-speaker	0	0
Bamileke	8	4
Ewondo	6	13
Fufulde	2	3
Sawa	1	5
Bulu	1	8
Eton	11	5
Others	6	3

Note: NTFPs: Non Timber Forest Products, 1 Dollar = 500 FCFA; 1 Euro = 650 FCFA

Table 3. Ingredients, their quantities and roles that made it possible to obtain the final syrup

Ingredients	Quantities per 100 g	Quantities per 2,062.5 g for 100g x K	Functions (roles)
Simple syrup	99.57 g	2,053.63g	Taste, viscosity
Vanilla	0.03 g	0.618g	Flavoring
Sodium benzoate	0.4 g	8.25g	Antimicrobial reservative
Coconut water	175 g	612.5 g	Active ingredient

Evaluation of antibacterial activity

Sensitivity tests in solid media

Regardless of the bacterial strain, it is observed that the reference antibiotic (CIP) shows the largest zone of inhibition (36 mm) (Table 4). Regarding the extracts used, CWC (Coco water cure) does not present any zone of inhibition (00 mm) with three strains (*E. coli*, *S. dysenteriae* and *S. enteritidis*). In comparison, it presents a zone of inhibition with the bacterial strain *A. baumannii* with a value of 10 mm. According to Asif et al. (2019), the scientific community pays particular attention to *A. baumannii* because of its resistance to the latest wave of antimicrobials. It is a Gram-negative bacteria is multi-resistant to several antibiotics and causes nosocomial infections (Reina et al. 2022). The results obtained could be explained as follows. Coconut water (CWC) consists of: simple syrup (95.25 mL), flavoring (0.03 mg), preservative (0.4 mg) and active ingredient (4.32 mL). The active compounds present in this medicinal syrup (lauric acid, etc.) can explain its action on *A. baumannii* which would not have been the case with the three other strains. It should be remembered that lauric acid is a fatty acid. Fatty acids (oleic acid, etc.) inhibit *A. baumannii* (Khadke et al. 2021). The impact of fatty acids on the physiology of *A. baumannii* has been reported (Zang et al. 2022). *Escherichia coli* and *S. dysenteriae* are enterobacteria similar in terms of biochemical characteristics (gram-negative bacillus, mobile or immobile, aerobic-anaerobic). According to Ragupathi et al. (2018), molecular studies are needed to differentiate between these two bacteria. *S. enteritidis*, also an enterobacterium, differs from the two previous strains regarding antigenic characteristics. The genus *Salmonella* has antigens. This difference in their structure and biological compounds may explain why only *A. baumannii* reacted and not the other three strains. The active compounds in coconut water, particular lauric acid, have an antimicrobial effect on *A. baumannii* by disrupting its cell membrane (Marion et al. 2018). Lauric acid has a lipid structure that can penetrate the bacteria's cell membrane and disrupt its stability, leading to a leak of essential substances inside the bacteria, which leads to its death. Also, lauric acid can interfere with the bacteria's metabolic pathways, disrupting its function and survival. However, it should be noted that the effectiveness of lauric acid may vary depending on various factors, such as concentration, exposure time, individual resistance of the bacteria, etc.

Table 4. Values of the inhibition zones of bacterial strains depending on the reference antibiotic (CIP) and the 3 extracts (CWC, COV Med and HA)

Bacterial strains	Reference antibiotic CIP	Extracts used		
		CWC	COV Med	HA
E. coli	36 mm	0	0	0
	32 mm	0	0	0
	32 mm	0	0	0
S. dysenteriae	36 mm	0	0	0
	30 mm	0	0	0
	32 mm	0	0	0
S. enteritidis	30 mm	0	0	0
	30 mm	0	0	0
	26 mm	0	0	0
A. baumannii	26 mm	0	0	0
	30 mm	0	0	0
	30 mm	10mm	0	0

Note: E.C: *Escherichia coli*, CIP: Ciprofloxacin, CWC: Coco Water Cure, COV Med: COVID medicinal syrup, HA: Almond oil, 0: no inhibition zones observed

Figure 2. Syrup bottles after labeling

Table 5. MIC and CMB of the antibiotic and the three extracts on the four strains used

	Priority pathogens	Bacterial strains tested							
		S. dysenteriae		*S. enteritidis*		*A. baumannii*		*E. coli*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Extracts used	H.A (µL/mL)	-		-		-		-	
	Cov Med (µL/mL)	-		-		-		-	
	CWC (µL/mL)	0.25	0.25	0.25	0.25	-		-	
Reference antibiotic	CIP (µg/mL)	3.96	3.96	3.96	3.96	3.96	3.96	3.96	3.96

Note: Indeterminate: (-), MIC: Minimum Inhibitory Concentration, CMB: Minimum Bactericidal Concentration, µL: microliter, mL: milliliter, HA: Almond Oil, CWC: Coconut water cure

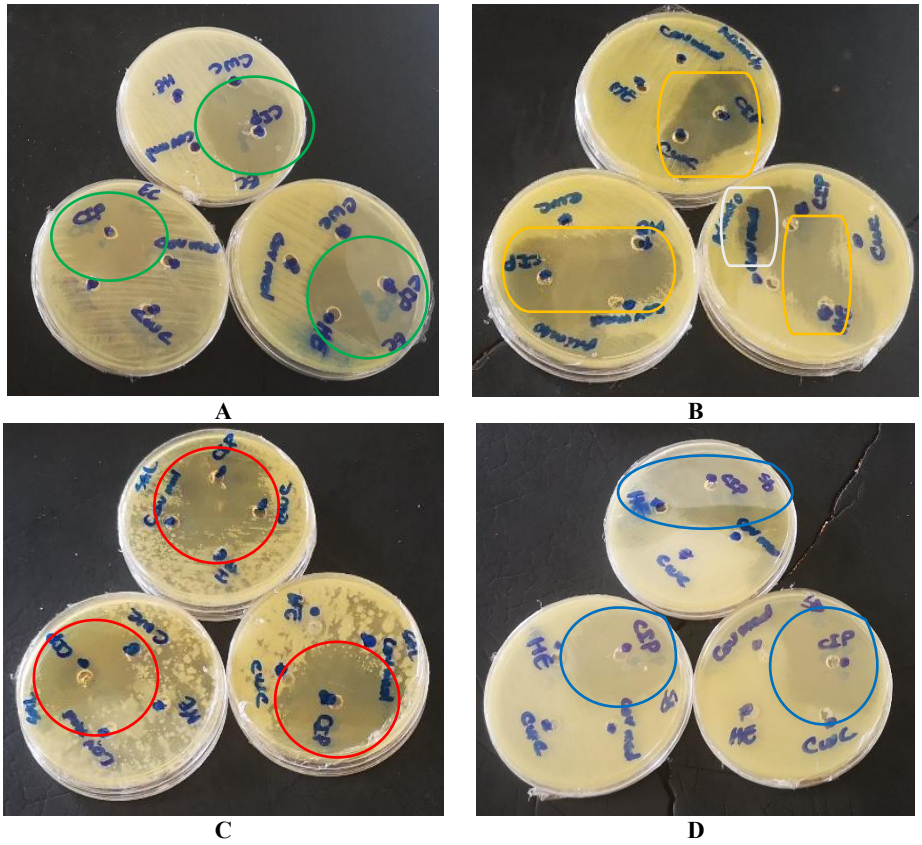


Figure 3. Three respective replicates for A. *E. coli*, B. *A. baumanii*, C. *S. enteritidis* and D. *S. dysenteriae* strains. Note: Circle/rectangular: Halo zone. White rectangular: Delimitation of CWC extract inhibition zone

Figure 3 shows the inhibition zones formed by the different bacterial strains in contact with the extracts and the reference antibiotic. As for the behavior of the *E. coli* bacterial strain (Figure 3.A), regardless of the petri dishes used (repetition), only the reference antibiotic (CIP) presents a zone of inhibition delimited by the box green in color because no zone of inhibition is observed with the extracts used (CWC, Cov Med and HA). Regarding the behavior of the bacterial strain *A. baumanii* (Figure 3.B), whatever the petri dishes used (repetition), the reference antibiotic (CIP) presents an inhibition zone delimited by the yellow box. However, with the extracts used, only the CWC extract presents a zone of inhibition delimited by the white box. Concerning the bacterial strain *S. enteritidis* (Figure 3.C), whatever the petri dishes used (repetition), only the reference antibiotic (CIP) presents a zone of inhibition delimited by the red box. Therefore, no zone of inhibition is observed with the extracts used (CWC, COV Med and HA). Regarding the behavior of the bacterial strain *S. dysenteriae* (Figure 3.D), regardless of the petri dishes used (repetition) only the reference antibiotic (CIP) presents a zone of inhibition delimited by the blue box because no zone of inhibition is observed with the extracts used (CWC, COV Med and HA).

Sensitivity tests in liquid media

Table 5 on the 3 (CWC, COV Med and HA) and reference antibiotic (CIP) show that of the four (4) bacterial strains tested, only two (2) of them (*S. enteritidis* and *S. dysenteriae*) were sensitive. Indeed, *S. enteritidis* and *S. dysenteriae,* respectively, show a MIC of 0.25 µL/mL, were much more sensitive to the CWC extract. These results reflect that in CWC extracts ≤ 0.25 µL/mL concentration, the bacteria *S. enteritidis* and *S. dysenteriae* are sensitive and not sensitive to the other bacterial strains (*A. baumannii* and *E. coli*). Indeed, these CWC extract compounds would act on *S. enteritidis* and *S. dysenteriae* due to more biochemical and bacteriological similarities. Unlike *A. baumannii* and *E. coli*, which are similar in constitution and biochemistry (El-Housseiny et al. 2017). Specifically for *S. dysenteriae*, fatty acids, including lauric acid, interact with certain proteins to abolish their transcription and promotion activities (Trirocco et al. 2023). In addition, all the bacterial strains tested on the reference antibiotic have an identical MIC and CMB value (3.96 µg/mL). These results reflect that all the bacterial strains tested are sensitive for a value ≤ 3.96 µg/mL of extract. MIC values for ciprofloxacin may vary depending on the type of bacteria targeted. Generally, the MIC of ciprofloxacin for Gram-negative bacteria is around 0.015 to 0.25 µg/mL, while for Gram-positive bacteria, it can be around 0.015 to 0.5 µg/mL. However, these values may vary depending on the bacterial strain and the testing method. When a bacteria is inhibited or killed at ciprofloxacin MICs greater than 1 µg/mL, this indicates that this bacteria is resistant to ciprofloxacin MICs less

than 1 µg/mL (Grillon et al. 2016). In other words, the bacteria requires a higher concentration of ciprofloxacin to be inhibited or killed, indicating that ciprofloxacin treatment is less effective against that specific bacterial strain. Additionally, those four bacterial strains tested are in the list of "priority pathogens" made public by the WHO in 2017. The results of the antibacterial ITM from coconut water of Center, Cameroon, on these strains, indicate that this water is eligible for research into new antibiotics.

In conclusion, the initial hypothesis confirms that coconut water has traditional antibacterial uses in Center, Cameroon, and improved traditional antibacterial medicine (ITM) has been made from this water. The evaluation of the antibacterial activity of this ITM on four bacterial strains (*A. baumannii*, *E. coli*, *S. enteritidis* and *S. dysenteriae*) shows an effect on two of them. Only the bacteria *S. enteritidis* and *S. dysenteriae* were observable in a MIC and MBC of 0.25 µg/mL with the antibacterial ITM extract; therefore, *C. nucifera* water of Center, Cameroon is eligible for research into new antibiotics. In perspective, it would be interesting to extend the evaluation of the antibacterial activity of this ITM on other bacterial strains. Other methods of evaluating antibacterial activity should also be explored.

ACKNOWLEDGEMENTS

The authors would like to thank the populations of Center, Cameroon for sharing its local knowledge. They thank the Laboratory of Botany and Traditional Medicine of the Institute of Medical Research and Medicinal Plants Studies (IMPM), Cameroon. As well as the laboratory of Galenic Pharmacy and Pharmaceutical Legislation (PGLP) from the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I, Cameroon for allowing them to handle easily.

REFERENCES

Agnieszka C, Katarzyna S. 2018. Campylobacteriosis, Salmonellosis, Yersiniosis, and Listeriosis as Zoonotic Foodborne Diseases: A review. Intl J Environ Res Public Health 15: 863. DOI: 10.3390/ijerph15050863.

Amujoyegbe OO, Idu M, Agbedahunsi JM, Erhabor JO. 2016. Ethnomedicinal survey of medicinal plants used in the management of sickle cell disorder in Southern Nigeria. J Ethnopharmacol 185: 347-360. DOI: 10.1016/j.jep.2016.03042.

Asif M, Alvi IA, Rehman SU. 2019. Insight into *Acinetobacter baumannii*: Pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities. Infect Drug Resist 11: 1249-1260. DOI: 102147/IDR.S166750.

Asokan GV, Ramadhan T, Ahmed E, Sanad H. 2019. WHO global priority pathogens list: A bibliometric analysis of medline-PubMed for knowledge mobilization to infection prevention and control practices in Bahraim. Omam Med J 34 (3): 184-193. DOI: 10.5001/omj.2019.37.

Assa RR, Konan JL, Agbo N, Prades A, Nemlin J. 2007. Caractéristiques physico-chimiques de l'eau des fruits de quatre cultivars de cocotier (*Cocos nucifera* L.) en Côte d'Ivoire. Agron Afr 19 (1): 41-51. DOI: 10.4314/aga.v19i1.1701.

Begert L. 2015. Le conditionnement des médicaments: Un élément essentiel de protection des patients. Sci Pharm hal-01731903.

Clinical and Laboratory Standards Institute (CLSI). 2011. Performance Standards for Antimicrobial Susceptibility Testing, 21st Informational Supplement. CLSI Document M100-S21. Clinical and Laboratory Standards Institute, Wayne.

David T, Wessel C. 2022. The State of Innovation in Antibacterial Therapeutics. BIO Industry Analysis, Washington DC, USA.

Dénou A, Diallo D, Koumaré M. 2021. Mise au point d'une technique d'amélioration de la conservation des sirops d'extraits de *Guiera senegalensis* J.F. Gmel (Combretaceae). J Appl Biosci 165: 17110-17119. DOI: 10.35759/JABs.165.7.

Dougnon TV, Déguénon E, Fah L, Lègba B, Hounmanou YMG, Agbankpè J, Amadou A, Koudokpon H, Fabiyi K, Aniambossou A, Assogba P, Hounsa E, de Souza M, Dougnon TJ, Gbaguidi F, Boko M, Bankolé HS, Baba-Moussa L. 2017. Traditional treatment of human and animal salmonelloses in Southern Benin: Knowledge of farmers and traditherapsis. Vet World 10 (6): 580-592. DOI: 10.14202/vetworld.2017.580-592.

El-Housseiny GS. 2017. Antibacterial effects of lauric acid against *Acinetobacter baumanii*. J Adv Med Pharm Sci 13 (2): 1-9.

Gianluigi F, Annarita F, Stefania G, Luciana P, Mahendra R, Giancarlo M, Massimiliano G. 2015. Silver nanoparticles as potential antibacterial agents. Molecules 20: 8856-8874. DOI: 10.3390/molecules20058856.

Grillon A, Schramm F, Kleinberg M, Jehl F. 2016. Comparision activity of Ciprofloxacin, Levofloxacin and Moxifloxacin against *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* assessed by minmum inhibitory concentration and time-kill studies. PloS ONE 11 (6): e0156690. DOI: 10.1371/journal.pone.0156690.

IRAD. 2008. Deuxième rapport sur l'état des ressources phytogénétiques pour l'alimentation et l'agriculture au Cameroun. Institut de Recherche Agricole pour le Développement. Yaoundé, Cameroun.

Jiounandan LL. 2019. Les plantes médicinales utilisées par les descendants d'engagés indiens en Guadeloupe: étude bibliographique et enquête de terrain. Sciences du Vivant [q-bio].

Khadke SK, Lee JH, Kim YG, Raj V, Lee J. 2021. Assessment of antibiofilm potencies of nervonic and oleic acid against *Acinetobacter baumannii* using in vitro and computational approaches. Biomedicines 9: 1133. DOI: 10.3390/biomedicines9091133.

Kheraro J. 1975. La médecine et la pharmacopée traditionnelles sénégalaises. ORSTOM, France.

Konan BR, Agnememel AB, Akely PMT, Assa RR, Konan KJL, Amani NG. 2016. Variation des paramètres biochimiques de l'eau de coco (*Cocos nucifera* L.) issu de la culture in vitro pendant la période de stockage. Intl J Biol Chem Sci 10 (3): 957-965. DOI: 10.4314/ijbcs.v10i3.4.

Lamy LGM, Meli KP, Talba D, Amougou AC, Dona A, Zambou ZL, Ndjib R, Fawa G, Nzweundji GJ, Donfagsiteli TN, Agbor AG, Wadjiri PBJV, Wackilou. 2020. Reciprocity in ethnobotanical research: Case of a study carried out in the Mbe plain of Adamawa, Cameroon. Ethnobot Res Appl 20 (38): 1-11. DOI: 1032859/era.20.38.1-11.

Lamy LGM., Nnanga LS, Mahama, Ossongo AF, Elomo LB, Tagne SR, Mbo AJ, Likeng NLJ, Nnanga NE, Harmsen K. 2023. Medicinal syrup for children from the association of significant parts of anti-COVID-19 medicinal plants from the Centre, Cameroon. Asian J Nat Prod Biochem 21 (2): 58-66. DOI: 10.13057/biofar/f2110202.

Maertens de Noordhout C, Devleesschauwer B, Haagsma JA, Havelaar AH, Bertrand S, Vandenberg O, Quoilin S, Brandt PT, Speybroeck N. 2017. Burden of salmonellosis, campylobacteriosis and listeriosis: A time series analysis, Belgium, 2012 to 2020. Euro Surveill 22 (38): 30615. DOI: 10.2807/1560-7917.ES.2017.22.38.30615.

Marion N, Luizet AB, Skogman M, Jouene T, Salcedo SP, Dé E. 2018. Unsaturated fatty acids affect quorum sensing communication system and inhibit motility and biofilm formation of *Acinetobacter baumannii*. Intl J Mol Sci 19 (214): 1-10. Doi: 10.3390/ijms19010214

O'neill J. 2016. The Review on Antimicrobial Resistance. Tracking Drug-Resistant Infections Globally: An Overview of Our Work. Wellcome Trust, UK.

Ouedraogo S, Yoda J, Traoré KT, Nitiema M, Sombie BC, Diawara ZH, Yameogo BG, Djande A, Belemnaba L, Kini FB, Ouédraogo S, Semde R. 2021. Production de matières premières et fabrication des médicaments à base de plantes médicinales. Intl J Biol Chem Sci 15 (2): 750-772. DOI: 10.4314/ijbcs.v15i2.28.

Privalsky TM, Alexander MS, Jinhua W, Christopher TW, Gerard DW, Eric MG, Nathanael SG, Chaitan K. 2021. Prospects for antibacterial

discovery & development. J Am Chem Soc 143 (50): 21127-21142. DOI: 10.1021/jacs.1c10200.

Ragupathi NKD, Sethuvel DPM, Inbanathan FY, Veeraraghavan B. 2018. Accurate differentiation for *E. coli* and *Shigella* serogroups: Challenges and Strategies. New Microb New Infect 21: 58-62. DOI: 10.1016/j.nmni.2017.09.003.

Reina R, Léon-Moya C, Garnacho-Montero J. 2022. Treatment of *Acinetobacter baumannii* severe infections. Med Intensiva 46: 700-710. DOI: 10.1016/j.medine.2022.08.007.

Trirocco R, Pasqua M, Tramonti A, Grossi M, Colonna B, Paiardini A, Prosseda G. 2023. Fatty acids abolish Shigella virulence by inhibiting its master regulator, VirF. Microbiol Spectr 11 (3): e0077823. DOI: 10.1128/spectrum.00778-23.

Zang M, Adams FG, Hassan KA, Eijkelkamp BA, 2022. The impact of omega-3 acids on the evolution of *Acinetobacter baumannii* drug resistance. Microbiol Spectr 9: e0145521. DOI : 10.1128/Spectrum.01455-21.

ASIAN J NAT PROD BIOCHEM
Volume 22, Number 1, June 2024
Pages: 35-42

P-ISSN: 2775-4189
E-ISSN: 2775-4197
DOI: 10.13057/biofar/f220105

Ethanol leaf extract of *Alchornea cordifolia* (Euphorbiaceae) effects on reproductive dysfunctions in streptozotocin-induced diabetic male Wistar rats

SUNDAY AUGUSTINE EJEH[1,♥], HYACINTH ADAKOLE ABU[2], PATRICK AZUIBIKE ONYEYILI[3], JERRY NGUTOR ABENGA[4], SAMSON ENEOJO ABALAKA[5], NDIDI GLORY ENEFE[1], ISHAKU EUGIENE[6]

[1]Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Abuja. PMB 117, Abuja, Nigeria. Tel.: +234-807-940-236-2, ♥email: sunday.ejeh@uniabuja.edu.ng

[2]Department of Veterinary Physiology and Biochemistry, College of Veterinary Medicine, Joseph Sarwuan Tarka University. P.M.B 2373 Makurdi, Benue State, Nigeria

[3]Department of Veterinary Pharmacology and Toxicology, College of Veterinary Medicine, Joseph Sarwuan Tarka University. P.M.B 2373 Makurdi, Benue State, Nigeria

[4]Department of Veterinary Pathology, College of Veterinary Medicine, Joseph Sarwuan Tarka University. P.M.B 2373 Makurdi, Benue State, Nigeria

[5]Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Abuja. PMB 117, Abuja, Nigeria

[6]Department of Public Health, Faculty of Veterinary Medicine, University of Abuja. PMB 117, Abuja, Nigeria

Manuscript received: 23 March 2024. Revision accepted: 29 May 2024.

Abstract. *Ejeh SA, Abu HA, Onyeyili PA, Abenga JN, Abalaka SE, Enefe NG, Eugiene I. 2024. Ethanol leaf extract of* Alchornea cordifolia *(Euphorbiaceae) effects on reproductive dysfunctions in streptozotocin-induced diabetic male Wistar rats. Asian J Nat Prod Biochem 22: 35-42.* Diabetes and some of its treatment agents reportedly induce male reproductive dysfunction in male Wistar rats. Therefore, the study investigated the therapeutic effects of ethanol leaf extract of *Alchornea cordifolia* (Schumach. & Thonn.) Müll. Arg. on diabetic male Wistar rats. Twenty-five male Wistar rats were used, and 50 mg/kg freshly prepared streptozotocin in cool citrate buffer single intraperitoneal injection was used to induce diabetes mellitus. The rats were grouped into 5 of 5 rats each. Group 1 was normal + distilled water; group II was diabetic untreated; group III was diabetic + 100 mg/kg metformin HCl; and IV and V rats were diabetic and received 100 and 200 mg/kg of the extract orally, respectively. Treatment was done for 28 days; after that, reproductive parameters such as extrapyramidal sperm parameters (sperm motility, count, concentrations, viability, morphology, and acrosome integrity) and hormonal assay (FSH, LH, and Testosterone) were evaluated using a standard protocol. Testicular and epididymal histopathological changes were analyzed using standard procedure. The results revealed a marked impairment in all the parameters evaluated in the untreated diabetic group. However, groups exposed to the ethanol leaf extract of *A. cordifolia* recorded a significant ($p < 0.05$) improvement in the above-mentioned reproductive parameters, including the restorations of the collapsed epididymal tubules observed in the diabetic untreated group. Therefore, the ethanol leaf extract of *A. cordifolia* can potentially facilitate and restore reproductive dysfunctions associated with diabetes mellitus complications, hence a possible alternative to synthetic antidiabetic agents.

Keywords: *Alchornea cordifolia*, diabetes mellitus, histopathology, morphometry, sperm parameters

INTRODUCTION

Diabetes mellitus is a non-infectious endocrine, metabolic condition with persistent hyperglycemia resulting from deficient insulin secretion or the inability of insulin receptors to function effectively or both. The global estimate of people affected with diabetes mellitus stood at 463 million, with about 1.5 million deaths annually (American Diabetes Association (ADA 2022)). Diabetes mellitus is a highly devastating disease with increased morbidity and mortality (Lotti and Maggi 2023) if not properly managed and could lead to increased complications such as retinopathy, neuropathy, and foot ulceration (ADA 2021) and macrovascular complications and several other systemic disorders (Zawada et al. 2018). Studies have shown that diabetes mellitus affects the health status of an individual, leading to poor quality of life by enhancing and modulating several risk factors associated with the condition and a consequential increase in several complications such as microangiopathy and macrovascular diseases, resulting in higher morbidity and mortality (E-Garcia et al. 2018; Yun and Ko 2021). Besides the various complications recorded in diabetes, the recent rise in cases of infertility among diabetic patients has increased the awareness of many researchers regarding the role of diabetes in reproductive failure (Ventimiglia et al. 2017). According to He et al. (2021), there is a growing incidence of diabetes mellitus among male couples worldwide compared to their female counterpart, prompting an insinuation that most infertility in couples may be caused by male factor-induced infertility. Investigations into the possible mechanisms of diabetes-induced reproductive dysfunctions revealed a serious change in semen/sperm quality owing to an increase in mitochondria DNA fragmentation, apoptosis, and DNA integrity decrease (Shi et al. 2017). Oxidative stress induced by hyperglycemia has been implicated in the pathophysiology of diabetes reproductive dysfunctions (Shoorei et al. 2019), resulting in

increased DNA sperm damage and decreased sperm motility, viability, and count. Increased oxidative stress-induced reproductive dysfunction in diabetic patients has also been attributed to the impairment of the hypothalamic-pituitary-gonadal axis, leading to hormonal disorders (Temidayo and du Plessis 2018). Several treatment protocols have been used in the management of diabetes and its complications, including the use of oral antidiabetic agents such as Metformin, which is considered the first-line drug of choice (Fatima et al. 2018). However, exposure of diabetes patients to these agents has been shown to exert a negative effect on the reproductive system, resulting in reduced testosterone levels, sexual motivation, libido, and erectile dysfunctions (Al-Kuraishy and Al-Gareeb 2016).

Recent scientific improvement has led to the development of several treatment protocols for managing diabetes and its complications. However, side effects such as hepatic and gastrointestinal disorders, hypoglycemic coma, and lactic acidosis have been associated with the use of current antidiabetic agents (Chaudhury et al. 2017). Interestingly, the World Health Organization recommends alternative plant-based medicine to treat the disease (da Rocha Fernandes et al. 2016). Therefore, there is a need for a search for alternative remedies for the therapeutic management of diabetes and its reproductive complications that will be readily accessible, available, and potent.

The use of plants as alternatives to synthetic antidiabetic agents has continued to attract researchers' concerns, given the presence of important bioactive molecules with therapeutic properties (Solati et al. 2021). It has become a major source of disease management among the rural population in sub-Saharan Africa (Solati et al. 2021). *Alchornea cordifolia* (Schumach. & Thonn.) Müll. Arg. is a dioecious evergreen plant belonging to the family Euphorbiaceae. It is a widely used plant in both tropical and sub-Saharan African traditional medicine for managing diverse disease conditions ranging from respiratory, gastrointestinal, wound healing, infectious diseases, and inflammatory conditions (Noundou et al. 2016). Several reports have attributed *A. cordifolia* medicinal actions to the presence of vital phytochemical compounds such as flavonoids, polyphenols, alchornedine, triterpenes, quercetin, steroids, and protocatechnic acids (Kouakou-Siransy et al. 2010; Osadebe et al. 2012) Pharmacological activities associated with *A. cordifolia* have also been reported, including; antimicrobial, anti-inflammatory, antidiarrheal, antidiabetic, and antioxidant activity (Agbor et al. 2004; Manga et al. 2004; Effo et al. 2017).

Although the antidiabetic and reproductive enhancing potentials of *A. cordifolia* have been investigated (Mohammed et al. 2013; Ngaha-Njile et al. 2019), its ameliorative activity on reproductive dysfunctions associated with diabetic complications remains poorly understood. Hence, this work aimed to evaluate the therapeutic effects of *A. cordifolia* on reproductive dysfunctions in streptozotocin-induced diabetic Wistar rats.

MATERIALS AND METHODS

Ethical clearance was sought and received from the University of Abuja Ethics Committee on Animal Use (UAECAU), Nigeria with approval number UAECAU/2022/005. It was performed according to national guidelines on the use of animals for scientific investigation.

Plant collection

The *A. cordifolia* leaf was collected from a farm in Alkpali, Ugbokolo, Okpokwu Local Government Area, Benue State, Nigeria during the dry season. Dr Okoh Thomas identified and authenticated the leaf at the herbarium of the Department of Botany, Faculty of Biological Sciences, Joseph Sarwuan Tarka University Makurdi, Nigeria, where the voucher's number (FUAM/BOT/HERB/02781) was deposited for future reference.

Extract preparation

The harvested leaves were washed in running tap water to remove sand and other solid particles/impurities and dried under laboratory conditions before pulverization into a fine powder using an electric blender. Next, 500 g of the leaves powder was loaded into the thimble and extracted in 1000 mL ethanol using the Soxhlet apparatus and concentrated at 40°C for three (3) hours in a rotary evaporator (Shanghai Yarong Re-52aa/52CS, China) before evaporating it to dryness using a water bath at 40°C to obtain a yellow-brown Ethanol Leaf Extract of *A. cordifolia* (ELEAC) residue. The percentage yield recorded was 28.8%.

Induction of diabetes mellitus in male albino Wistar rats

Diabetes mellitus was injected by intraperitoneal injection of 50 mg/kg of streptozotocin in freshly prepared 0.1M cold citrate buffer at a pH of 4.5 after eighteen (18) hours of fasting. The rats were then exposed to clean water and feed before measuring their blood glucose levels using blood from the tail vein on an Accu-chek® glucometer (Accu-chek® GB, Roche Mannheim Germany) after forty-eight (48) hours of treatment. Male Wistar rats with fasting blood glucose above 200 mg/dL concentrations were considered diabetic and selected for the study.

Testicular and epididymal morphometric evaluation

Morphometric parameters such as weight, length, width, and volume of the epididymis and testes were measured using conventional instruments such as a ruler, scientific weighing scale, measuring cylinder, and ropes.

Evaluation of epididymal sperm motility

Sperm motility was determined using the swim-out technique (Veen and Preeti 2017). After exteriorization and trimming of the cauda epididymis to remove fat and tissue debris, a small incision was made through it and then placed in 1 mL of normal saline for a few minutes to allow the spermatozoa to swim into the fluid. After that, a drop of the suspension was placed on a pre-warmed grease-free

glass slide, and a cover slip was placed on it, which was viewed at X400 magnification. Individual sperm motility was assessed by counting progressive motile sperm across different fields per unit area. The values were presented as percentages of motile spermatozoa.

Evaluation of epidydimal sperm viability

Sperm viability was assessed using the method of (Blom 1973). Briefly, about 0.1 mL of sperm suspension was mixed with 0.1 mL of eosin-nigrosin stain on a grease-free microscope slide and allowed to stand for a few seconds. Next, a smear was made, air-dried, and viewed at X400 magnification. After that, 200 spermatozoa were counted across different fields, and the percentage of live spermatozoa was recorded.

Evaluation of epididymal sperm concentration

The method of (Robb et al. 1978) was used to evaluate epididymal sperm concentration. Briefly, a drop of sperm suspension prepared in 0.05% formol-saline was placed on a pre-warmed improved hemocytometer and counted to determine the epididymal sperm concentration.

Evaluation of epididymal sperm morphology

The effect of the extract on sperm morphology was evaluated using sperms from the cauda epididymis, following the method of (Linder et al. 1992). Next, 0.1 mL sperm suspension was placed on a grease-free slide to make a thin smear. The smear was air-dried, fixed in methanol, and stained with Giemsa. After that, the slide was washed in running water, dried, and viewed at X40 magnification. Abnormal spermatozoa were counted in different fields and expressed as a percentage of the normal spermatozoa.

Assessment of the effects of the extract on acrosome integrity of spermatozoa of streptozotocin-induced diabetic male albino Wistar rats

In this study, the method of (De-Oliveira et al. 2011) was used to assess the effects of the extract of *A. cordifolia* on the acrosome integrity of spermatozoa in Streptozotocin-induced diabetic rats. Briefly, 0.1 mL of sperm suspension was placed on a pre-warmed glass slide, and a smear was made on it. The smear slides were fixed in methanol for 10 minutes and rinsed for a few minutes in running tap water. After that, the slides were stained in buffer Giemsa for three hours, rinsed in running water, dried, and examined at X400 magnification. Next, 200 cells were counted under the microscope, and the number of intact acrosome, which is characterized by purple head, was calculated by dividing its number by the total number of spermatozoa multiplied by 100%

Evaluation of the effects of ethanol leaves extract of *A. cordifolia* on male hormones of streptozotocin-induced diabetic Wistar rat

Male hormones [testosterone, luteinizing hormone (LH), and Follicle-Stimulating Hormone (FSH)] were evaluated using commercial ELISA kits obtained from Monobind Inc. (Lake Forest, CA 92630, USA) following the manufacturer's instructions. The values were extracted from the plotted absorbance curve versus the concentrations and recorded accordingly.

Histopathological analysis

The testis and the epididymis were harvested after the last day of treatment following the humane sacrifice of the experimental Wistar rats. For histopathological preparation according to standard procedures (Bancroft and Cook 1994), combined ketamine (0.1 mg/kg)/xylazine (0.5 mg/kg) was administered after fixation in 10% formalin for histopathological evaluations.

Statistical analysis

Data obtained from this study were analyzed and represented as mean ± standard error of the mean (Mean ± SEM). Data were subjected to a One-way analysis of variance, and tests of significance between treated groups and control were evaluated using Dunnett's multiple post hoc test. All statistical analyses were done using GraphPad Prism version 7 software (Graph Pad Prism Inc., San Diego, CA, USA), and values of $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Testicular and epidydimal morphometry

The results of testicular and epididymal morphometry revealed a significant ($p < 0.05$) increase in the weight of the epididymis compared with the diabetic untreated group. However, no significant changes in the other parameters were measured (Table 1).

Table 1 The effects of ethanol leaf extract of *Alchornea cordifolia* on testicular and epidydimal morphometry of streptozotocin-induced diabetic male albino Wistar rats

Groups	Testes weight (g)	Testes length (cm)	Testes width (cm)	Testes volume (cm)	Epididymal weight (g)	Epididymal length (cm)
Control	1.31±0.21	1.81±0.13	1.08±0.25	1.21±0.21	0.33±0.03[a]	4.10±0.14
Diabetic contr.	1.22±0.13	2.03±0.02	1.09±0.03	1.28±0.12	0.36±0.02	4.47±0.18
Extract (100 mg/kg)	1.21±0.13	1.88±0.12	1.08±0.06	1.28±0.11	0.38±0.06	3.84±0.36
Extract (200 mg/kg)	1.24±0.04	1.89±0.05	1.03±0.01	1.31±0.06	0.51±0.02[b]	4.54±0.10
Metformin (5 mg/kg)	1.33±0.04	1.98±0.02	1.13±0.04	1.27±0.07	0.39±0.01	4.60±0.10

Note: Different superscripts within columns are statistically significant ($p<0.05$) compared to the control after post hoc Dunnett's multiple comparison test

Table 2. Effects of ethanol leaf extract of *A. cordifolia* on sperm parameters of streptozotocin-induced diabetic male albino Wistar rats

Groups	Epididymal sperm count (x10^6)	Percent sperm motility	Percent sperm viability	Percent abnormal sperm	Percent acrosome integrity
Control	84.63±12.62^a	80.60±3.37	85.00±2.20^a	1.88±0.43^a	72.50±4.47^a
Diabetic control	44.44±16.31	59.00±5.10	64.25±2.78	6.00±0.96	22.90±3.83
Extract (100 mg/kg)	66.50±11.52	72.00±11.25	83.80±4.74^b	4.10±1.16	36.00±4.09
Extract (200 mg/kg)	111.6±9.27^b	85.60±7.13	90.20±2.35c	3.70±0.56^b	40.80±4.03^b
Metformin (5 mg/kg)	74.15±16.81	79.00±5.34	87.20±2.52^d	2.90±0.29^c	30.50±3.66

Note: Different superscripts within columns are statistically significant ($p<0.05$) compared to the control after post hoc Dunnett's multiple comparison test

Table 3. Effects of ethanol leaf extract of *A. cordifolia* on male hormones of Streptozotocin-induced diabetic Wistar rats

Groups	LH (ng/mL)	FSH (ng/mL)	Testosterone (ng/mL)
Control	8.96±3.12	4.90±1.12	62.10±16.63^a
Diabetic control	6.22±1.90	3.30±1.16	42.50±16.22
Extract (100 mg/kg)	7.16±2.64	5.14±1.76	61.20±3.46
Extract (200 mg/kg)	7.52±1.96	3.50±1.18	63.96±6.07^b
Metformin (5 mg/kg)	8.72±3.56	3.0±0.52	68.10±5.14^c

Note: Different superscripts within columns are statistically significant ($p<0.05$) compared to the control after post hoc Dunnett's multiple comparison test

Effects of ethanol leaf extract of *A. cordifolia* on sperm parameters of streptozotocin-induced diabetic male albino Wistar rats.

The results of the ethanol leaf extract of *A. cordifolia* on sperm parameters such as epididymal sperm count, percentage viability, motility, percentage acrosome integrity, and morphological abnormality percentage were presented in Table 2. Diabetes was observed to induce marked acrosome damage and increased morphological abnormality. However, treatment with the extract of *A. cordifolia* at 200 mg/kg significantly ($p<0.05$) improved the integrity of the acrosome (40.80±4.03^b). It markedly decreased (3.70±0.56^b) the percentage of sperm damage observed in the diabetic control group (6.00±0.96). Though no significant ($p>0.05$) changes were noted in the groups treated with 100 mg/kg (36.00±4.09) and standard antidiabetic drug (30.50±3.66) (Metformin hydrochloride), there was an improvement in the level of acrosome damage compared with the diabetic control (22.90±3.83). There were also no significant ($p>0.05$) changes in the morphological abnormality of the treated groups compared to the diabetic untreated group. However, a non-significant ($p>0.05$) reduction in the percentage morphological abnormality was observed in the 200 mg/kg treated group and standard antidiabetic drug (Metformin hydrochloride). There was also a significant ($p<0.05$) increase in the percentage viability of the sperm cells of extract-treated groups compared to the diabetic control. Decreased epididymal sperm count induced by diabetes was significantly ($p<0.05$) increased at 200 mg/kg exposure. However, there was no significant change in the other treatment groups compared to the diabetic untreated group.

Effects of ethanol leaves extract of *A. cordifolia* on hormones male reproductive in streptozotocin-induced diabetic male albino Wistar rats.

The results of the effects of the extract of *A. cordifolia* on male hormones are presented in Table 3. The results showed a significant ($p<0.05$) increase in serum testosterone level at 200 mg/kg extract treated groups compared to the diabetic control. Despite Luteinizing hormone (7.16±2.64; 7.52±1.96) and follicle-stimulating hormone (5.14±1.76; 3.50±1.18) levels elevations in the study, the values were not statistically significant ($p>0.05$) when compared to the diabetic control.

Histopathological findings

There were no obvious cellular changes in the testes of male Wistar rats exposed to streptozotocin alone (45 mg/kg) (Figure 1. B) and in conjunction with either 5 mg/kg, 100 mg/kg, or 200 mg/kg (Figure 1. C, 1.D, and 1. E, respectively) of the ethanol extract of *A. cordifolia* by gavage. However, there were collapsed epididymal tubules within a fibrous stroma (33%) in the diabetic untreated group (Figure 2. B), which was greatly ameliorated by the extract-treated groups (100 and 200 mg/kg) (Figure 2c and 2d) and the standard antidiabetic drug used in this study (Figure 2. E).

Discussion

The presence of numerous bioactive compounds and the array of natural plant communities (Ezeonu and Ejikeme 2016) across sub-Saharan Africa has enhanced the herbal medicine use as an undisputable alternative to synthetic antidiabetic agents in diabetes management and its complications, especially those associated with male functions and fertility. Diabetes mellitus, a disease associated with disorders of carbohydrates, proteins, and lipids metabolism, is known to greatly impair reproductive functions, including male fertility (Jangir and Jain 2014). The use of streptozotocin as a chemical model for the induction of experimental diabetes mellitus in animal species continues to be relevant in diabetology due to its ability to destroy pancreatic beta cells selectively (Shi et al. 2017).

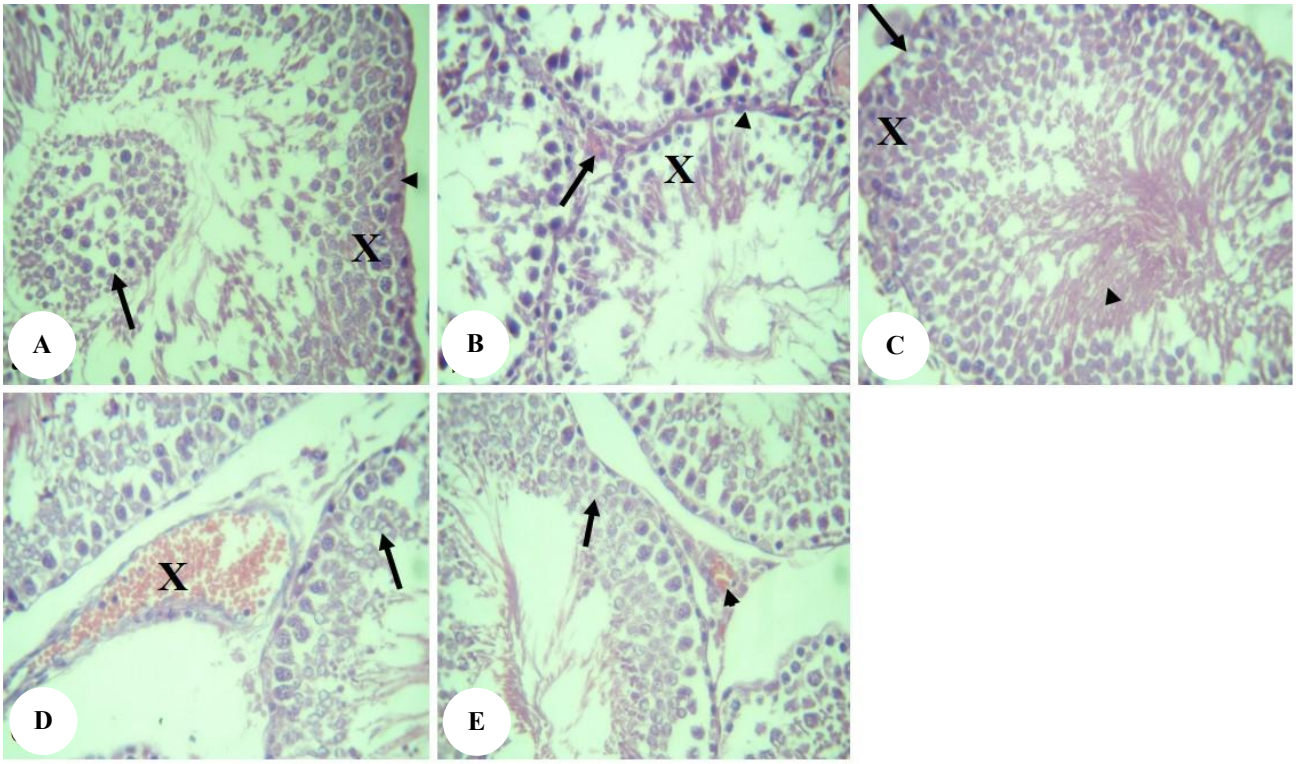


Figure 1. A-D: Photomicrograph of a section of the testis of streptozotocin-induced diabetic male Wistar rats exposed to various ethanol leaf extracts of *A. cordifolia* (100 mg/kg, 200 mg/kg) and Metformin (5 mg/kg). A: Control; note the intact basement membrane of the seminiferous tubule (arrowhead) with the spermatogonia spermatocytes (X) and spermatozoa (arrow). B: Diabetic untreated. Note the inter-tubular congestion (arrow) and intact basement membrane (arrowhead) with necrosis of the spermatogonia, primary spermatocytes, secondary spermatocytes, and the spermatids C: Diabetic + drug; note the seminiferous tubule with intact basement membrane (arrow) containing spermatogonia and spermatocytes (X) and spermatids (arrowheads) D: Diabetic + 100 mg/kg extract. E: Diabetic + 200 mg/kg. Note the inter-tubular congestion (arrowhead) with the necrosis of the primary spermatocyte (arrow) and secondary spermatocyte (double arrowheads). H and E: x 400

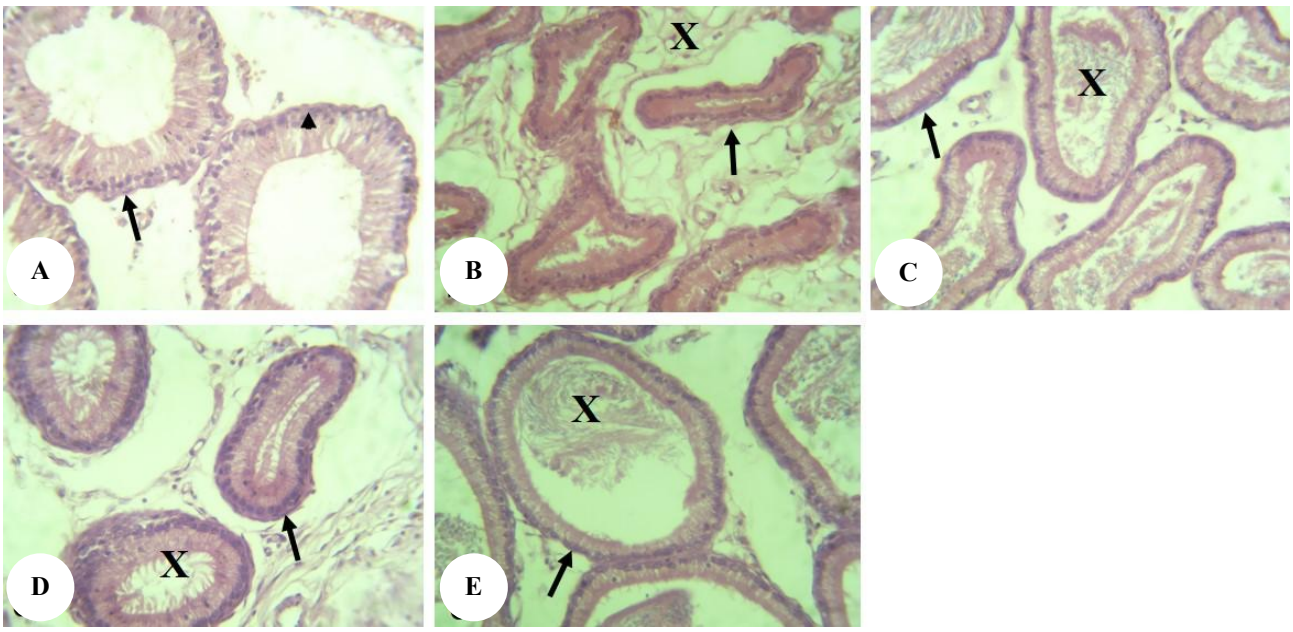


Figure 2. A-D: Photomicrograph of a section of the epididymis of streptozotocin-induced diabetic male Wistar rats exposed to various ethanol leaf extracts of *A. cordifolia* (100 mg/kg, 200 mg/kg) and Metformin (5 mg/kg). A: Note the epididymal tubules (arrows) with pseudostratified columnar epithelial cells (arrowheads). B: Diabetic untreated. Note the collapsed epididymal tubules (arrows) within a fibrous stroma (X), C: Diabetic + drug, Note the epididymal tubules (arrows) containing spermatozoa, D: Diabetic + 100 mg/kg extract, Note the epididymal tubules (arrows) containing spermatozoa. E: Diabetic + 200 mg/kg. Note the epididymal tubules (arrows) containing spermatozoa (X). H and E: x 400

This study evaluated the effects of ethanol leaf extract of *A. cordifolia* on male reproductive dysfunctions induced by diabetes. Although the morphometric analysis of the testes and the epididymis revealed diabetes-induced organ weight reduction (1.22±0.13; 0.36±0.02), significant ($p<0.05$) increases were recorded in groups exposed to the various doses of the extract as well as in the epididymis weight ($0.51±0.02^b$) compared to the diabetic untreated group. The increased epididymal weight could be attributable to the presence of more spermatozoa in the epididymal tubular lumen, as evidenced in the photomicrographs (Figure 2. C-D). The finding was consistent with the report of Allassane et al. (2021), who associated the gain in epididymal weight with the androgenic activity of the plant extract since testicular growth and secretions are strictly under the influence of androgens (Bakloul et al. 2016). Allassane et al. (2021) also reported significantly ($p<0.05$) increased testicular weight and volume in streptozotocin-induced diabetic rats coadministered with hydroalcoholic extract of *Alpinia officinarum* leaf extract (Heidari et al. 2021).

The significantly ($p<0.05$) increased extrapyramidal sperm count and enhanced sperm motility and viability suggested that the exposure of those diabetic rats to ethanol leaf extract of *A. cordifolia* greatly improved the hyperglycemia-induced damages. The ability of the ethanol leaf extract of *A. cordifolia* to enhance the evaluated reproductive parameters could be linked to the presence of their inherent important bioactive phytoconstituents, according to Solati et al. (2021). Several studies have demonstrated that important phytochemicals such as polyphenols and flavonoids could restore damaged sperm parameters and hormonal disturbances associated with disease conditions because of their potent antioxidant activity (Jangir and Jain 2014).

Oxidative stress damage has been implicated as the main instigator of complications in diabetes (Nna et al. 2019), including male infertility (Alsenosy et al. 2019). This is because increased oxidative stress induced by hyperglycemia could disrupt the hypothalamic-pituitary-gonadal system to trigger hormonal disorder with a consequential decrease in male fertility as a result of lower gonadotropins, sperm motility, count, and viability (Abbasihormozi et al. 2019). The positive impact of this plant extract could be attributed to the presence of vital phytochemical constituents such as phenol and flavonoids earlier reported by Ejeh et al. (2023), which possess strong radical scavenging abilities (Kaushik et al. 2011).

This study revealed that streptozotocin diabetes induction lowered serum gonadotropins (6.22±1.90; 3.30±1.16) and testosterone levels (42.50±16.22), which agreed with the findings of Arikawe et al. (2012), Jangir and Jain (2014), and Soliman et al. (2019), who recorded similarly decreased hormonal values. The findings showed the ability of diabetes to disrupt the synthesis of these hormones and cause Leydig and Sertoli cell functional alterations with subsequent impairment of spermatogenesis (Jangir and Jain 2014). However, the exposure of diabetic male Wistar rats to ethanol leaf extract of *A. cordifolia* in this study significantly ($p<0.05$) elevated serum testosterone levels (63.96±1.96) and insignificantly ($p>0.05$) increased FSH (5.14±1.76) and LH levels (7.52±1.96). Although the LH and FSH values were not statistically significant ($p>0.05$), they might be clinically relevant as both hormones influence Leydig and Sertoli's cellular functions. The finding was consistent with the work of (Ngaha-Njile et al. 2019), who reported an increased testosterone concentration in male Wistar rats exposed to various doses of *A. cordifolia*. The elevated testosterone level might be responsible for the improved sperm motility, viability, counts, and acrosome integrity recorded in the present study.

According to Tian et al. (2020), testicular and epididymal damage resulting in defective spermatozoa production and maturation has occurred in diabetes-induced oxidative stress. Histopathological findings in the present study revealed complete epididymal tubular collapse with seminiferous cellular damage. Heidari et al. (2021) have reported testicular damage with a manifested tubular atrophy, reduction in seminiferous tubular diameter, and thickness of the seminiferous epithelium in streptozotocin-induced diabetic animals. The reported testicular damage could probably be due to elevated oxidative stress damage.

In conclusion, the study has demonstrated that streptozotocin-induced diabetes causes serious damage to male reproductive structures and parameters. However, the exposure of diabetes-induced Wistar rats to various doses of the ethanol leaf extract of *A. cordifolia* improved the evaluated reproductive structure and profile of the exposed Wistar rats. Therefore, the ethanol leaf extract of *A. cordifolia* could be employed as an alternative to synthetic antidiabetic agents in managing male reproductive dysfunctions associated with diabetic complications.

ACKNOWLEDGEMENTS

This research work was supported by the Tertiary Education Trust fund (TETFund) under her Institutional Based Research, Nigeria grant with grant number TETFUND/UNIABUJA/2023. The authors acknowledge the management of the University of Abuja, Nigeria, for facilitating the financial assistance received and TETFund for their financial support. SAE, HAA, and PAO contributed to the study's conceptualization and design. SAE, SEA, and NGE collected, analyzed, and interpreted the data, while JNA participated in the histopathological slide preparations. SAE wrote the first draft of the manuscript, and all authors commented on it. All authors read and approved the final manuscript. The authors declare no conflict of interest.

REFERENCES

Abbasihormozi SH, Babapour V, Kouhkan A, Naslji AN, Afraz K, Zolfaghary Z, Shahverdi AH. 2019. Stress hormone and oxidative stress biomarkers link obesity and diabetes with reduced fertility potential. Cell J 21 (3): 307-313. DOI: 10.22074/cellj.2019.6339.

Agbor GA, Léopold T, Jeanne NY 2004. The antidiarrhoeal activity of *Alchornea cordifolia* leaf extract. Phytother Res 18 (11): 873-876. DOI: 10.1002/ptr.1446.

Al-Kuraishy HM, Al-Gareeb AI 2016. Erectile dysfunction and low sex drive in men with Type 2 DM: The potential role of diabetic pharmacotherapy. J Clin Diagn Res 10 (12): FC21-FC26. DOI: 10.7860/JCDR/2016/19971.8996.

Allassane K, Goueh G, Daouda D, Koffi K 2021. Effect of an aqueous extract of *Alchornea cordifolia* (Euphorbiacea) leaves on sperm parameters and reproductive function of male Wistar rats. Eur Sci J 17 (17): 236. DOI: 10.19044/esj.2021.v17n17p236.

Alsenosy AA, El-Far AH, Sadek KM, Ibrahim SA, Atta MS, Sayed-Ahmed A, Al Jaouni SK, Mousa S A. 2019. Graviola (*Annona muricata*) attenuates behavioural alterations and testicular oxidative stress induced by streptozotocin in diabetic rats. PloS One 14 (9): e0222410. DOI: 10.1371/journal.pone.0222410.

American Diabetes Association (ADA). 2021. Microvascular complications and foot care: Standards of medical care in diabetes. Diabetes Care 44 (Suppl 1): S151-S167. DOI: 10.2337/dc21-S011.

American Diabetes Association (ADA). 2022. Professional Practice Committee. 2. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2022. Diabetes Care 45 (Suppl 1): S17- S38. DOI: 10.2337/dc22-S002.

Arikawe AP, Daramola AO, Udenze IC, Akinwolere MF, Olatunji-Bello II, Obika LF 2012. Comparison of streptozotocin-induced diabetic and insulin-resistant effects on spermatogenesis with Proliferating Cell Nuclear Antigen (PCNA) immunostaining of adult rat testis. J Exp Clin Med 29 (3): 209-214. DOI: 10.5835/jecm.omu.29.03.008.

Bakloul F, Jakhlal N, Elghazoui A, Karmouni T, El Khader K, Koutani A, Ibnattya AA. 2016. Kyste de la vésicule séminale: À propos d'un cas [Seminal vesicle cyst: About a case]. Pan Afr Med J 25: 68. DOI: 10.11604/pamj.2016.25.68.8240. [French]

Bancroft JD, Cook HC 1994. Manual of Histological Techniques and Their Diagnostic Application. Churchill Livingstone, London.

Blom E. 1973. Ultrastructure af nogle karakteristiske sperimedefekter og forslag til etnyt klassificerings-system for tyrens sperimiogram [The ultrastructure of some characteristic sperm defects and a proposal for a new classification of the bull spermiogram (author's transl)]. Nord Vete Med 25 (7): 383-391. [Danish]

Chaudhury A, Duvoor C, Reddy Dendi VS, Kraleti S, Chada A, Ravilla R, Marco A, Shekhawat NS, Montales MT, Kuriakose K, Sasapu A, Beebe A, Patil N, Musham CK, Lohani GP, Mirza W. 2017. Clinical review antidiabetic drugs: Implications for type 2 diabetes mellitus management. Front Endocrinol 8: 6. DOI: 10.3389/fendo.2017.00006.

da Rocha Fernandes J, Ogurtsova K, Linnenkamp U, Guariguata L, Seuring T, Zhang P, Cavan D, Makaroff LE. 2016. IDF diabetes atlas estimates of 2014 global health expenditures on diabetes. Diabetes Res Clin Pract 117: 48-54. DOI: 10.1016/j.diabres.2016.04.016.

De-Oliveira VP, Marques MG, Simoes R, Assumpeao MEOD, Visintin JA. 2011. Influence of caffeine and chondroitin sulfate on swin sperm capacitation and in vitro embryo production. Acta Sci Vet 39 (2): 960.

Effo KE, Kouakou SL, Irie-N'Guessan G, Kouakou-Stransy NG. 2017. Antioxidant activity and hepatoprotective effects of an aqueous extract of *Alchornea cordifolia* leaves. Pharmacol Pharm 8: 369-380. DOI: 10.4236/pp.2017.811027.

E-Garcia D, Narvaez-Mendez M, Morgan S, Coronado-Malagon M, Arce-Salinas CA, Barajas A, Arenas I, Svarch A. 2018. Biomarkers through the development, progression and chronic complications of diabetes mellitus: A mini-review. J Endocrinol Diabetes 5: 1-7. DOI: 10.15226/2374-6890/5/6/001121.

Ejeh SA, Abu HA, Onyeyili PA, Abenga JN, Ogbe RJ, Abalaka SE. 2023. Phytochemical, metal analysis, and in-vitro antioxidant activity of ethanol leaf extract of *Alchornea cordifolia* (Schumach and Thonn) Müll Arg (Euphorbiaceae). Trop J Nat Prod Res 7 (9): 4070-4075. DOI: 10.26538/tjnpr/v7i9.34.

Ezeonu CS, Ejikeme CM. 2016. Qualitative and quantitative determination of phytochemical contents of indigenous Nigerian softwoods. New J Sci 9: 5601327. DOI: 10.1155/2016/5601327.

Fatima M, Sadeeqa S, Nazir SUR 2018. Metformin and its gastrointestinal problems: A review. Biomed Res 29: 2285-2289. DOI: 10.4066/biomedicalresearch.40-18-526.

He Z, Yin G, Li QQ, Zeng Q, Duan J 2021. Diabetes mellitus causes male reproductive dysfunction: A review of the evidence and mechanisms. In vivo 35 (5): 2503-2511. DOI: 10.21873/invivo.12531.

Heidari H, Abdollahi M, Khani S, Nojavan F, Khani S. 2021. Effect of *Alpinia officinarum* extract on reproductive damages in streptozotocin-induced diabetic male rats. J Diabetes Metab Disord 20 (1): 77-85. DOI: 10.1007/s40200-020-00711-0.

Jangir RN, Jain GC. 2014. Diabetes mellitus induced impairment of male reproductive functions: A review. Curr Diabetes Rev 10 (3): 147-157. DOI: 10.2174/157339 9810666140606111745.

Kaushik D, Yadav J, Kaushik P, Sacher D, Rani R. 2011. Current pharmacological and phytochemical studies of the plant *Alpinia galanga*. Chin J Integr Med 9 (10): 1061-1065. DOI: 10.3736/jcim20111004.

Kouakou-Siransy G, Sahpaz S, Irié Nguessan G, Datté JY, Brou J.K, Gressier B, Bailleul F 2010. Effects of *Alchornea cordifolia* on elastase and superoxide anion produced by human neutrophils. Pharm Biol 48: 128-133. DOI: 10.3109/13880200903051609.

Linder RE, Strader LF, Slott VL, Suarez JD 1992. Endpoints of spermatotoxicity in the rat after short duration exposures to fourteen reproductive toxicants. Reprod Toxicol 6 (6): 491-505. DOI: 10.1016/0890-6238 (92)90034-q.

Lotti F, Maggi M. 2023. Effects of diabetes mellitus on sperm quality and fertility outcomes: Clinical evidence. Andrology 11 (2): 399-416. DOI: 10.1111/andr.13342.

Manga HM, Brkic D, Marie DE, and Quetin-Leclercq J. 2004. In vivo anti-inflammatory activity of *Alchornea cordifolia* (Schumach. & Thonn.) Müll. Arg. (Euphorbiaceae). J Ethnopharmacol 92 (2-3): 209-214. DOI: 10.1016/j.jep.2004.02.019.

Mohammed RK, Ibrahim S, Atawodi SE, Eze ED, Suleiman JB, Ugwu MN, Malgwi IS. 2013. Antidiabetes and haematological effects of n-butanol fraction of *Alchornea cordifolia* leaf extract in streptozotocin-induced diabetic Wistar rats. Sci J Biol Sci 2 (3): 45-53.

Ngaha-Njile NMI, Lembe DM, Koloko BL, Meng GY, Ebrahimi M, Awad EA, Baiee FH, Kenmogne H, Hambe M, Mandengue SH. 2019. Sperm parameters quality and reproductive effects of methanolic extract of *Alchornea cordifolia* leave on senescent male rats. Andrologia 51: e13359. DOI: 10.1111/and.13359.

Nna VU, Abu Bakar AB, Ahmad A, Eleazu CO, Mohamed M. 2019. Oxidative Stress, NF-κB-Mediated inflammation and apoptosis in the testes of streptozotocin-induced diabetic rats: Combined protective effects of Malaysian propolis and metformin. Antioxidants 8 (10): 465. DOI: 10.3390/antiox8100465.

Noundou XS, Krause R, Van Vuuren S, Ndinteh DT, Olivier D. 2016. Antibacterial effects of *Alchornea cordifolia* (Schumach. and Thonn.) Müll. Arg extracts and compounds on gastrointestinal, skin, respiratory and urinary tract pathogens. J Ethnopharmacol 179: 76-82. DOI: 10.1016/j.jep.2015.12.043.

Osadebe PO, Okoye FB, Uzor PF, Nnamani NR, Adiele IE, Obiano NC. 2012. Phytochemical analysis, hepatoprotective and antioxidant activity of *Alchornea cordifolia* methanol leaf extract on carbon tetrachloride-induced hepatic damage in rats. Asian Pac J Trop Med 5 (4): 289-293. DOI: 10.1016/S1995-7645(12)60041-8.

Robb GW, Amann RP, Killian GJ. 1978. Daily sperm production and epididymal sperm reserves of pubertal and adult rats. J Reprod Fertil 54: 103-107. DOI: 10.1530/jrf.0.0540103.

Shi GJ, Zheng J, Wu J, Qiao HQ, Chang Q, Niu Y, Sun T, Li YX, Yu JQ. 2017. Protective effects of *Lycium barbarum* polysaccharide on male sexual dysfunction and fertility impairments by activating hypothalamic-pituitary-gonadal axis in streptozotocin-induced type-1 diabetic male mice. Endocr J 64 (9): 907-922. DOI: 10.1507/endocrj.EJ16-0430.

Shoorei H, Khaki A, Khaki AA, Hemmati AA, Moghimian M, Shokoohi M. 2019. The ameliorative effect of carvacrol on oxidative stress and germ cell apoptosis in testicular tissue of adult diabetic rats. Biomed Pharmacother 111: 568-578. DOI: 10.1016/j.biopha.2018.12.054.

Solati K, Karimi M, Rafieian-Kopaei M, Abbasi N, Abbaszadeh S, Bahmani M. 2021. Phytotherapy for wound healing: The most important herbal plants in wound healing based on Iranian ethnobotanical documents. Mini-Rev Med Chem 21: 500-519. DOI: 10.2174/1389557520666201119122608.

Soliman GA, Saeedan AS, Abdel-Rahman RF, Ogaly HA, Abd-Elsalam RM, Abdel-Kader MS. 2019. Olive leaf extract attenuates type II diabetes mellitus induced testicular damage in rats: Molecular and biochemical study. Saudi Pharm J 27 (3): 326-340. DOI: 10.1016/j.jsps.2018.11.015.

Temidayo SO, du Plessis SS. 2018. Diabetes mellitus and male infertility. Asian Pac J Reprod 7 (1): 6-14. DOI: 10.4103/2305-0500.220978.

Tian Y, Song W, Xu D, Chen X, Li X, Zhao Y. 2020. Autophagy induced by ros aggravates testis oxidative damage in diabetes via breaking the feedforward loop linking p62 and Nrf2. Oxid Med Cell Longev 2020: 7156579. DOI: 10.1155/2020/7156579.

Ventimiglia E, Capogrosso P, Serino A, Boeri L, Colicchia M, La Croce G, Scano R, Papaleo E, Damiano R, Montorsi F, Salonia A. 2017. Metabolic syndrome in White-European men presenting for secondary couple's infertility: An investigation of the clinical and reproductive burden. Andrology 4 (5): 944-951. DOI: 10.1111/andr.12232.

Yun JS, Ko SH. 2021. Current trends in the epidemiology of cardiovascular disease and cardiovascular risk management in type 2 diabetes. Metabolism 123: 154838. DOI: 10.1016/j.metabol.2021.154838.

Zawada AE. Moszak M. Skrzypczak, D. Grzymisławski M. 2018. Gastrointestinal complications in patients with diabetes mellitus. Adv Clin Exp Med 27 (4): 567-572. DOI: 10.17219/acem/67961.

ASIAN J NAT PROD BIOCHEM
Volume 22, Number 1, June 2024
Pages: 43-50

P-ISSN: 2775-4189
E-ISSN: 2775-4197
DOI: 10.13057/biofar/f220106

Antibacterial activity against multidrug-resistant *Salmonella*, toxicity and biochemical effects of *Moringa oleifera* leaf extracts in mice

MOSES NJUTAIN NGEMENYA[1,2,♥], LAMSON OTTOTOH ITOE[1], LAUPY ANNE AWAH[1], RODOLPH ASONGANA[1], RITA AYUK NDIP[1]

[1]Department of Biochemistry and Molecular Biology, Faculty of Science, University of Buea. P.O. Box 63, Buea, South West Region, Cameroon
[2]Department of Medical Laboratory Science, Faculty of Health Sciences, University of Buea. P.O. Box 63 Buea, South West Region, Cameroon
Tel.: +237-654-72-63-88, ♥email: njutain.moses@ubuea.cm

Manuscript received: 5 March 2024. Revision accepted: 10 June 2024.

Abstract. *Ngemenya MN, Itoe LO, Awah LA, Asongana R, Ndip RA. 2024. Antibacterial activity against multidrug-resistant* Salmonella*, toxicity and biochemical effects of* Moringa oleifera *leaf extracts in mice. Asian J Nat Prod Biochem 22: 43-50.* The emergence of multidrug resistance has significantly compromised the treatment of *Salmonella* infections. The anti-*Salmonella* activity, toxicity, and biochemical effects of *Moringa oleifera* Lam. leaf crude extracts were studied by disc diffusion and microdilution against Multidrug-Resistant (MDR) strains. Cytotoxicity was investigated on monkey kidney epithelial cells (LLCMK2) and acute toxicity in BALB/c mice. Both hexane (MO_{HEX}) and methanol (MO_{MET}) extracts produced small zones of inhibition of 8mm at 1 mg and 2 mg per disc, indicating weak activity, but the minimum inhibitory concentration showed high (0.0625 mg/mL) to low activity (10 mg/mL) activity. No minimum bactericidal concentration was recorded at the concentrations tested. The 50% cytotoxic concentration (CC_{50}) for methanol and hexane extracts were 2524 µg/mL and 5004 µg/mL, respectively, indicating a low risk of toxicity. No mortality or adverse effects were recorded in the acute toxicity test. Both extracts had no significant effects ($p < 0.05$) on renal function and one liver enzyme (alanine aminotransferase), but MO_{MET} significantly increased aspartate aminotransferase ($p = 0.04$), suggesting possible liver toxicity. The study shows that *M. oleifera* leaves possess bacteriostatic activity against multidrug-resistant *Salmonella* and are non-toxic; hence, it is a potential alternative treatment against multidrug-resistant *Salmonella*. Further studies of fractions and pure natural products of the extracts should be pursued.

Keywords: Antibacterial, biochemical, *Moringa oleifera*, multidrug-resistance, *Salmonella*, toxicity

INTRODUCTION

The morbidity and mortality of *Salmonella* Lignieres, 1900 infections, including typhoidal and non-typhoidal, are persistently high, particularly in developing countries. The global burden of *Salmonella* infections has been reported to be very high, resulting in over 10% mortality of all cases (He et al. 2023). In situations of poor hygiene, food and water are easily contaminated with *Salmonell*a, leading to the contraction of the infection via the oral and fecal routes. Managing severe infection cases requires antibiotics, given that a suitable preventive vaccine is unavailable.

Antibiotics initially used to treat *Salmonella* infections included penicillins, sulphamethoxazole–trimethoprim, chloramphenicol, and some macrolides. Due to the emergence of multidrug resistance in *Salmonella* to these antibiotics, other antibiotics, including fluoroquinolones, third-generation cephalosporins, and azithromycin, are currently being used to treat infections caused by multidrug-resistant *Salmonella* strains. However, recently, there have been reports of increasing resistance to the currently used antibiotics (Tack et al. 2020; Ndip et al. 2022) in both typhoidal and non-typhoidal *Salmonella* (Matic et al. 2018; Marchello et al. 2020; Park et al. 2021). Multidrug-Resistant (MDR) *Salmonella* is increasing with high levels in Africa and Asia, where extensively resistant strains have been detected (Marchello et al. 2020). Meanwhile, there is a high burden of invasive non-typhoidal *Salmonella* infections in sub-Saharan Africa mainly due to *Salmonella enterica* serovar Typhimurium with emerging extensive and pan-drug resistance (Puyvelde et al. 2023). Pursuing the discovery and development of efficacious anti-*Salmonella* therapies is imperative in this context. The approaches employed include synthetic medicinal chemistry, drug repurposing, combination therapy, study of natural products, and synthetic biology (Vila et al. 2020; Ahmed et al. 2023a).

Moringa oleifera Lam. (Moringaceae) is a tropical tree indigenous to India but has spread to Africa, Asia, and Southern America (van den Berg and Kuipers 2022). It is used in Indian traditional medicine in poultices and ointments to treat wounds, infections, and abscesses, and its leaves are a rich source of proteins. It is also used to improve memory and alertness, treat respiratory system ailments (asthma, sore throat), fevers, urinary tract infections, and diarrhea, as a food preservative and water purification; hence, it is termed a miracle tree due to its numerous properties (van den Berg and Kuipers 2022). An ethnobotanical survey in South Benin revealed various medicinal uses for infectious and non-communicable diseases involving all plant parts, with the leaves being the most used (Agoyi et al. 2017). Several parts of the plant, particularly the leaves, are widely used as food and in food recipes across Africa due to their rich nutrient content

(Matic et al. 2018). Studies of the plant have demonstrated anti-cancer, anti-diabetic, plasma lipid-lowering, tissue-protective, anti-inflammatory, and antimicrobial effects. These effects are due to its rich phytochemistry and nutrient contents (van den Berg and Kuipers 2022). Several studies have reported wide-ranging antibacterial activity for various parts of *M. oleifera*. A review of its antibacterial activity by (van den Berg and Kuipers 2022) reported that the leaf extract showed weak to moderate activity against Gram-negative bacteria (*Klebsiella pneumoniae* (Schroeter, 1886) Trevisan, 1887, *Proteus vulgaris* B, *Escherichia coli* E, *Pseudomonas aeruginosa* A); however, the methanol extract showed the best activity among the three solvents analyzed. A study reported high antibacterial activity against pyogenic multidrug-resistant bacteria isolated from camel abscesses (Fouad et al. 2019). Significant inhibition of the growth of food-borne pathogens, including *S. enterica* serovar Typhimurium, in preserved food by an aqueous extract of the leaves has been reported (Abdallah et al. 2023). In other studies, the aqueous and ethanolic extracts of *M. oleifera* leaves showed high activity against control strains of *Salmonella typhi* (Schroeter, 1886) Warren & Scott, 1930 and *S. enterica* (Ahmed et al. 2023b), while moderate activity was recorded for the ethanol extract only against multidrug-resistant clinical *Salmonella* isolates (Enerijiof et al. 2021). However, reports of studies conducted on characterized clinical multidrug-resistant bacteria, including *Salmonella*, are very rare. Considering the well-documented use of *M. oleifera* in ethnomedicine against a wide range of infectious diseases, this study aimed to investigate the activity of *M. oleifera* leaf crude extracts against multidrug-resistant clinical strains of *Salmonella*, which has not been extensively investigated. In addition, the investigation of the toxicity of the extracts was among the objectives of the study.

MATERIALS AND METHODS

Plant collection and preparation of extracts

The leaves of *M. oleifera* were collected in Kumba in the South West region of Cameroon. A voucher specimen was taken to the Limbe Botanic Garden, where it was authenticated by a botanist using the voucher specimen number SCA 7706. The fresh leaves were dried under a shaded area for 4 weeks and ground to a fine powder. The powder was weighed, giving a mass of 700.9 g. It was macerated sequentially, first by submerging it in hexane and then kept for 48 hours with occasional stirring. Then, the mixture was filtered through Whatman filter paper No.1, and the residue was dried at room temperature to constant mass to remove the hexane solvent. The filtrate was concentrated at 69°C in a rotary evaporator (BUCHI Rotavapor R-200, Switzerland). The dried residue was similarly macerated in methanol and concentrated at 40°C. The two extracts were dried to constant mass at room temperature to remove residual solvent and then stored at -20°C. Amounts of extract required for use were weighed from the stored stock.

Screening of phytochemical constituents

Standard chemical tests were done to detect the presence of phytochemicals (flavonoids, glycosides, saponins, tannins, steroids, phenols, alkaloids) in the extracts and their relative amounts, based on the color intensity of the test reaction mixture as described (Pant et al. 2017; Shaikh and Patil 2020).

Antibacterial screening

Sixteen (16) multidrug-resistant clinical isolates of *Salmonella* and two (2) control strains were used in this research. The clinical strains were isolated and characterized using culture, biochemical, and molecular analyses and stored in 50% glycerol in Muller Hinton (MH) broth (Liofilchem, Italy) at -20°C (Ndip et al. 2022). All assays were conducted under sterile conditions.

The antibacterial screening was initially done using a disc diffusion test as described (Mbah et al. 2012), with some modifications. Briefly, 100 and 200 mg of each extract were dissolved in 1mL of 0.5% dimethyl sulfoxide (DMSO), giving 100 and 200 mg/mL solutions. Each *Salmonella* strain was first cultured on nutrient agar to obtain a pure culture. Paper discs (6 mm in diameter) were cut out from Whatman filter paper No. 1 and sterilized by autoclaving, then 10 µL of extract solution containing 1 and 2 mg of extract were transferred onto separate discs and kept to dry. A 0.5 McFarland bacterial suspension (1.5×10^8 CFU/mL) was prepared by adding a pure colony to about 1 mL, then mixed and diluted to the required density with saline. Then, 100 µL of the suspension was spotted and spread on the surface of Mueller Hinton (MH) agar culture plates, followed by gently placing the extract discs on the bacterial spread. Positive control discs of gentamycin (10 µg) and ciprofloxacin (5 µg) and a negative control disc (10 µL of 0.5% DMSO) were included. The plate was incubated at 37°C (DHP-1952, England incubator) for 24 hours; the inhibition zone around each disc was first observed for the absence of any colonies, and the diameters of clear zones were measured using a millimeter scale.

The Minimum Inhibitory Concentration (MIC) was determined by microdilution (Ngemenya et al. 2022). Briefly, 40 mg of each extract was dissolved in 1 mL of 5% DMSO and then diluted to 20 mg/mL in MH broth. The extract solution was diluted to give double the required concentrations, then 75 µLof each solution was added into a 96-well microtitre plate in duplicate wells, followed by 75 µL of bacterial suspension (1×10^6 CFU/mL) in broth to obtain final extract concentrations of 10, 8, 6, 4, 2, 1, 0.5, 0.25, 0.125 and 0.0625 mg/ mL and cell density of 5×10^5 CFU/mL. Gentamycin or ciprofloxacin (50 µg/mL) and DMSO (2.5%) were positive and negative controls, respectively. The plate was observed visually, and optical densities (ODs) were read at 595 nm (Emax microplate reader, Molecular Devices, USA). The plate was incubated at 37°C for 24 hours, and the ODs were recorded after 24 hours. The percentage inhibition was calculated from the change (Δ) in OD using the formula (Ngemenya et al. 2022):

$$\%\text{Inhibition} = \frac{\Delta\text{OD Negative control} - \Delta\text{OD extract}}{\Delta\text{OD Negative control}} \times 100$$

MIC was taken as the lowest concentration of extract that produced at least ≥ 50% inhibition of bacterial growth. Minimum Bactericidal Concentration (MBC) was determined by spotting 10 µL of inhibited MIC well contents on nutrient agar on culture plates and incubating them at 37°C for 24 hours. The lowest concentration of the corresponding well without growth was considered the MBC.

Cytotoxicity test

This was done on monkey kidney epithelial cells, LLCMK2 Original (ATCC® CCL7™), (Virginia, USA), by the method of Ngemenya et al. (2019). Briefly, a complete culture medium, RPMI-1640 medium (CCM), was prepared with the addition of $NaHCO_3$, 25 mM Herpes, 0.3 g γ-irradiated L-glutamine powder, 10% heat-inactivated newborn calf serum, 200 units/mL penicillin and 200 µg/mL streptomycin and 0.25 µg/mL amphotericin B, and pH adjusted to 7.4. An incomplete medium (ICM, no calf serum) was also prepared similarly. The extract solution (2 mg/mL) was prepared in 4% DMSO in CCM. The cells were cultured in a T-shaped flask in a Heracell 150i (USA) incubator under conditions of 5% CO_2 and humidified air at 37°C. When cells grew to confluence, the medium was decanted, and the flask was washed off with ICM two times. The cells were dislodged with trypsin and centrifuged at 1000 rpm for 10 min (Eppendorf 5810R, Germany). Then, they were re-suspended in ICM and counted using a microscope (Nikon Eclipse TS100, China). The cells were diluted with CCM such that 3,000 cells in 100 µL were added in duplicate in a 96-well flat bottom microtitre plate. This was followed by incubation under the same conditions for 3 days for the cell to reach confluence. The extract was tested at 1 mg/mL by adding 100 µL of test solution. Wells containing auranofin (30 µM) and 2% DMSO were included as positive and negative controls, respectively. The plates were incubated at the same conditions for 5 days while well contents were examined for dead cells using a microscope daily to check for toxicity. An extract that showed toxicity was diluted to 15-1000 µg/mL and re-tested to determine the CC_{50}. After incubation, the medium was discarded, ICM was added to all wells, and the plate was shaken (IKA Labortechnik KS125 basic shaker) at 600 rpm for 5 minutes to wash off any colored solution. Then, 100 µL the cytotoxicity test reagent MTT formazan (5 mg/mL in ICM) was added into the required wells and then incubated for 30 minutes. The formazan precipitate formed in the MTT reduction reaction was dissolved by adding 100 µL DMSO.

Well contents were gently mixed by shaking, ODs were read at 595 nm, and percentage inhibitions were calculated using the formula below (Ngemenya et al. 2019):

$$\text{Percent inhibition (\%)} = \frac{\text{OD of control} - \text{OD of treatment}}{\text{OD of control}} \times 100$$

Acute toxicity

A stock solution of hexane extract was prepared by dissolving 25 mg in 60 µL of acetone to dissolve partially, and 250 µL DMSO was added for complete dissolution, followed by 690 µL of distilled water to give 25 mg/mL. The stock solution of the methanol crude extract was prepared similarly by adding 25 mg/mL in 1 mL of 2% DMSO (SIGMA, USA) and vortexed for complete dissolution. Corresponding control solutions without extract were prepared similarly. All solutions were stored at 4°C. The University of Buea granted the study ethical approval by the Institutional Animal Care and Use Committee (No. UB–IACUC No 13/2021). The guidelines of the Organization for Economic Cooperation and Development version 423 (OECD 2002) were followed concerning the dosage and handling of animals during the acute toxicity test, which was conducted according to the procedure of (Ngemenya et al. 2019). Briefly, nine-week-old mice were placed in three groups of five animals (the control and two test groups, with three females per group); the mice were acclimatized to the study conditions for one week before the test.

One animal per test group was weighed, fasted overnight with access to water only, and administered 2,000 mg/kg body weight extract orally in a 1 mL/100 g body weight volume using an oral gavage needle with a 22G ball tip. The treated animals were fasted further for 2 hours and observed for 24 hours with access to food. Following the survival of treated animals, the others were treated as above, and control solutions were also administered to corresponding animals. All animals were observed for gross changes as outlined (Ngemenya et al. 2019) for 14 days with access to food and water. After that, animals were weighed, fasted overnight, anesthetized with ketamine/xylazine (90/10 mg/kg), and blood was collected by retro-orbital bleeding into a dry Eppendorf tube. The blood was kept for 30 minutes to solidify, centrifuged at 2,000 rpm for 15 minutes (Eppendorf centrifuge 5702R), and the serum was analyzed for liver enzymes (alanine aminotransferase, ALT, and aspartate aminotransferase, AST) using commercial test kits (CHRONOLAB, Spain), following manufacturer's instructions. Kidney function tests (urea and creatinine) were performed similarly using Biorex Diagnostics (United Kingdom) kits.

Data and statistical analysis

A plot of the percentage inhibition of growth of bacteria against extract concentration in the microdilution assay was done using GraphPad Prism 5.0 (GraphPad Prism INC. USA) software, where the MIC (lowest concentration corresponding to 50% inhibition) was interpolated. The CC_{50} (cytotoxic concentration for 50% of cells) was determined using the same method as the MIC. Biochemical parameters of control and test animals were compared using GraphPad Prism 5.0 software, and an unpaired two-tailed t-test was used to check for any significant difference ($P < 0.05$).

RESULTS AND DISCUSSION

Yield and phytochemical composition of extracts

The amount of the hexane extract (30.9 g) obtained from the powder of the dried *M. oleifera* leaves gave a

higher yield of 4.4% (a percentage of the 700.9 g of powder used for the extraction) compared to the methanol extract (8.2 g), which gave a yield of 1.17%. From the phytochemical screening, both extracts had a similar content of secondary metabolites, with relatively high amounts of saponins and steroids and small amounts of tannins; smaller amounts of flavonoids and phenols were detected in the methanol extract only.

Antibacterial activity of extracts

In the disc diffusion test, both extracts produced small zones with diameters of 8 mm to 11 mm at 1 and 2 mg, respectively, which indicated weak activity against the 16 MDR clinical and control *Salmonella* strains. On the contrary, the gentamycin and ciprofloxacin positive control produced large inhibition zones ranging from 25 to 35 mm and 29 to 35 mm, respectively.

The MIC values of the hexane extract ranged from 0.0625 to 10 mg/mL against different *Salmonella* strains. In comparison, MIC values of the methanol extract ranged from 0.125 to 10 mg/mL (Table 1), showing varied activity against the MDR strains. According to an activity scale by (Cos et al. 2006), the hexane extract (MO_{HEX}) showed high activity (MIC = 0.0625 to 2 mg/mL) against 8 out of 18 strains of *Salmonella*, while the methanol extract (MO_{MET}), showed high activity against 4 strains only (Table 1). Hence, the hexane extract was more active than the methanol extract. The content of the MIC wells for both crude extracts, which showed inhibition, also showed bacterial growth (CFUs) on solid nutrient agar. Hence, no MBC was recorded within the concentration range tested.

Toxicity of extracts

Cytotoxicity

The CC_{50} on monkey kidney cells for both the hexane and methanol extracts of *M. oleifera* was 5004 and 2524 µg/mL, respectively, much higher than the cut–off value for lack of toxicity (CC_{50} > 30 µg/mL) (Ogbole et al. 2017). The relative selectivity of the extracts based on the lowest MIC values and the CC_{50} (determined from CC_{50}/MIC in µg/mL) is 80.0 and 40.3 for the hexane and methanol extracts, respectively.

Acute toxicity

Animals treated with methanol extract were sluggish and dizzy, whereas those treated with hexane extract had irritation of the eyes and nose within the first 2 hours post-treatment. No mortality was recorded on day 14, and food and water intake was similar to that of control mice. No adverse effects were observed. The average weights of mice increased in test and control groups for both extracts, with no significant difference for mice treated with methanol extract (p = 0.497) and hexane extract (p = 0.2838).

Table 1. Minimum inhibitory concentrations of *Moringa oleifera* leaf extracts against multidrug-resistant *Salmonella* isolates

MIC Value (mg/mL)	No. of Isolates MO_{HEX}	No. of Isolates MO_{MET}	Activity
0.0625	1	0	High
0.125	1	3	
0.25	1	0	
1	1	0	
2	4	1	
4	0	6	Moderate
6	3	5	
8	2	2	Low
10	5	1	
Total	18	18	

Note: Interpretation of MIC values (mg/mL): ≤ 2: High activity; > 2 and < 6: Moderate activity; > 6: low activity (Cos et al. 2006). Extracts: MO_{HEX} and MO_{MET}: Hexane methanol extracts of *M. oleifera*. MICs: Minimum Inhibitory Concentrations

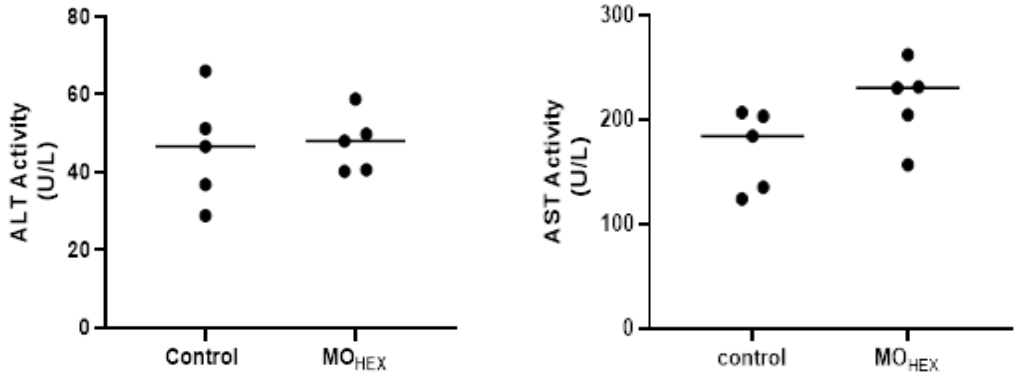


Figure 1. Effect of 2000 mg/kg hexane extract of *Moringa oleifera* (MO_{HEX}) on mouse liver enzyme activity in BALB/c ALT: Alanine aminotransferase (P = 0.831); AST: Aspartate aminotransferase (P = 0.0976)

Effect of extracts on liver and renal functions

The hexane extract (MO_{HEX}) did not affect alanine aminotransferase (ALT) but caused an increase in aspartate aminotransferase (AST). However, there was no significant difference in ALT and AST levels between the control mice and those treated with the hexane extract (p = 0.831 and 0.0976, respectively), as shown in Figure 1. The AST: ALT ratio of the mean enzyme activity for the test group was 0.22 (< 1), indicating no adverse effect in the liver. The methanol extract (MO_{MET}) produced the same pattern of effects on liver enzymes as MO_{HEX}. Still, there was also no significant difference in ALT levels between the control mice and the test group administered the methanol extract (MO_{MET}) (p = 0.824). However, there was a significant difference in AST level (p = 0.045) (Figure 2) and AST: ALT ratio of 5.7, which indicates adverse effects on the liver.

MO_{HEX} caused a slight increase in urea and a decrease in creatinine. Still, the difference between treated mice and the control group was not significant (p = 0.639 and 0.051, respectively), as shown in Figure 3. Meanwhile, MO_{MET} produced an increase in both urea and creatinine. Still, there was no significant difference in urea and creatinine levels between the treated (MO_{MET}) and a control group of mice, with p-values of 0.394 and 0.118, respectively (Figure 4).

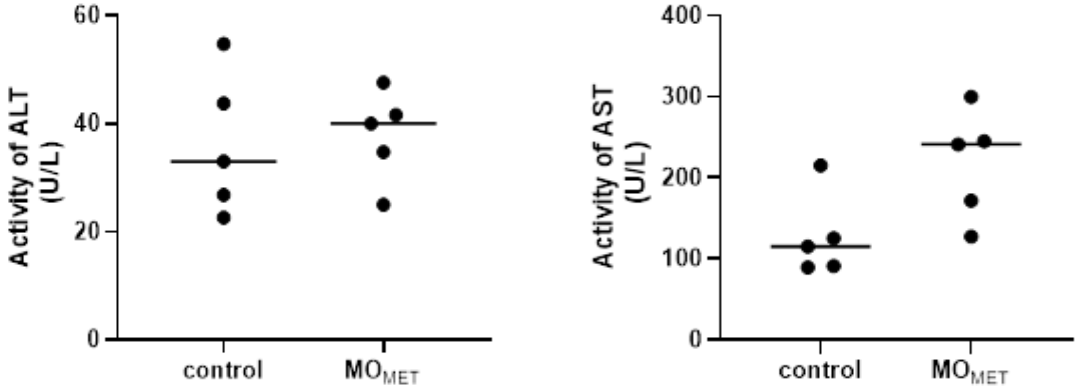


Figure 2. Effect of 2000 mg/kg methanol extract of *Moringa oleifera* (MO_{MET}) on mouse liver enzyme activity in BALB/c. ALT: Alanine aminotransferase (P = 0.824); Aspartate aminotransferase (P = 0.0456)

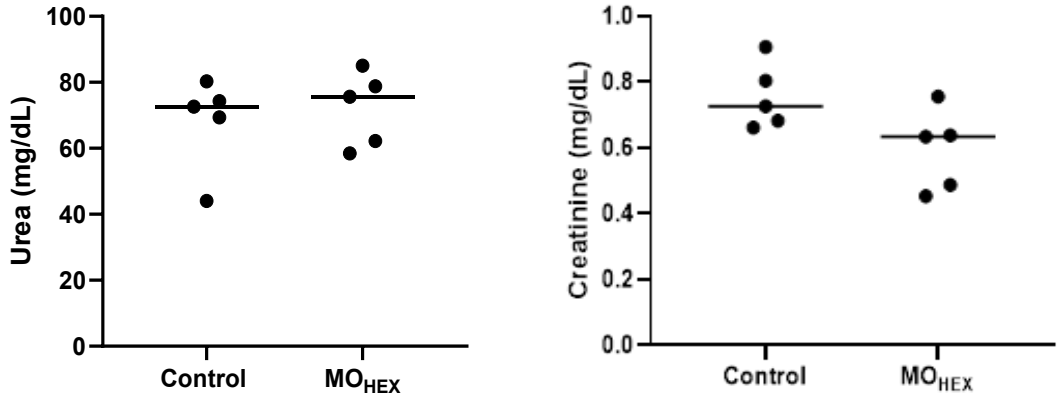


Figure 3. Effect of 2000 mg/kg hexane extract of *Moringa oleifera* (MO_{HEX}) on renal function in BALB/c mice. Urea (P = 0.639); Creatinine (P = 0.051)

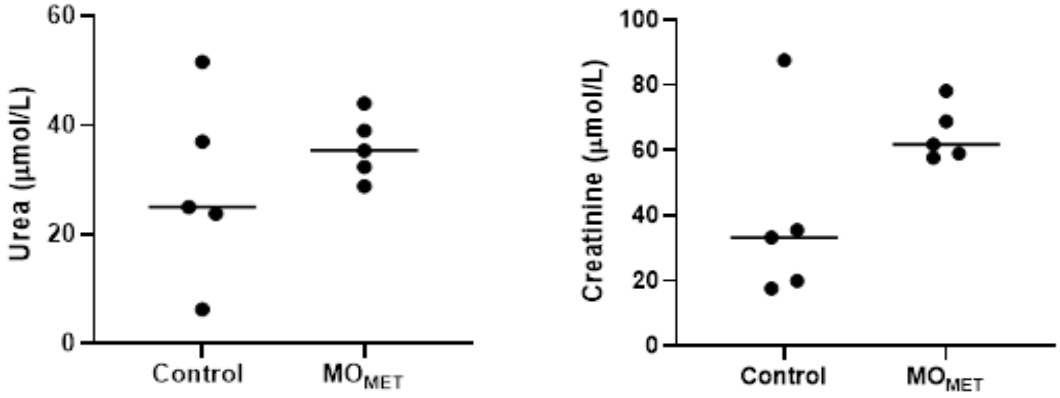


Figure 4. Effect of 2000 mg/kg methanol extract of *Moringa oleifera* (MO_{MET}) on renal function in BALB/c mice. Urea (P = 0.394); Creatinine (P = 0.118)

Discussion

As mentioned above, *M. oleifera* is widely used in traditional medicine to treat bacterial infections. Several reports on the plant's antibacterial activity support the traditional use. However, studies on its activity against MDR *Salmonella* are very rare. This study found high bacteriostatic activity against some clinical strains of MDR *Salmonella* and no adverse toxicity of the leaf extracts in mice. These findings constitute evidence to support the use of *M. oleifera* leaves in traditional medicine to treat infections caused by different bacteria. Furthermore, the result suggests that this plant's leaves are a potential alternative treatment to counter resistant *Salmonella* infections and, therefore, require further exploration. The hexane extract produced the highest activity against a higher number of MDR *Salmonella* isolates in the microdilution assay compared to the methanol extract; hence, the hexane extract carries a higher potential to counter resistance in *Salmonella*. This high activity is likely due to non-polar secondary metabolites present in the extract. Both extracts showed dose-dependent activity as more isolates were inhibited at higher MICs, and all 18 isolates were inhibited within the extract concentration range tested. No MBC was recorded within the concentration range tested, suggesting that the extracts are bacteriostatic; this points to the mechanism of action by inhibition of protein synthesis in the bacterial cell (Halawa et al. 2024). However, the actual mechanism of action needs to be investigated. The low activity recorded in the disc diffusion test could be due to several factors, including a low amount of bioactive secondary metabolites in the extract embedded in the disc or due to the well-known limitations of the disc test, such as poor diffusion of secondary metabolites in the agar medium among others (Bubonja-Šonje et al. 2020). The wide range in the MIC values is likely due to differences in the magnitude of resistance of the clinical *Salmonella* strains used; the strains were multidrug-resistant but had differences in their resistance gene as reported following molecular characterization (Ndip et al. 2022). Some studies have reported high antibacterial activity of *M. oleifera* leaf extract against *Salmonella*. In one study (Abdallah et al. 2023), an aqueous extract of the leaves significantly reduced the counts of spoilage bacteria in meatballs and other bacteria, including *S. typhimurium* inoculated in meatballs, making it a potentially natural, safer alternative preservative for meatballs as opposed to chemical preservatives. On the contrary (Anzano et al. 2022) reported that both polar and apolar extracts of the leaves and seeds showed weak activity against Gram-negative bacteria, *P. aeruginosa* and *S. enterica*, but the apolar extract of the seeds showed high activity against Gram-positive pathogens. However, unlike in this study, the two studies cited above did not specify the antibiotic susceptibility or resistance of the bacterial strains used. To explore the activity of M. oleifera fully, in vivo efficacy and further toxicity studies should be conducted to obtain more data that support its use. Studies in combination with other medications or treatments may reveal enhanced activity. To generalize these findings, the extracts should also be screened against strains of *Salmonella* found in other populations or regions.

Both extracts had very high selectivity index values, indicating that they are not likely to be toxic at relatively high doses required to inhibit the most resistant strains with the highest MIC values. The acute toxicity test did not show any adverse toxicity both macroscopically and in terms of biochemical changes, except for the significant increase in AST with a high AST:ALT ratio recorded for the methanol extract, which suggests severe liver damage. However, no mortality occurred in the mice treated with the methanol extract, suggesting any liver damage that occurred was reversible. Overall, the toxicity findings suggest that the leaves are not likely to produce adverse toxicity in humans when used in traditional medicine, although this depends on the dose administered. A work by (Asare et al. 2012) reported that the aqueous extract of the leaves was cytotoxic at a high dose of 20 mg/mL on human peripheral blood mononuclear cells, which is much higher than the higher CC_{50} of 5 mg/mL and the highest MIC of

10 mg/mL recorded in this study. Interestingly, in this same study, an acute toxicity test of the extract did not cause liver or kidney toxicity and no hematological abnormalities at up to 3,000 mg/kg in Sprague-Dawley rats. Still, the leaf extract was genotoxic at this high dose. Also, de Barros et al. (2022) reported no abnormality in an acute toxicity test in Swiss female albino mice for *M. oleifera* leaf infusion or powder at 5,000 mg/kg. Still, they observed liver and kidney damage in a 28-day repeated dose toxicity test at a low dose of 500 mg/kg. The differences in the findings of other studies are likely due to the different cell lines, animal species used, doses and types of toxicity tests. However, similar to other studies, there is a likelihood of negligible toxicity at relatively low potent doses with a high selectivity index. Even though *M. oleifera* did not show adverse toxicity in the liver and kidney, the toxicity in other organs needs to be investigated.

In terms of strength, to the best of our knowledge, this study is likely the first to report on the antibacterial activity of the leaves of *M. oleifera* against MDR *Salmonella* strains. The findings suggest that *M. oleifera* could be used as an alternative treatment with the potential impact of decreasing the morbidity and mortality of *Salmonella* infections. However, the use of this plant as an alternative medicine should be controlled by experts in phytomedicine to avoid the emergence of resistance. As a limitation, other parts of the plant were not studied; this should be done by including factors that may affect its activity, such as environmental conditions, harvesting and processing techniques. Also, MDR strains of *Salmonella* found in other populations or regions, as well as those of other bacterial species, should be studied.

In conclusion, this study has shown considerable bacteriostatic anti-*Salmonella* activity of the leaves of *M. oleifera* against MDR strains and a low risk of toxicity at effective doses. Hence, the leaves are a potential alternative treatment for antibiotic-resistant infections of *Salmonella* and a possible source of an antibacterial lead. The findings support the use of this plant in the traditional treatment of bacterial infections; hence, further studies should be carried out on other resistant bacteria, and the anti-*Salmonella* activity should be investigated in an *in vivo* model.

ACKNOWLEDGMENTS

We acknowledge the Biotechnology Unit of the Faculty of Science, University of Buea, Cameroon, for technical and material assistance.

REFERENCES

Abdallah R, Mostafa NY, Kirrella GAK, Gaballah I, Imre K, Morar A, Herman V, Sallam KI, Elshebrawy HA. 2023. Antimicrobial effect of *Moringa oleifera* leaves extract on food-borne pathogens in ground beef. Foods 12: 766. DOI: 10.3390/foods12040766.

Agoyi EE, Okou FAY, Assogbadjo EA, Sinsin B. 2017. Medicinal uses of *Moringa oleifera* in Southern Benin (West Africa). Acta Hortic 1158: 303-308. DOI: 10.17660/ActaHortic.2017.1158.34.

Ahmed M, Marrez DA, Abdelmoeen NM, Mahmoud EA, Ali M A-S, Decsi K, Tóth Z. 2023b. Proximate analysis of *Moringa oleifera* leaves and the antimicrobial activities of successive leaf ethanolic and aqueous extracts compared with green chemically synthesized Ag-NPs and crude aqueous extract against some pathogens. Intl J Mol Sci 24: 3529. DOI: 10.3390/ijms24043529.

Ahmed S, Ahmed MZA, Rafique S, Almasoudi SE, Shah M, Jalil NAC, Ojh SC. 2023a. Recent approaches for downplaying antibiotic resistance: Molecular mechanisms. BioMed Res Intl 2023: 5250040. DOI: 10.1155/2023/5250040.

Anzano A, de Falco B, Ammar M, Ricciardelli A, Grauso L, Sabbah M, Capparelli R, Lanzotti V. 2022. Chemical analysis and antimicrobial activity of *Moringa oleifera* Lam. leaves and seeds. Molecules 27: 8920. DOI: 10.3390/molecules27248920.

Asare GA, Gyan B, Bugyei K, Adjei S, Mahama R, Addo P, Otu-Nyarko L, Wiredu EK, Nyarko A. 2012. Toxicity potentials of the nutraceutical *Moringa oleifera* at supra-supplementation level. J Ethnopharmacol 139 (1): 265-272. DOI: 10.1016/j.jep.2011.11.009.

Bubonja-Šonje M, Knežević S, Abram M. 2020. Challenges to antimicrobial susceptibility testing of plant derived polyphenolic compounds. Arh Hig Rada Toksikol 71: 300-311. DOI: 10.2478/aiht-2020-71-3396.

Cos P, Vlietinck AJ, Berghe DV, Maes L. 2006. Anti-infective potential of natural products: How to develop a stronger *in vitro*' proof-of-concept'. J Ethnopharmacol 106: 290-302. DOI: 10.1016/j.jep.2006.04.003.

de Barros MC, Silva AGB, Souza TG-S et al. 2022. Evaluation of acute toxicity, 28-day repeated dose toxicity, and genotoxicity of *Moringa oleifera* leaves infusion and powder. J Ethnopharmacol 296: 115504. DOI: 10.1016/j.jep.2022.115504.

Enerijiof KE, Akapo FH, Erhabor JO. 2021. GC–MS analysis and antibacterial activities of *Moringa oleifera* leaf extracts on selected clinical bacterial isolates. Bull Natl Res Cent 45: 179. DOI: 10.1186/s42269-021-00640-9.

Fouad EA, Elnaga ASMA, Kandil MM. 2019. Antibacterial efficacy of *Moringa oleifera* leaf extract against pyogenic bacteria isolated from a dromedary camel (*Camelus dromedarius*) abscess. Vet World 12 (6): 802-808. DOI: 10.14202/vetworld.2019.802-808.

Halawa EM, Fadel M, Al-Rabia MW, Behairy A, Nouh NA, Abdo M, Olga R, Fericean L, Atwa AM, El-Nablaway M, Abdeen A. 2024. Antibiotic action and resistance: updated review of mechanisms, spread, influencing factors, and alternative approaches for combating resistance. Front Pharmacol 14: 1305294. DOI: 10.3389/fphar.2023.1305294.

He Y, Wang J, Zhang R, Chen L, Zhang H, Qi X, Chen J. 2023. Epidemiology of food-borne diseases caused by *Salmonella* in Zhejiang Province, China, between 2010 and 2021. Front Public Health 11: 1127925. DOI: 10.3389/fpubh.2023.1127925.

Marchello CS, Carr SD, Crump JA. 2020. A systematic review on antimicrobial resistance among *Salmonella typhi* worldwide. Am J Trop Med Hyg 103 (6): 2518-2527. DOI: 10.4269/ajtmh.20-0258.

Matic I, Guidi A, Kenzo M, Mattei M, Galgani A. 2018. Investigation of medicinal plants traditionally used as dietary supplements: A review on *Moringa oleifera*. J Public Health Afr 9: 841. DOI: 10.4081/jphia.2018.841.

Mbah JA, Ngemenya MN, Abawah AL, Babiaka SB, Nubed LN, Nyongbela KD, Lemuh ND, Efange SMN. 2012. Bioassay-guided discovery of antibacterial agents: In vitro screening of *Peperomia vulcanica*, *Peperomia fernandopoioana* and *Scleria striatinux*. Ann Clin Microbiol Antimicrob 11:10. DOI: 10.1186/1476-0711-11-10.

Ndip RA, Awah LA, Ghogomu SM, Cho-Ngwa F, Ngemenya MN. 2022. Isolation and molecular identification of *Salmonella* with high multidrug resistance to first line typhoid antibiotics in Southwest Cameroon. Microbes Infect Dis 3 (4): 988-997. DOI: 10.21608/mid.2022.122411.1249.

Ngemenya MN, Asongana R, Zofou D, Ndip RA, Itoe LO, Babiaka SB. 2022. In vitro antibacterial potential against multidrug-resistant *Salmonella*, cytotoxicity, and acute biochemical effects in mice of *Annona muricata* leaf extracts. J Evid Based Complement Altern Med 2022: 3144684. DOI: 10.1155/2022/3144684.

Ngemenya MN, Djeukem GGR, Nyongbela KD, Bate PNN, Babiaka SB, Monya E, Kanso RK. 2019. Microbial, phytochemical, toxicity analyses and antibacterial activity against multidrug-resistant bacteria of some traditional remedies sold in Buea Southwest Cameroon. BMC Complement Altern Med 19: 150. DOI: 10.1186/s12906-019-2563-z.

Ogbole OO, Segun PA, Adeniji AJ. 2017. In vitro cytotoxic activity of medicinal plants from Nigeria ethnomedicine on Rhabdomyosarcoma

cancer cell line and HPLC analysis of active extracts. BMC Complement Altern Med 17: 494. DOI: 10.1186/s12906-017-2005-8.

Organization for Economic Cooperation and Development (OECD). 2002. Test No. 423: Acute oral toxicity - acute toxic class method. OECD Guidelines for the Testing of Chemicals, OECD Publishing, Paris. DOI: 10.1787/9789264071001-en.

Pant DR, Pant ND, Saru DB, Yadav UN, Khanal DP. 2017. Phytochemical screening and study of antioxidant, antimicrobial, anti-diabetic, anti-inflammatory and analgesic activities of extracts from stem wood of *Pterocarpus marsupium* Roxburgh. J Intercult Ethnopharmacol 6: 170-176. DOI: 10.5455/jice.20170403094055.

Park SE, Pham DT, Pak GD, Panzner U, Maria Cruz Espinoza L, von Kalckreuth V, Im J, Mogeni OD, Schütt-Gerowitt H, Crump JA, Breiman RF, Adu-Sarkodie Y, Owusu-Dabo E, Rakotozandrindrainy R, Bassiahi Soura A, Aseffa A, Gasmelseed N, Sooka A, Keddy KH, May J, Aaby P, Biggs HM, Hertz JT, Montgomery JM, Cosmas L, Olack B, Fields B, Sarpong N, Razafindrabe TJL, Raminosoa TM, Kabore LP, Sampo E, Teferi M, Yeshitela B, El Tayeb MA, Krumkamp R, Dekker DM, Jaeger A, Tall A, Gassama A, Niang A, Bjerregaard-Andersen M, Løfberg SV, Deerin JF, Park JK, Konings F, Carey ME, Van Puyvelde S, Ali M, Clemens J, Dougan G, Baker S, Marks F. 2021. The genomic epidemiology of multidrug resistant invasive non-typhoidal *Salmonella* in selected Sub-Saharan African countries. BMJ Glob Health 6: e005659. DOI: 10.1136/bmjgh-2021-005659.

Puyvelde SV, de Block T, Sridhar S, Bawn M, Kingsley RA, Ingelbeen B, Beale MA, Barbé B, Jeon HJ, Mbuyi-Kalonji L, Phoba MF, Falay D, Martiny D, Vandenberg O, Affolabi D, Rutanga JP, Ceyssens PJ, Mattheus W, Cuypers WL, van der Sande MAB, Park SE, Kariuki S, Otieno K, Lusingu JPA, Mbwana JR, Adjei S, Sarfo A, Agyei SO, Asante KP, Otieno W, Otieno L, Tahita MC, Lompo P, Hoffman IF, Mvalo T, Msefula C, Hassan-Hanga F, Obaro S, Mackenzie G, Deborggraeve S, Feasey N, Marks F, MacLennan CA, Thomson NR, Jacobs J, Dougan G, Kariuki S, Lunguya O. 2023. A genomic appraisal of invasive *Salmonella* Typhimurium and associated antibiotic resistance in sub-Saharan Africa. Nat Commun 14: 6392. DOI: 10.1038/s41467-023-41152-6.

Shaikh JR, Patil MK. 2020. Qualitative tests for preliminary phytochemical screening: An overview. Intl J Chem Stud 8 (2): 603-608. DOI: 10.22271/chemi.2020.v8.i2i.8834.

Tack B, Jolien Vanaenrode J, Verbakel JY, Toelen J, Jacobs J. 2020. Invasive non-typhoidal *Salmonella* infections in sub-Saharan Africa: A systematic review on antimicrobial resistance and treatment. BMC Med 18: 212. DOI: 10.1186/s12916-020-01652-4.

van den Berg J, Kuipers S. 2022. The antibacterial action of *Moringa oleifera*: A systematic review. S Afr J Bot 151: 224233. DOI: 10.1016/j.sajb.2022.09.034.

Vila J, Moreno-Morales J, Balleste-Delpierre C. 2020. Current landscape in the discovery of novel antibacterial agents. Clin Microbiol Infect 26 (5): 596-603. DOI.org/10.1016/j.cmi.2019.09.015.