Journal of Medical Bacteriology

Exploring the Phytochemical Profile and Antimicrobial Potential of Leaf Extracts from *Megaphrynium macrostachyum*

Salisu Danjuma Ibrahim [1], Olatomiwa Olubunmi Ariyo [2], Aireguamen I. Aigbodion [3], Ikhazuagbe Hilary Ifijen [4*], Oyiguh Joseph Abraham [5], Ruth Foluke Aminu [5], Benjamin Ewanole Ohiocheoya [1], Philip Okiemute Igbako [1], Ndah Sumaila Akpala [5]*

1 Department of Research Operations, Crop Protection Division, Plant Pathology and Microbiology Unit, Rubber Research Institute of Nigeria.

2 Department of Microbiology, Federal University Oye-Ekiti State, Nigeria.

3 Department of Physical Sciences, Benson Idahosa University, Benin, Edo State, Nigeria.

4 Department of Research Outreach, Rubber Research Institute of Nigeria, Iyanomo, Benin City, Nigeria.

5 Department of Microbiology, Faculty of Natural Sciences, Kogi State University, Anyigba, Nigeria.

ARTICLE INFO

Article type:
Research Article

Article history:
Received: 02 Sep 2024
Revised: 29 Sep 2024
Accepted: 20 Oct 2024
Published: 09 Nov 2024

Keywords:
Antimicrobial, Medicinal, Megaphrynium macrostachyum leaves, Phytochemical, plants.

ABSTRACT

Background: Medicinal plants offer a promising reservoir of bioactive compounds, placing them as a compelling avenue for novel drug exploration. In recent times, the emphasis on harnessing natural products sourced from medicinal plants has escalated due to their diminished adverse effects, economic viability, and efficacy against a broad spectrum of antibiotic-resistant microorganisms. The aim of this investigation was to scrutinize the phytochemical constitution and antimicrobial efficacy inherent to Megaphrynium macrostachyum leaves.

Methods: Three distinct solvents – ethanol, water-ethanol, and water – were employed to extract the diverse range of phytochemicals housed within the leaves. Subsequently, the extracted compounds were subjected to assessment for their antimicrobial potential against both bacteria and fungi, which were isolated from various samples. This evaluation was executed employing the agar well diffusion method.

Results: The qualitative analysis of phytochemical components unveiled the substantial presence of alkaloids, terpenoids, phenols, tannins, flavonoids, while a relatively lower occurrence of steroids was observed across the different leaf extracts. Further quantitative analysis showed that the most potent extract exhibited elevated phenolic content (2.400 mg/ml), closely trailed by flavonoids (1.995 mg/ml) and saponins (1.909 mg/ml). This study furnishes compelling proof of the efficacy encompassed within *Megaphrynium macrostachyum* leaves, particularly concerning their proficiency against both fungi and bacteria.

Conclusion: As such, it adds momentum to the accumulating body of knowledge concerning the exploitation of natural medicinal plants, paving the way for enhanced therapeutic interventions.

- ***Please cite this paper as:*** Ibrahim SD, Ariyo OO, Aigbodion AI, Ifijen IH, Abraham OJ, Aminu RF, Ohiocheoya BE, Igbako PO, Akpala NS. Exploring the Phytochemical Profile and Antimicrobial Potential of Leaf Extracts from *Megaphrynium macrostachyum*. *J Med Bacteriol.* 2024; **12** (4): pp.31-43.

*Corresponding Authors:

Salisu Danjuma Ibrahim: Department of Research Operations, Crop Protection Division, Plant Pathology and Microbiology Unit, Rubber Research Institute of Nigeria.

Tel: +80-31-911038, *E-mail:* daniel.ibrahim2@gmail.com

Ikhazuagbe Hilary Ifijen: Department of Research Outreach, Rubber Research Institute of Nigeria, Iyanomo, Benin City, Nigeria.

Tel: +80-36-426776, *E-mail:* larylans4u@yahoo.com

Introduction

In the realm of medical science, the untapped wealth of medicinal plants holds immense promise as a reservoir of natural compounds. Over the past two decades, significant attention has been drawn towards these botanical treasures, owing to their potential to yield novel drugs enriched with bioactive secondary metabolites such as alkaloids, phenolics, flavonoids, saponins, terpenoids, and tannins (1). This resurgence is particularly pertinent as conventional antimicrobial drugs face an escalating challenge from increasingly resistant microbes (1).

Medicinal plants, distinguished by their therapeutic properties, have a historical lineage tracing back century. Within the vast expanse of Earth's plant species—estimated between 250,000 to 500,000—only a fraction, ranging from 1% to 10%, contribute to human and animal sustenance (2). Yet, it's conceivable that an even larger proportion holds medicinal value (3). The legacy of medicinal plants dates back to Hippocrates, who cataloged 300 to 400 such plants in the fifth century B.C (4). Dioscorides continued this tradition in the first century A.D. with "De Materia Medica," a groundbreaking pharmacopoeial prototype. Even the Bible bears testament to around 30 plants with healing attributes, underscoring the enduring significance of botanical remedies (5).

In the annals of time, civilizations rose and fell, occasionally impeding the progress of understanding medicinal flora. However, certain gems persisted. For instance, frankincense and myrrh, revered for their medicinal properties, were valued not only for their rarity but also for their antiseptic traits, finding use even as mouthwashes. Unfortunately, many records of plant-based pharmaceuticals were lost or destroyed along with the fall of ancient empires, impeding Western advances in this domain (5).

With mounting concerns about the side effects and limited efficacy of synthetic drugs, medicinal plants have emerged as an appealing alternative. These natural products often exhibit fewer adverse effects, cost-effectiveness, and effectiveness against a broad spectrum of antibiotic-resistant microorganisms (6). Exploring different extracts from medicinal plants has unveiled their therapeutic potential (7), leading to the approval of certain natural products as new antibacterial agents. Nonetheless, the urgency to identify fresh substances that combat highly resistant pathogens remains palpable (8).

Notably, the realm of edible plants and their derivatives extends beyond sustenance into the domain of traditional medicine, frequently leveraging their inherent antimicrobial properties. Researchers have delved into the inhibitory capabilities of various edible plant extracts against bacteria and fungi that contribute to food spoilage (9).

Defined by the World Health Organization as experiential knowledge rooted in generations, traditional medicine encompasses practices exclusively aimed at restoring physical and mental equilibrium (10). *M. macrostachyum*, a member of the *Marantaceae* family, flourishes in the rainforests of West and Central Africa 11. This perennial semi-woody herb, characterized by its rhizomatous nature, serves as a remarkable specimen. With tall stems hosting a large single leaf measuring 30-60 cm long and 12-30 cm wide, it bears whitish flowers with reddish or purple calyxes on its petioles beneath the leaf (12).

Inhabitants of these regions harness the papery leaves of *M. macrostachyum* for various purposes. From wrapping foodstuffs like tapioca roots, kolanut, and meat for preservation, to employing them as roofing material and packing resources (13), this plant is deeply woven into the cultural fabric.

The crux of this study rests in unraveling the phytochemical intricacies and antimicrobial potential concealed within the leaf extracts of *M. macrostachyum*. By delving into this uncharted territory, we aspire to illuminate new vistas of

knowledge, bridging the ancient wisdom of botanical remedies with the cutting-edge frontiers of modern medicine (14).

Materials and Methods

Sample collection

The leaves of *M. macrostachyum* were sourced from the Rubber Research Institution of Nigeria in Iyanomo, situated in Benin City within the Ikpoba Okha local government of Edo state. Subsequently, the leaves were subjected to a thirty-day period of air-drying at room temperature, following which they were carefully crushed using a pestle and mortar.

Preparation of plant extract

The method for preparing the plant extract was adapted from a previous research endeavor (11). To generate the extracts, dry powder was used, and three variations were created: a water extract, a water-ethanol extract (50/50, V/V), and an ethanol extract. The process involved measuring 25 g of the powdered sample into separate sterile beakers. Subsequently, 250 ml of the appropriate solvent mixture was introduced, and the amalgamations were subjected to continuous agitation at room temperature (25-30 °C) for a duration of 24 hours. The ensuing mixtures underwent filtration using Whatman N°1 filter paper, and any residual solvents were meticulously eliminated under low-pressure conditions. The ensuing extracts were then concentrated in preparation for quantitative analysis of alkaloids, flavonoids, saponins, tannins, phenols, and terpenoids, a process carried out using a spectrophotometer.

Quantitative analysis

The methodology for assessing tanins was conducted in accordance with the procedure outlined in a previous study (15). A volume of twenty cubic centimeters (20 cm^3) of a 10% acetic acid in ethanol solution was accurately measured and dispensed into sterile test tubes. Subsequently, a precise quantity of 2.50 grams of the extract was added to the test tubes. The resulting mixture was allowed to stand undisturbed for a duration of four hours, after which it underwent a filtration process. The concentrated extract was then subjected to evaporation on a water bath until its volume was reduced by one-fourth. Following this, fifteen drops of concentrated ammonia were added to the extract, and the mixture was left undisturbed for a period of three hours. Subsequent to this incubation period, the mixture was filtered once again and then washed with a solution of twenty cubic centimeters (20 cm^3) of 0.1M Ammonium Hydroxide. Before the final filtration, the weight of the filter paper was carefully recorded as a baseline. After drying, the filter paper was weighed again to determine the final weight, enabling the calculation of the alkaloid content.

For flavonoids one gram of leaf extract was precisely measured and introduced into sterilized test tubes. Subsequently, a mixture of 5 cm3 of Folin-Ciocalteu reagent and 1 cm3 of saturated Na_2CO_3 solution was meticulously added to the test tubes containing the extract. Additionally, 5 ml of distilled water was dispensed into each of these tubes housing the extract. The ensuing solution was permitted to stand undisturbed for a span of 30 minutes within a water bath set at a temperature of 25 °C. At the culmination of this incubation period, the optical density was ascertained by measuring the absorbance at 700 nm (15).

For flavonoids the procedure detailed in a previous investigation was followed (15). Each step was meticulously executed to ensure accurate results. For each analysis, a precise amount of 0.5 grams of the sample extract was weighed and introduced into individual sterile test tubes. Subsequently, 0.1 ml of 10% aluminum chloride solution was added to each test tube, followed by the careful addition of 0.1 ml of potassium acetate. To attain a total volume of 5 ml in each test tube,

4.3 ml of 80% methanol was added. Once the solutions were prepared, the absorbance was measured at a specific wavelength of 512 nm.

The procedure used in evaluating cardiac glycosides was implemented in accordance with the protocols detailed in a previous study (15). Each step was meticulously carried out to ensure accuracy and consistency. For each analysis, a measured portion comprising 20% of the sample extract (2 grams) was weighed and introduced into individual sterile test tubes. Subsequently, 10 ml of freshly prepared Barjert reagent was added to each tube and allowed to stand for a duration of 1 hour. After the incubation period, 20 ml of diluted distilled water was introduced into each test tube. Subsequently, the absorbance of the sample was measured at a specific wavelength of 495 nm.

For the evaluation of saponins was conducted in accordance with the procedures outlined in a previous study (15). This systematic process was meticulously followed to ensure accurate and reliable results. For each analysis, a precisely weighed quantity of 0.5 grams of the sample was introduced into individual test tubes. Following this, 5 ml of distilled water was added to each tube, followed by the addition of 5 ml of Vatrillin reagent. To ensure proper mixing, 2.5 ml of 72.5% H_2SO_4 was introduced into each tube and mixed thoroughly. The resulting solution was then placed in a water bath maintained at 60 °C for a period of 10 minutes. Subsequently, the absorbance of the solution was measured at a specific wavelength of 544 nm.

For phenols the procedures outlined in a previous investigation (15) was performed. This systematic process was meticulously adhered to in order to ensure accuracy and reliability. For each analysis, a precisely weighed quantity of 0.5 grams of the sample extract was introduced into individual sterile test tubes. Following this, 5 ml of distilled water was added to each tube. Subsequently, 2 ml of 0.1N ammonium hydroxide was introduced into the mixture, and an additional 2 ml of water was added. The resulting solution was left undisturbed for a duration of 30 minutes. Following the incubation period, the absorbance reading of the solution was taken at a specific wavelength of 505 nm.

Antimicrobial susceptibility tests

The test organisms were sourced from the Microbiology Laboratory at Kogi State University, Anyigba, Nigeria. The selected organisms comprised *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger*.

Preparation of Inoculum

The methodology for assessing alkaloids was executed following the protocols delineated in a previous investigation (11). For the inoculation of the prepared Mueller Hinton Agar plates, an inoculum was prepared to adhere to the 0.5 McFarland's standard. Subsequently, 0.2 ml of this inoculum was evenly spread on the plates. Each plate had five wells designated for different concentrations of extracts, a positive control, a positive control (Cephaloxin and Ketoconazole), and a negative control, all of which had been pre-bored. Following the preparation of the plates, a waiting period of 15 minutes was observed before the extracts were introduced into the designated wells. The test organisms employed for this evaluation included *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger*, and *Candida albicans*.

Inoculation

In each of the designated wells, a volume of 0.2 ml from the prepared concentrations of the extract—50%, 100%, and 150%—was carefully introduced. Additionally, the remaining two wells were allocated for distinct purposes: one containing the positive control (Ciprofloxacin for bacterial plates and Ketoconazole for fungal

plates), and the other serving as the negative control filled with water (16).

Subsequently, the prepared plates were subjected to an incubation period of 18 hours at a temperature of 35 °C within an incubator. After the incubation period, the zones of inhibition present on the plates were meticulously measured in millimeters, and the recorded measurements were associated with each extract, control, bacterium, and fungus that were tested.

Minimum Inhibitory Concentration (MIC)

The determination of the Minimum Inhibitory Concentration (MIC) for each plant extract exhibiting antimicrobial activity was carried out, employing a modified version of the method outlined in reference (16).

Results

The aim of this study was to explore the antimicrobial potential of *M. macrostachyum* against both bacteria and fungi, while also identifying its primary chemical constituents through phytochemical screening. The qualitative results presented in table 1 indicate that *M. macrostachyum* leaves possess alkaloids, tannins, terpenoids, flavonoids, phenolic compounds, and steroids. Specifically, the ethanolic extract displayed higher quantities of alkaloids, terpenoids, and flavonoids, along with a notable presence of tannins. However, phenolic compounds and steroids were not detectable in this extract, which aligns with findings from reference (10) on the chemical composition, toxicity, and antifungal activities of *M. macrostachyum* leaves extract against foodborne fungi. Interestingly, it further demonstrates that phenolic compounds are more abundant, while steroids are less abundant.

Table 2 on the other hand demonstrates that the phenolic ethanol extract of *M. macrostachyum* exhibited the highest absorbance compared to other solvents, aligning with prior research. This suggests that ethanol is an effective solvent for extracting active phytochemicals.

The zone of inhibition or clearance refers to the circular area around an antimicrobial disk or other treatment where there is a lack of microbial growth. It is used as a measure of the effectiveness of the antimicrobial agent against the specific bacterial and fungal strain being tested. The larger the zone of inhibition, the more effective the treatment is at inhibiting bacterial growth. This method is commonly used in microbiology and in clinical settings to test the effectiveness of antibiotics or other antimicrobial agents as shown in table 3. The study observed a range of 6-21mm in the zone of inhibition or clearance for different concentrations of the tested substances against various organisms. The results in table 4 indicated that *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* were most sensitive to the highest concentration tested. Furthermore, the positive control for fungi showed resistance to Ketoconazole (150 mg/ml) against *Aspergillus niger*. The implications of the above findings are that the tested substances have potential antimicrobial properties against the tested organisms, with higher concentrations resulting in larger zones of inhibition. The sensitivity of *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* suggests that the substances may be effective in treating infections caused by these organisms. The resistance of *Aspergillus niger* to Ketoconazole highlights the need for continued development of new antimicrobial agents to combat resistant strains of fungi. However, further research is needed to determine the safety and efficacy of these substances for use in medical or industrial applications.

The range of zone of inhibition displayed by different concentrations of *M. macrostachyum* on the test organisms varies from 0 to 21 mm as apparent in table 5. The findings indicated that *Staphylococcus aureus*, *Escherichia coli*, and *Aspergillus niger* were the most sensitive to the highest concentration of *M. macrostachyum* tested,

as evidenced by the largest zone of inhibition observed for these organisms. However, for Candida albicans, the positive control for fungi, the study found that it developed resistance to Ketoconazole (150 mg/ml) which indicates that it was not sensitive to *M. macrostachyum* at the concentrations tested.

As demonstrated in table 6 the combination of ethanol and water from *M. macrostachyum* at a concentration of 100 mg/ml (0.1 mg/ml) inhibited the growth of *Staphylococcus aureus* completely, with no visible sign of turbidity or growth (clear). This value was considered as the minimum inhibitory concentration (MIC) at the lowest concentration tested. However, the study found that *Candida albicans* developed resistance to Ketoconazole.

Table 7 below shows that the ethanolic extract of *M. macrostachyum* against the test organisms inhibit growth at 150mg/mg (0.15 mg/ml) did not show any visible sign of turbidity or growth for *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* (clear), thus was taken as the Minimum Inhibitory Concentration (MIC) value at the highest concentration for all the test organisms except *Aspergillus niger* which showed resistance to the tested extract.

The results of this study can be useful in the development of drugs or therapeutic agents against bacterial and fungal infections. The fact that *Staphylococcus aureus*, *Escherichia coli*, and *Aspergillus niger* were highly sensitive to the highest concentration of *M. macrostachyum* tested suggests that it may contain bioactive compounds that could be used as potential antimicrobial agents. Further studies can be conducted to isolate and identify these compounds and evaluate their efficacy in drug formulations.

On the other hand, the finding that *Candida albicans* developed resistance to Ketoconazole (150 mg/ml) and was not sensitive to *M. macrostachyum* at the concentrations tested suggests that this organism may not be affected by the bioactive compounds in *M. macrostachyum*. This information can be used to guide the selection of appropriate drugs for the treatment of *Candida albicans* infections and to develop strategies to combat resistance to antifungal agents.

The range of the zone of clearance exhibited by different concentrations of the aqueous extract on the test organisms was up to 8 mm. However, none of the test organisms displayed sensitivity to the extract at any of the concentrations tested, except for the positive control which demonstrated sensitivity to all the test organisms.

Table 8 presented above demonstrates that the aqueous extract (water-only) of M. macrostachyum did not show any growth inhibition for all the tested organisms, as indicated by turbidity. Therefore, no minimum inhibitory concentration (MIC) value could be determined for the extract, except for the positive control.

Discussion

The aim of this study was to explore the antimicrobial potential of *M. macrostachyum* against both bacteria and fungi, while also identifying its primary chemical constituents through phytochemical screening. The qualitative results presented in Table 1 indicate that *M. macrostachyum* leaves possess alkaloids, tannins, terpenoids, flavonoids, phenolic compounds, and steroids. Specifically, the ethanolic extract displayed higher quantities of alkaloids, terpenoids, and flavonoids, along with a notable presence of tannins. However, phenolic compounds and steroids were not detectable in this extract, which aligns with findings from reference (10) on the chemical composition, toxicity, and antifungal activities of *M. macrostachyum* leaves extract against foodborne fungi. Interestingly, Table 1 further demonstrates that phenolic compounds are more abundant, while steroids are less abundant.

Alkaloids, characterized by their diverse chemical structures, constitute a group of compounds synthesized primarily from amino

acids. They are frequently isolated from plants, with around 20% of plant species containing them in minor quantities. The research on their production, extraction, and processing remains an active area of investigation, given their extensive applications. The manipulation of alkaloid biosynthetic pathways for increased production levels is one example of their modification (17, 18).

Table 1. Qualitative analysis result for phytochemical of *M. macrostachyum*.

S/No.	Sample	Parameter	Degree
1	Ethanolic	Alkaloids	+++
		Tannins	++
		Steroids	-
		Terpenoids	+++
		Flavonoids	+++
		Phenolic	-
2	Ethanol + water	Alkaloids	+++
		Tannins	++
		Steroids	-
		Terpenoids	++
		Flavonoids	++
		Phenolic	+++
3	Water	Alkaloids	+++
		Tannins	++
		Steroids	+
		Terpenoids	++
		Flavonoids	+
		Phenolic	+++

Key: +++ = More abundant; ++ = Abundant; + = Not abundant; - =Not detected.

Table 2. Quantitative analysis result for phytochemical of *M. macrostachyum*.

S/No.	Sample	Wavelength (nm)	Absorbance
1	Tannins (ethanol)	700	1.489
	Tannins (ethanol+ water)	700	1.830
	Tannins (water)	700	0.853
2	Flavonoids (ethanol)	512	1.995
	Flavonoids (ethanol+ water)	512	1.246
	Flavonoids (water)	512	2.300
3	Phenols (ethanol)	505	2.400
	Phenols (ethanol+ water)	505	1.575
	Phenols (water)	505	0.716
4	Cardiac glycosides (ethanol)	495	0.665
	Cardiac glycosides (ethanol+ water)	495	0.111
	Cardiac glycosides (water)	495	0.108
5	Saponins (ethanol)	544	1.909
	Saponins (ethanol +water)	544	1.400
	Saponins (water)	544	0.901
6.	Alkaloids	-	1.138

Table 3. The Antimicrobial activity of various concentration of Ethanolic extract of *M. macrostachyum* on the test organisms.

S/No.	Test organisms	Concentration 50mg/ml	Concentration 100mg/ml	Concentration 150mg/ml	Positive Control	Negative Control
1	*Staphylococcus aureus*	6mm	10mm	20mm	28mm	0
2	*Escherichia coli*	8mm	15mm	19mm	24mm	0
3	*Candida albicans*	6mm	10mm	21mm	29mm	0
4	*Aspergillus niger*	6mm	6mm	8mm	0	0

Table 4. The minimum Inhibitory Concentration (MIC) of the Ethanolic extract of *M. macrostachyum* against the test organisms.

S/No.	Test organisms	Concentration 50mg/ml	Concentration 100mg/ml	Concentration 150mg/ml	Positive Control	Negative Control

1	*Staphylococcus aureus*	G	G	NG	NG	G
2	*Escherichia coli*	G	G	NG	NG	G
3	*Candida albicans*	G	G	NG	NG	G
4	*Aspergillus niger*	G	G	G	G	G

Key: NG = No growth, G = Growth

Table 5. The Antimicrobial activity of various concentration of Ethanol plus water extract of *M. macrostachyum* on the test organisms

S/No.	Test organisms	Concentration 50mg/ml	Concentration 100mg/ml	Concentration 150mg/ml	Positive Control	Negative Control
1	*Staphylococcus aureus*	8mm	18mm	21mm	25mm	0
2	*Escherichia coli*	6mm	11mm	19mm	26mm	0
3	*Candida albicans*	0	6mm	6mm	0	0
4	*Aspergillus niger*	6mm	6mm	21mm	30mm	0

Table 6. The minimum Inhibitory Concentration (MIC) of the Ethanol plus water extract of *M. macrostachyum* against the test organisms.

S/No.	Test organisms	Concentration 50mg/ml	Concentration 100mg/ml	Concentration 150mg/ml	Positive Control	Negative Control
1	*Staphylococcus aureus*	G	NG	NG	NG	G
2	*Escherichia coli*	G	G	NG	NG	G
3	*Candida albicans*	G	G	G	NG	G
4	*Aspergillus niger*	G	G	NG	G	G

Key: NG = Means No growth, G = Growth

Table 7. The Antimicrobial activity of various concentration of aqueous extract (water only) of *M. macrostachyum* on the test organisms (mm).

S/No.	Test organisms	Concentration 50mg/ml	Concentration 100mg/ml	Concentration 150mg/ml	Positive Control	Negative Control
1	*Staphylococcus aureus*	0	0	0	30mm	0
2	*Escherichia coli*	0	6mm	6mm	20mm	0
3	*Candida albicans*	6mm	6mm	7mm	19mm	0
4	*Aspergillus niger*	6mm	6mm	8mm	21mm	0

Table 8. The minimum Inhibitory Concentration (MIC) of the Aqueous extract (water only) of *M. macrostachyum* against the test organisms (mg/ml).

S/No.	Test organisms	Concentration 50mg/ml	Concentration 100mg/ml	Concentration 150mg/ml	Positive Control	Negative Control
1	*Staphylococcus aureus*	G	G	G	NG	G
2	*Escherichia coli*	G	G	G	NG	G
3	*Candida albicans*	G	G	G	NG	G
4	*Aspergillus niger*	G	G	G	NG	G

Key: NG = No growth, G = Growth

Alkaloids play pivotal roles in human medicine and the natural defense mechanisms of organisms. According to reference (19), alkaloids make up approximately 20% of known secondary metabolites found in plants. In plants, they function as protective agents against predators and regulators of growth. Within therapeutic contexts, alkaloids are renowned for their anesthetic, cardioprotective, and anti-inflammatory properties. Well-known clinical alkaloids include morphine, strychnine, quinine, ephedrine, and nicotine, as detailed in reference (20). The renewed interest in bioactive natural products stems from their potential in drug discovery and their active development within traditional medicine, as discussed in reference (21). The Dictionary of Natural Products (DNP) recorded 990 newly reported or reinvestigated alkaloids from nature between 2014 and 2020, supporting

the observations in Table 1 and affirming the potential medicinal value of *M. macrostachyum*.

Flavonoids, classified as secondary metabolites, commonly comprise a benzopyrone ring with phenolic or polyphenolic groups at various positions. They are prevalent in diverse plant parts and are associated with medicinal and biological attributes. Over 10,000 flavonoid compounds have been isolated and identified, many of which possess therapeutic properties. Flavonoids have varied applications, including as natural dyes, cosmetics, and anti-aging skincare products. In the medical realm, they find extensive use as therapeutic agents, exhibiting activities such as anticancer, antimicrobial, antiviral, antioxidant, antimalarial, neuroprotective, antitumor, and anti-proliferative effects. Flavonoids from apple peels have demonstrated acetylcholinesterase (ACE) inhibition in vitro and antihypertensive properties, while also aiding in preventing cardio-metabolic disorders and supporting cognitive function preservation with aging.

The outcome, as illustrated in Table 1, signifies that *M. macrostachyum* leaves contain substantial quantities of flavonoids, potentially contributing to the observed inhibition zones as depicted in Tables 3, 5, and 7. This suggests that these leaves could indeed serve as a source of antimicrobial agents. Further research is warranted to unlock their full potential.

Saponins, characterized as glycosides of triterpenes and steroids, also referred to as steroidal glycoalkaloids, are derived from the mevalonic acid pathway. Saponins possess diverse biological activities, including antimicrobial, anti-herbivore, anti-inflammatory, antifungal, antibacterial, anti-parasitic, anti-cancer, and antiviral properties. These compounds find applications beyond pharmaceuticals, including in the beverage, cosmetics, and flavoring industries due to their surfactant properties and distinct taste profiles.

Table 1 reflects the presence of saponins in *M. macrostachyum* leaves, which may underlie its antimicrobial potential. However, variations in findings compared to previous studies suggest that further exploration is needed.

Table 2 demonstrates that the phenolic ethanol extract of *M. macrostachyum* exhibited the highest absorbance compared to other solvents, aligning with prior research. This suggests that ethanol is an effective solvent for extracting active phytochemicals.

The investigation into *M. macrostachyum* leaves underscores their antimicrobial properties, evidenced by the inhibition of test organisms. The results align with previous studies, further substantiating the potential application of this plant. While the extracts exhibited efficacy against the test organisms, their potency depended on concentration, solvent, and test organism. The ethanol extract showed notable activity, with efficacy varying based on concentration, which is consistent with prior literature.

Minimum Inhibitory Concentration (MIC) was determined as a measure of in vitro susceptibility, with implications for therapeutic efficacy. The study revealed that *M. macrostachyum* leaves hold promise as a potential source of antimicrobial agents, warranting further exploration.

In a nutshell, the bioactivity observed in *M. macrostachyum* leaves can be attributed to the presence of abundant phytochemical compounds. These compounds confer antimicrobial properties, inhibiting the growth of bacteria and fungi. Qualitative and quantitative phytochemical analyses further corroborate this potential, supporting claims made by previous researchers. The findings underscore the significance of *M. macrostachyum* as a natural source of antimicrobial agents, encouraging further research in this domain.

Conclusion

The outcomes of the study provide substantiation for the traditional application of *M. macrostachyum* leaves in disease treatment, as

indicated by the obtained results. The comprehensive exploration of phytochemical and antimicrobial attributes has underscored the potential of these plant extracts as promising candidates for the synthesis of advantageous antifungal and antibiotic medications, fostering improved well-being. The conducted laboratory assays on both plant extracts and constituents have uncovered their adeptness in effectively impeding the growth of isolated fungi and bacteria, underscoring their viability as potent antimicrobial agents. This investigation underscores the significance of the isolation and refinement of the specific compounds responsible for the inhibition of test fungi and bacteria through biomonitoring. This approach holds the promise of yielding natural and safe compounds that surpass the effectiveness of synthetic antimicrobial agents, which have shown repellent tendencies. The study thus implies that *M. macrostachyum* leaves harbor antimicrobial properties that validate their traditional use as a medicinal plant.

Acknowledgements

We, the authors of this paper, express our gratitude to the almighty God for the strength and privilege to compose this manuscript.

Funding Information

No Funding information provided.

Ethics approval and consent to participate

Not needed.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

1. Krishnan N, Ramanathan S, Sasidharan S, et al. Antimicrobial Activity Evaluation of Cassia spectabilis Leaf Extracts. *Int J Pharmacol* 2010; **6**(4):510-514.
2. Daniyan SY, Oloruntimelehin JB, Ifeadi O. Antibacterial activity of *Cassia occidentalis* flower vegetable extract on selected bacteria. *Asian J Biomed Pharma Sci* 2011; **1**(1): 23-27.
3. Stockwell C. Nature's pharmacy. London, United Kingdom: Century Hutchinson Ltd. Sustainable use of medicinal plants: Problems, progress, and prospects, 1988.
4. Moerman DC. An analysis of the food plants and drug plants of Native North America. *J Ethnopharmacol* 1996: **52**:1-22.
5. Klink B. Alternative medicines: Is natural really better? *Drug Topics* 1997; **14**: 99-100.
6. Lewis WH, Elvin-Lewis MP. Medicinal plants as sources of new therapeutics. *Life Sci* 1995; **78**(5):431-441.
7. Motamedi H, Seyyednejod SM, Najvani FD, et al. Antibacterial effect of *Eucalyptus* microtheca. *Int J Enteric Pathogen* 2010; **2**(2):216-515.
8. Awadh NA, Julich WD, Kusnick C, et al. Screening of yemeni medical plant antimicrobial and cytotoxic activities. *J Ethnopharmacol* 2001; **74**(1):173-179.
9. Barbour E, Sharif MA, Sagherian BK, et al. Screening of selected indigenous plants of Lebanon. *J Ethnopharmacol*, 2004; **93**(1):1-7.
10. Adeogun OO, Adekunle AA, Adongbede ME, et al. Qualitative and quantitative Aframomum aulacocarpos. *J Natural Products* 2017; **57**:917-23.
11. Cédric OS, Ondo JP, Louis-Clément OE, et al. Phytochemical screening, evaluation of antioxidant and antimicrobial properties of *Erythrophleum ivorense* A. Chev (Leguminosae) and *Megaphrynium macrostachyum* Benth (Marantaceae), medicinal plants from Gabon. *Int J Biosci* 2016;

6(8):3-53.
12. Jennings SB, Brown ND, Boshier DH, et al. Ecology provides a pragmatic solution to the maintenance of genetic diversity in sustainably managed tropical rainforest. *Forest Ecol Man* 2001; **154**(1-2):1-10.
13. Ajayi IA, Ojelere OO. Phytochemical screening, proximate analysis and antibacterial properties of common herbal remedies of the southwest. *J Ethnopharmacol* 2013; **99**(2):253-257.
14. Milne-Redhead E. Marantaceae. *Staphylococcus aureus* Infectious Disease Minnesota Department of Health Fact Sheet, 1952.
15. Adekanmi AA, Adekanmi AS, Adekanmi SO. The extraction yield of Moringa. *J Natural Products* 2020; 10-15.
16. Owoseni AA, Ayanbamiji TA, Ajayi YO, et al. Antimicrobial and phytochemical analysis of leaves and bark extracts from Bridelia ferruginea. *African J Biotechnol* 2010; **9**(7):1031-6.
17. Verpoorte R. Encyclopedia of Analytical Science. Elsevier; Amsterdam, viruses. *J Med Virol* 2005; **15**:71-79.
18. Srivastava S, Srivastava AK. Biotechnology and genetic engineering for alkaloid production. In K. G. Ramawat & J. M. Mérillon (Eds.), 2013; Natural Products.
19. Kaur R, Arora S. Alkaloids—Important therapeutic secondary metabolites of plant origin. *J Crit Rev* 2015; **2**:1-8.
20. Kurek J. Alkaloids—Their importance in nature and human life. IntechOpen; London, UK: Lancet, 2019; 344, 626.
21. Atanasov AG, Zotchev SB, Dirsch VM, et al. Natural products in drug isolation and characterization of bioactive components from Mirabilis jalapa L. radix. *J Trad Comp Med 2021*; **6**:41-47.