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Qualitative and Quantitative Phytochemical Constituents, Antioxidant Activities, and Antimicrobial Studies of Methanol Extract of *Morinda citrifolia* Stem

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Abstract: This study evaluated the qualitative and quantitative phytochemical constituents, antioxidant potential, and antimicrobial properties of *Morinda citrifolia* stem. The stem extract was prepared using the maceration method with 99.8% methanol for three days at room temperature. Phytochemical and proximate compositions were analyzed using standard methods, while selected minerals were determined by atomic absorption spectrophotometry. Antioxidant activity was assessed using the DPPH free radical scavenging assay, and antimicrobial activity was examined by the agar-well diffusion method. Phytochemical screening revealed the presence of phenolics, glycosides, steroids, terpenoids, alkaloids, tannins, flavonoids, and eugenols. Proximate analysis showed moisture, lipid, protein, ash, fiber, and carbohydrate contents of 18.99%, 9.12%, 4.72%, 6.40%, 37.90%, and 22.87%, respectively. The concentrations of Na, Mg, K, Ca, Fe, Cu, and Zn were 7.51 ± 1.60, 126.67 ± 9.43, 91.12 ± 16.12, 7.59 ± 0.63, 3.50 ± 0.65, 0.02 ± 0.003, and 0.26 ± 0.086 mg/kg, respectively. The extract exhibited strong antioxidant activity with an IC_{50} of 49.48 µg/mL, comparable to ascorbic acid (46.56 µg/mL). Antimicrobial testing showed inhibition of *Staphylococcus aureus* growth, with minimum inhibitory and bactericidal concentrations of 500 mg/mL and 50–200 mg/mL, respectively. These results demonstrate that *M. citrifolia* stem methanol extract possesses significant antioxidant and antimicrobial activities, supporting its potential as a natural therapeutic and its traditional medicinal applications.

Introduction

Medicinal plants have long been a foundation for human health and well-being, offering a vast collection of bioactive compounds with therapeutic capability (1, 2). These plants contain phytochemicals, in other words, secondary metabolites such as glycosides, phenolics, alkaloids, and flavonoids that serve as natural agents to help prevent, treat, and manage various ailments (3-5), that is why they have been used as anti-inflammatory, anti-mutagenic, anti-carcinogenic, antimicrobial, and antioxidant drugs (6, 7). Present-day medications are mainly derived from plant-based sources, which reinforces their substantial role in pharmaceutical practice (8).

Morinda citrifolia, commonly known as the *Noni* plant, exists naturally in Tonga and Southeast Asia, together with French Polynesia, Africa, the Pacific Islands, and parts of Australia (9). *M. citrifolia* is a tropical, perennial shrub or small tree that commonly attains a height between 10 and 15 feet (3 and 4.5 meters). Its stem is usually straight, displaying colors ranging from gray to brown with a slightly knobby surface, and its thickness can vary, ranging from a few inches to approximately a foot in diameter, contingent upon the plant's age and environmental factors (10). To better illustrate the morphological characteristics discussed above, the **Figure 1** presents a view of the *M. citrifolia* leaf and stem.

Traditionally, *M. citrifolia* has been used in medical treatments to address inflammation as well as provide pain relief while enhancing wound healing and supporting immune response (12). The therapeutic benefits of the *M. citrifolia* depend on its fruit production and leaf growth, together with root system development and bark utilization. Scientific interest has developed because traditional communities use *M. citrifolia* and its bioactive constituents. Among the Polynesians, the fruit from the *M. citrifolia* is said to be in high demand due to its purpose to serve as an alternative medicine for various ailments such as menstrual difficulties, arthritis, high blood pressure, headache, heart diseases, cancer, diabetes, senility, muscle pain and aches, and blood vessel problems (10, 13). Research has discovered that *M. citrifolia* contains multiple elements within its

Figure 1. (A) Leaf and (B) stem of *M. citrifolia*. Adapted from (11) licensed: CC BY 4.0.

chemical framework, consisting of phytochemicals together with vitamins and minerals, which contribute to the wide range of medicinal properties of the plant. Four major phytochemical compounds in the M. citrifolia blossom were identified as flavonoids-(A) quercetin-3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopy-ranoside, (B) kaempferol-3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside, and iridoids (C) asperulosidic acid, (D) deacetylasperulosidic acid, (14). Some other vital components found in *M. citrifolia* include alkaloids, terpenes, and fatty acids (10).

In addition, *M. citrifolia* also stands out due to its antioxidant-filled composition, which includes vitamins C and E as well as beta-carotene and polyphenols (15, 16). The body needs antioxidants in order to eliminate destructive free radicals, thereby protecting cells with antioxidants that help prevent chronic diseases. Studies have revealed that when determining the antioxidant properties of medicinal plants through methods such as ABTS scavenging assay, DPPH radical scavenging, and NO scavenging activity, the methanol solvent extract possesses a greater affinity when compared to water and ethyl acetate extracts (17). The leaves, seed, and fruit extracts of *M. citrifolia* have also been reported to exhibit extensive activities against microorganisms like bacteria, such as *Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus,* and fungi, such as *Candida albicans,* and *Aspergillus niger,* because of the bioactive constituents present in the plant (9, 18). While extensive studies have explored the chemical composition and bioactive constituents of various parts of *M. citrifolia,* particularly its fruit and leaves, the stem remains significantly under-investigated. Existing literature lacks comprehensive data on the phytochemical profile, antioxidant potential, and antimicrobial efficacy of stem extracts. Hence, this study aims to evaluate qualitative and quantitative phytochemical constituents, antioxidant activities, and antimicrobial properties of *M. citrifolia* stem using methanol as the solvent for extraction.

Materials and Methods

Materials

Reagents

The reagents used during this study were of analytical grade, and solutions were prepared using distilled water as solvent. These are; Methanol (99.8% purity, Sigma-Aldrich, USA), Hydrochloric acid (35% purity, Merck, Germany), Glacial Acetic acid (99.5% purity, BDH Chemicals, UK), Ferric chloride (99% purity, Loba Chemie, India), Sulphuric acid (97% purity,Sigma-Aldrich, USA), Gelatin solution (Fisher Scientific, USA), Sodium chloride (Merck, Germany), Lead acetate (Qualikems, India), Potassium hydroxide (Sigma-Aldrich, USA), Chloroform (99.8% purity, BDH Chemicals, UK), Folin-Ciocalteu reagent (Sigma-Aldrich, USA), Tannic acid (Merck, Germany), 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma-Aldrich, USA), Sodium carbonate (Fisher Scientific, USA), Ethanol (99.7% purity, BDH Chemicals, UK), Ammonium hydroxide (Loba Chemie, India), Ammonia (Merck, Germany), Ammonium chloride (Sigma-Aldrich, USA), Sodium acetate (BDH Chemicals, UK), Folin-Denis reagent (Sigma-Aldrich, USA), Selenium tablet (Qualikems, India), Boric acid solution (Merck, Germany), Methyl red indicator (Sigma-Aldrich, USA), Sodium hydroxide (pellets, BDH Chemicals, UK), Petroleum ether (Fisher Scientific, USA), Nitric acid (65% purity, Merck, Germany), Perchloric acid (60% purity, Sigma-Aldrich, USA). Whatman No. 1 filter paper (Whatman™, Cytiva, USA)

Instrumentation

UV-Vis spectrophotometer (model: Jenway 6100, Dunmow, Essex, UK) and Atomic Absorption Spectrophotometer (AAS Model 210 VGP).

Sample Collection and Preparation

Fresh *M. citrifolia* stem bark used in this study was ethically obtained at the naturally occurring population in Benin City, Edo State, Nigeria, and was chosen because of the region's ecological suitability and known biodiversity status. This site is recommended so as to have a representative sample with minimum seasonal variation since the site grows the species regularly throughout the year. The appropriate collection permissions were established with reference to local and institutional guidelines. To prove the authenticity of the collected sample, it was identified at the Department of Plant Biology and Biotechnology, University of Benin and issued a voucher specimen with identifier UBH M427 for traceability and future reference. The preparation for analysis began by separating the leaves from the stem of the *M. citrifolia* plant, and washing the stem several times with running water, then rinsing it with distilled water 3 times before cutting it into irregular and small pieces (length within 3-5 cm). It was then air-dried under ambient conditions for seven days on the lab

bench. The dried stem pieces were ground manually using a mortar and pestle and later then processed in an electric blender (AKAI electric blender, 28000 RPM bare motor speed) into fine powder, which was of consistent particle size distribution within the acceptable range, suitable for extraction procedures.

Extraction Process

Into a clean 1-liter glass beaker, 100 g of finely powdered *M. citrifolia* stem bark were weighed, and then 500 mL of analytical grade methanol (99.8% purity) was added. The mixture was stirred well using a sterile glass rod and then covered with aluminum foil to reduce evaporation and prevent foreign contaminants. The beaker was placed to stand at ambient temperature in the laboratory for a period of 3 days of maceration. Gentle agitation was carried out twice a day during this period in order to increase the contact between the solvent and the sample. The macerated solution was filtered with muslin fabric, and then it was filtered with Whatman No. 1 filter paper to get a clear extract. The filtrate obtained was then evaporated to completion by placing it in a rotary evaporator (Model: re52-2) at 40 °C for 1 h. The resulting crude extract was then refrigerated in an amber-colored bottle and analyzed further.

Qualitative Phytochemical Screening

The phytochemicals present in the crude extract of *M. citrifolia* stem were qualitatively screened using standard methods described by (19).

Alkaloids

The first 2.0 mL of the extract was evaporated to dryness. Then the resultant residues were dissolved in 5mL of hydrochloric acid (2 mol/dm^3) and filtered. The filtrate was divided into two test tubes and labeled A and B. Into test tube A,two drops of Mayer's reagent were added, and into test tube B, two drops of Wanger's reagent were added, and the resulting change was recorded.

Glycoside

To 2.0 mL of the extract, 1.0 mL of glacial acetic acid and one drop of ferric chloride solution were added. 1.0 mL of concentrated sulfuric acid was added slowly down the side of the test tube, and the resulting change was noted.

Tannins

To 2.0 mL of the extract, 2.0 mL of distilled water and 1.0 mL of 1% gelatin solution containing sodium chloride were added, and the resulting change was noted

Phenols

To 1.0 mL of the extract, four drops of ferric chloride solution were added, and the resulting change was noted.

Saponins

The froth test method was used in the detection of saponins. 5.0 mL of the extract was diluted with distilled water to 20.0 mL, and this was shaken in a 50 mL graduated cylinder for 15 min using a flask shaker (Model: Cole-Parmer 230V).

Flavonoids

Using the lead acetate test method, 2.0 mL of the extract was treated with two drops of lead acetate solution, and the resulting change was noted.

Eugenols

The first 2.0 mL of the extract was mixed with 5 mL of 5% potassium hydroxide solution. The aqueous layers were separated and filtered, and three drops of hydrochloric acid were added to the filtrate. The resulting change was noted.

Steroid

To 2.0 mL of the extract, 2.0 mL of chloroform was added. 2.0 mL of concentrated sulfuric acid was added carefully, and the mixture was left to stand undisturbed. The resulting change was noted.

Terpeniod

To 2.0 mL of the extract, 2.0 mL of chloroform was added, and 3mL of sulfuric acid was carefully added to form a layer. The resulting change was noted.

Quantitative Phytochemical Screening

Determination of Total Phenolic Content

According to the method described by (20), but modified slightly, the research measured total phenolic content in *M. citrifolia* stem extract through Folin-Ciocalteu reagent analysis by using tannic acid as a standard solution. For the assay, 1.0 mL of the *M. citrifolia* stem extract at a concentration of 250 µg/mL was added to a test tube. Then, 1.0 mL of Folin-Ciocalteu reagent was added, and the content of the flask was mixed thoroughly. After 5 min, 15.0 mL of 20% sodium carbonate was added, and then the solution was left to stand for two h. The absorbance of the samples was measured at 760 nm through a UV-Vis spectrophotometer (model: Jenway 6100, Dunmow, Essex, UK). The tannic acid equivalent (TAE) amount of the total phenolic content was measured by using data obtained from the tannic acid calibration graph.

Determination of Total Alkaloids Content

The total alkaloid analysis followed the procedure described by (21), which required adding 100 mL of 20% acetic acid in ethanol solution to 5 g of *M. citrifolia* stem extract inside a 250 mL beaker. The solution was filtered, and the extract was obtained through water bath heating at 45 °C until it reached a quarter of its initial volume. A slow addition of concentrated ammonium hydroxide solution (30% w/w NH_3, 14.8 N) was performed on the extract until all the precipitation occurred. The entire solution settled before collecting the precipitate through filtration, while weighing the dried substance after washing it with 1% ammonia solution.

Determination of Total Flavonoid Content

Using methods outlined by (22), the total flavonoid content was evaluated from an aliquot of homogenous *M. citrifolia* stem extract (1.5 g). The determination utilized 30 µL aliquots of the methanol extract. 90 µL of methanol was used to dilute the sample; thereafter, 6 µL of 10% aluminum chloride, 6 µL of 1mol/L sodium acetate, and 170 µL of methanol were then added. The absorbance of the mixture was measured at 415 nm after 30 min using a UV-Vis spectrophotometer (model: Jenway 6100, Dunmow, Essex, UK). A standard measurement of Quercetin serves for calculating flavonoid content expressed in UQe/g.

Determination of Total Tannin Content

According to the methods described by (23), the total tannin content was determined. 0.20 mL of the sample was added

to 20 mL of methanol, and it was then placed in a water bath at a temperature ranging from 77 °C to 80 °C for a duration of 1 h, then shaken. The extract was then filtered using a double-layered Whatman No. 1 filter paper. To the filtrate, three solutions were combined: 2.5 mL of Folin-Denis reagent, 10 mL of 17% sodium carbonate, and 20 mL of distilled water; thereafter, the results were mixed and allowed to stand for 20 min at room temperature. Various standard tannic acid solutions were prepared using methanol, while their absorbance and all sample readings occurred at 760 nm wavelength after color development using a UV-Vis spectrophotometer (model: Jenway 6100, Dunmow, Essex, UK). The tannin content was determined from the calibration curve, and the concentration of the tannin in the sample was obtained.

Proximate Analysis

Using methods described by (19), parameters such as moisture content, ash content, crude fat, crude fiber, total carbohydrate content, and crude protein content were evaluated.

Moisture Content

Into an empty and clean pre-weighed ceramic crucible (W1), 5 g of the sample was weighed using an analytical balance (Model Ohaus PX224) and labeled (W2). Subsequently, the sample was subjected to a 24-hour drying process at 105 °C in a laboratory oven (Model Bov-D70) until it reached a constant weight. After cooling to room temperature within a desiccator, the sample's weight was accurately measured to establish its dry weight (W3) and the moisture content was calculated using **Equation 1**.

Ash Content

A clean crucible was placed in a laboratory oven (Model Bov-D70) at 105 °C for 45 min and then transferred to a desiccator until it cooled. Using an analytical balance (Model Ohaus PX224), the weight was noted (W1). 5 g of the sample was precisely weighed directly in the crucible as (W2). The sample was then dried in a boiling water bath at 105°C for 30 min and charred gradually over a hot plate at 250 °C in a fume cupboard until no more soot was emitted. Afterward, it was transferred into a muffle furnace for 6 h at 500 °C to obtain (W3), and the ash content was calculated using **Equation 2**.

Crude Protein

In a digestion flask, 1.0 g of the sample was placed, and five selenium tablets (Kjeldahl catalyst) and 20 mL of concentrated sulfuric acid were added. The mixture was then heated for 4 h at 450 °C in the Kjeldahl apparatus within a fume cupboard until no more soot was given off and the mixture turned pale yellow. It was then filtered using a Whatman filter paper into a 100 mL volumetric flask and diluted with distilled water to the mark. In a separate conical flask, 20 mL of 4% boric acid solution was added along with two drops of methyl red as an indicator. The sample was diluted with 75 mL of distilled water, and 10 mL of the digest was made alkaline with 20 mL of 20% sodium hydroxide and distilled for 15 min. The filtrate was then titrated against 0.1 N HCl, and the protein content was determined using **Equation 3**.

Fat Content

Into a thimble, 5 g of the sample were weighed and sealed with cotton wool and then placed in a Soxhlet extractor. Into a dry preweighed round-bottom flask, 200 mL of petroleum ether solvent and a few anti-bumping chips were added. It was then put into the Soxhlet extraction heating unit, and the Soxhlet extractor was attached. The extraction process was allowed to run for 4 h of reflux at 60 °C. Subsequently, the defatted thimbles and round-bottomed flasks were dried at 105 °C in an oven for 24 h, allowed to cool, and then reweighed. The percentage fat content was calculated using **Equation 4**.

Crude Fibre

A total of 2 g of the defatted samples was subjected to acid digestion using 200 mL of 1.25% sulfuric acid for 30 min. The residue was filtered using a clean muslin cloth and washed with distilled water till the filtrate became neutral. The residue was then transferred into a clean conical flask and subjected to alkali digestion using 200 mL of 1.25% sodium hydroxide for 30 min. The residue was then filtered using a clean muslin cloth and distilled water. It was then transferred into a dry pre-weighed crucible (W1) and dried in the oven for 2 h at 130 °C. It was then placed in the desiccator for 45 min and weighed (W2). Afterward, it was transferred to the muffle furnace and ashed at 600 °C for 30 min, which was then allowed to cool and weighed (W3). The percentage crude fibre was then calculated using **Equation 5**.

Carbohydrate Determination

The total carbohydrate content was calculated using **Equation 6**.

$$\%\,\mathrm{Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Equation 1

$$\%\,\mathrm{Ash} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Equation 2

$$\%\,\mathrm{Nitrogen} = \frac{T \times N_{acid} \times 0.014}{\mathrm{Sample\ weight}} \times 100$$

Equation 3 | T = titre value and N = normality of acid.

$$\%\,\mathrm{Fat} = \frac{\mathrm{Extracted\ crude\ fat}}{\mathrm{Sample\ weight}} \times 100$$

Equation 4

$$\%\,\mathrm{Crude\ fiber} = \frac{(W_2 - W_1) - (W_3 - W_1)}{\mathrm{Sample\ weight}} \times 100$$

Equation 5

$$\%\,\mathrm{Carbohydrate} = 100 - (\%\mathrm{Moisture} + \%\mathrm{Protein} + \%\mathrm{Fat} + \%\mathrm{Ash})$$

Equation 6

Mineral Analysis

According to the method described by (24), minerals such as magnesium (Mg), sodium (Na), calcium (Ca), potassium (K), zinc (Zn), and iron (Fe) were examined. A combined solution of 5 mL concentrated nitric acid, 1 mL concentrated sulfuric acid and 1 mL of concentrated perchloric acid was used to digest 1.0 g of oven-dried samples until white fumes appeared. A part of the digested solution was heated to reduce it to a semi-dry state, but stopped short of complete drying. The flask was gradually cooled, and then the content was diluted with distilled water and filtered into a 50 mL volumetric flask using a Whatman No. 1 filter paper. The mixture was then filled to the mark with distilled water and stored for later analysis of minerals present using an Atomic Absorption Spectrophotometer (AAS Model 210 VGP).

Determination of Antioxidant Potential

A stable DPPH standard method served to assess free radical scavenging activities of plant extracts, as described by (20), with slight alterations. Multiple reaction solutions were prepared from a variety of sample extract solutions combined with standard solutions for the analysis. The reaction mixtures with control and blank solutions received 30 min of dark incubation before measuring their absorbance at 517 nm wavelength using a UV/Visible Spectrophotometer (model: Jenway 6100, Dunmow, Essex, UK).

The %DPPH scavenging activity was plotted against the concentration of the sample, and from the plot, the concentration of the plant extract that produced 50% inhibition (IC50) was obtained using ascorbic acid as a standard.

Determination of Antimicrobial Activity

The antimicrobial sensitivity assay performed its measurements through the agar well diffusion method, which follows (19), with slight modification. *Pseudomonas aeruginosa, Aspergillus flavus, Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae,* and *Candida albicans* bacterial and fungal strains were obtained from the University of Benin Teaching Hospital (UBTH) in Benin City. Testing of *M. citrifolia* extract at 50 mg/mL, 100 mg/mL, 200 mg/mL, and 500 mg/mL concentrations was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Positive petri dish results from the minimum inhibitory concentration (MIC) were then transferred to determine the minimum bactericidal concentration (MBC).

Statistical Analysis

The values obtained from the analysis were analyzed as the mean (μ) and standard deviation (σ) of triplicate determinations. A one-way analysis of variance (ANOVA) was conducted using SPSS version 31.0 for Windows PC, and the Statistical significance was set at $p < 0.05$.

Results and Discussion

Qualitative Phytochemical Screening

The health benefits of *M. citrifolia* stem were evaluated by determining the presence of the bioactive constituents, and the results revealed the presence of glycosides, terpenoids, phenolic compounds, alkaloids, flavonoids, tannins, steroids, and eugenols, while saponins were not detected. These findings are consistent with previous studies on *M. citrifolia,* which showed the presence of alkaloids, steroids, tannin, carbohydrates, terpenoids, glycosides, and flavonoids in both the leaves and stem bark (25). The presence of these phytochemicals shows *M. citrifolia* stems possess antioxidant, antimicrobial, and anti-inflammatory properties, which help defend against cardiovascular diseases, bacterial and fungal infections (26). Phenolic compounds possess powerful antioxidant properties because they neutralize free radicals to stop lipid peroxidation and protect against cancer development (27, 28). Antioxidant, along with the antiviral and anti-inflammatory properties of flavonoids, have been confirmed by (29). Medical studies have demonstrated the significant therapeutic aspects of alkaloids because they provide multiple treatment benefits, including pain relief and antipyretic properties, as well as antihypertensive effects, antifungal activity, anti-inflammatory responses, and antibacterial abilities (30). According to (31), research proves that steroids function as antimicrobials. Tannins have also been reported to exhibit anti-inflammatory, antibacterial, antioxidant, antithrombotic, antiallergic, antimutagenic, antineoplastic, and antiviral activities (32).

Quantitative Phytochemical Screening

Table 1 shows the result for the total alkaloid content (TAC), total phenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC) of the methanol extract of *M. citrifolia* stem bark. TAC shows a statistically significant difference across samples ($p < 0.05$), suggesting a possible variation in bioactive compound concentration, while TPC, TFC, and TTC do not show significant differences. Previous studies by (23) have shown that *M. citrifolia* fruit contains a high level of total flavonoid content (197.03 mg/100 g) when compared to the stem (56.84 ± 0.11 mg/100 g). In comparison, the total tannin content of the fruit contains 3.56 mg/100 g, compared to the seed, which shows a slight difference of 3.03 ± 0.03 mg/100 g. According to (33), flavonoids together with tannins represent polymorphous phenolic compounds which function as primary antioxidants while displaying antimicrobial properties along with anti-inflammatory effects, anti-allergic agents, anticancer properties, antineoplastic potential and intestinal problem treatments. Among phytochemicals, alkaloids form the largest group, which aids in the development of potent painkiller drugs.

Proximate Analysis

The proximate evaluation results for *M. citrifolia* stem bark were presented in **Table 2** with astatistically significant difference of $p<0.01$. Plants possess a high crude fiber content because of their structural components, which include cellulose, hemicellulose, and lignin, which the plant uses to remain upright and transport nutrients (34-36). A plant needs high water content to support its water transport functions since stems contain xylem vascular tissue that allows water movement. Plants use their stems to store carbohydrates mainly as starch, together with water-soluble carbohydrates such as sucrose and fructose. The storage carbohydrates serve as fuel sources for plant development and reproduction, particularly under pressure or through resting states (37-39). Previous studies have shown that *M. citrifolia* seeds contain 8.37% moisture, 10.55% crude oil, 7.1% protein, 1.29% ash, and 25.20% carbohydrates (40). In our study, the results demonstrated that *M. citrifolia* stems had high levels of ash content alongside moisture content, which could be attributed to the soil and environmental factors, and by comparing the seed against the stem, it can

Table 1. Quantitative phytochemical profile for the methanol extract of *M. citrifolia* stem bark.

Parameters	Value
Total alkaloid content (TAC)	2.96 ± 0.12 %
Total phenolic content (TPC)	7.83 ± 0.09 mg/100 g of extract
Total flavonoid content (TFC)	56.84 ± 0.11 mg/100 g of extract
Total tannin content (TTC)	3.03 ± 0.03 mg/100 g of extract

Table 2. Proximate analysis of *M. citrifolia* stem bark.

Parameters	Value (%)
Moisture content	18.99 ± 0.12
Ash content	6.40 ± 0.20
Crude lipids	9.12 ± 0.08
Crude fiber	37.90 ± 0.20
Crude protein	4.72 ± 0.09
Total Carbohydrate content	22.87 ± 0.39

Table 3. Mineral analysis of *M. citrifolia* stem bark.

Elements	Values (mg/kg)
Natrium	7.51 ± 1.60
Kalium	91.12 ± 16.12
Calsium	7.59 ± 0.63
Magnesium	126.67 ± 9.43
Ferrium	3.50 ± 0.65
Cupper	0.02 ± 0.003
Zink	0.26 ± 0.086

Table 4. Free radical-scavenging potential of the crude extract of *M. citrifolia* stem bark.

Concentration (µg/mL)	%Inhibition	
	Ascorbic acid	Stem Extract
100	7.176	15.787
200	13.121	26.345
300	16.960	43.977
400	34.034	83.547
500	40.236	87.772

Table 5. IC_{50} value of stem extract of *M. citrifolia* and standard (ascorbic acid).

Sample	IC_{50} values expressed in µg/mL
Stem Extract	49.478 ± 0.39
Standard	46.564 ± 0.58

be observed that *M. citrifolia* stem has a low level of crude protein. The total carbohydrate content of *M. citrifolia* stems may be due to the higher fiber and ash content, which could displace some digestible carbohydrates. Nevertheless, it confirms *M. citrifolia* stem as a viable carbohydrate source, which provides significant diet-based advantages as a source of digestible dietary fiber and carbohydrates.

Mineral Analysis

The analysis shows the presence of minerals such as sodium, magnesium, potassium, iron, copper, calcium, and zinc, with potassium and magnesium showing elevated concentrations in *M. citrifolia* stem bark, as presented in **Table 3**. All elements show statistically significant differences ($p < 0.05$), and this suggests that the mineral composition varies meaningfully. These findings are consistent with previous studies on *M. citrifolia,* which reported that *M. citrifolia* fruit juice contains 66.75 mg/kg Na, 890.1 mg/kg K, 47.1 mg/kg Ca, 129.3 mg/kg Mg, 1.185 mg/kg Fe, 0.112 mg/kg Cu and 0.863 mg/kg Zn (41). This shows a high concentration of two crucial minerals, namely potassium and magnesium. The human body needs potassium to maintain muscle function and nerve signaling and fluid balance, yet requires magnesium to support muscle activity and biological processes within the bones as well as energy metabolism. The two essential minerals facilitate cardiovascular health through blood pressure regulation and disease prevention among heart diseases and diabetes (42, 43).

Antioxidant Potential

According to (1, 44), the DPPH assay serves as a standard method for analyzing free radical-scavenging studies due to its straightforwardness and simplicity. When an antioxidant is present, it allows the stable DPPH radical to undergo reduction. The DPPH measurement was at 517 nm, which corresponds to the maximum absorption wavelength in the UV spectrum, where it appears purple. The color change intensity shows the amount of antioxidant activity that occurs in a particular sample. **Table 4** shows the free radical-scavenging potential of the crude extract of *M. citrifolia* stem bark.

Table 5 shows the DPPH 50% inhibition (IC_{50}) value of the stem extract of *M. citrifolia*, using ascorbic acid as a standard. IC_{50} serves as the primary method for determining how well substances affect biological or metabolic processes. The antioxidant power of a substance increases when its IC_{50} value decreases because it makes it possible to achieve similar inhibitory levels using smaller extract concentrations (45). The ORAC and FRAP results proved that *M. citrifolia* stem bark possessed antioxidant properties similar to those reported by studies on different *M. citrifolia* plant parts (Assanga *et al*., 2013). According to reports by (46), pasteurized *M. citrifolia* fruit juice exhibited DPPH scavenging activity with an IC_{50} value of 57.90 ± 0.40 µg/mL and using Trolox, a synthetic antioxidant as standard, the IC_{50} value was 66.11 ± 0.20 µg/mL. In our study, an IC50 value analysis of *M. citrifolia* stem bark equals 49.478 ± 0.39 µg/mL, whereas ascorbic acid (standard) shows an IC_{50} of 46.564 ± 0.58 µg/mL. The biological effectiveness of substances during studies increases proportionally with their IC_{50} value (47). Based on the results, the statistically significant

difference suggests that while the stem extract is effective, it may require higher concentrations to achieve similar antioxidant effects as the standard (ascorbic acid) ($p < 0.001$). Future studies could explore synergistic effects with other plant parts or optimization of extraction methods to enhance efficacy.

Antimicrobial Activity

According to (48), the term "antimicrobial activity" refers to the ability of a substance to inhibit the growth of, or kill, microorganisms—including bacteria, fungi, viruses, and parasites. The size of the inhibition zone indicates the potency of the antimicrobial agent. In this study, the methanolic extract of *M. citrifolia* stem bark exhibited selective antibacterial activity. Among the tested organisms, only *S. aureus* showed measurable sensitivity, with an inhibition zone of 16 mm observed at the highest concentration tested (500 mg/mL). No inhibitory effect was detected against *P. aeruginosa*, *E. coli*, *S. pneumoniae*, *A. flavus*, or *C. albicans* at any concentration.

Using the modified agar well diffusion method with serial dilutions, the minimum inhibitory concentration (MIC) for *S. aureus* was determined to be 50 mg/mL, while the minimum bactericidal concentration (MBC) extended across the tested range of 50–200 mg/mL. This selective activity suggests that the stem extract is specifically effective against Gram-positive *S. aureus*, likely due to the absence of an outer membrane in Gram-positive bacteria, which facilitates the penetration of phytochemicals. Notably, not all Gram-positive bacteria (such as *S. pneumoniae*) are sensitive to plant-based antimicrobial agents (49).

In contrast, the Gram-negative bacteria tested, as well as the fungal isolates, were resistant to the extract. Previous studies have shown that methanolic extracts of *M. citrifolia* fruits possess some antifungal activity, although they were inactive against *C. albicans* and *A. flavus* (50). This finding is consistent with the present study, suggesting that *M. citrifolia* may lack the specific antifungal compounds required to inhibit these fungal isolates but is more effective as an antibacterial agent. Taken together, these results support the potential of *M. citrifolia* stem bark as a source of antibacterial compounds, particularly against *S. aureus*, while highlighting its limited spectrum of activity and lack of antifungal efficacy.

Conclusion

This research shows that *M. citrifolia* stem bark contains high amounts of bioactive constituents, including alkaloids, flavonoids, tannins, and phenolic compounds, among others, which make the plant possess antioxidants and antibacterial characteristics. The MIC and the MBC results were indicative of the highly potent antibacterial activity of the methanolic extract against *S. aureus* at a concentration of 50 mg/mL. This implies that *M. citrifolia* stem bark potentially holds good formulation potential for producing anti-microbial products and natural antioxidant products. Nevertheless, the IC_{50} value in the extract is relatively similar to that of ascorbic acid ($p < 0.001$), which suggests that a higher rate of the dosage might be needed to obtain similar effects in therapy, so the optimization of doses could play a significant role in further usage. Proximate and mineral compositions also contribute to the nutritional appeal of the stem bark, mainly because it contains much fiber, carbohydrates, and essential minerals such as potassium and magnesium, which offer cardiovascular and metabolic advantages. In conclusion, the findings validate the use of *M. citrifolia* stem bark as a potential natural source of antioxidants and antibacterial agents. It is however, suggested that further investigations can be carried out on its pharmacokinetics, formulation possibilities and *in vivo* efficacy to facilitate its incorporation in its therapeutic uses.

Declarations

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Conflict of Interest

The authors declare no conflicting interest.

Data Availability

All the neccessary data obtained during the analysis are available in the manuscript submitted.

Ethics Statement

Not applicable.

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Additional Information

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