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Comparative Phytochemical and Antimicrobial Analyses of *Siphonochilus aethiopicus* and *Monodora myristica*

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ABSTRACT. Medicinal plants are used as interventions and alternatives in many countries including Africa. The phytochemicals produced by plants possess potential antimicrobial activities against pathogens through various mechanisms of action. This study aimed to compare the phytochemical and antimicrobial constituents of rhizomes of *Siphonochilus aethiopicus* (African ginger) and seeds of *Monodora myristica* (African nutmeg). The rhizomes of *S. aethiopicus* and seeds of *M. myristica* were separately and thoroughly washed, peeled, sliced, room-dried and ground. The crude extracts of the plants were obtained using aqueous and methanol as solvents according to standard procedures while the phytochemical constituents were also evaluated using standard methods. The antimicrobial activities of the extracts were determined using the agar-well diffusion method. The bacterial species investigated were *Escherichia coli*, *Enterococcus faecalis*, *Klebsiellla pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, while the fungal species were *Aspergillus flavus, Aspergillus glaucus, Candida albicans, Candida tropicalis, Trichophyton mentagrophytes* and *Trichophyton rubrum*. The plant extracts contained alkaloids, saponins, phenols, flavonoid, tannin, phytate, terpenoids and cyanogenic glycoside in varying qualities and quantities. The extracts of the plants exerted antimicrobial effects against the test organisms. There was a significant difference between the antibacterial activities at 12.5 and 25 mg mL^{-1} concentrations ($p = 0.022$). The study revealed that the phytochemical components and antimicrobial properties of *M. myristica* extracts exhibit comparatively greater potency than those of *S. aethiopicus*, though the variances were not found to be statistically significant. The extracts of these plants could be purified, formulated and standardized for the production broad-spectrum antimicrobial agents.

Keywords: antimicrobials; antibiotic resistance; antifungal resistance; bacteria; fungi; medicinal plants.

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Introduction

The botanical realm stands as an expansive reservoir of biologically active compounds, boasting a rich tapestry of chemical structures. Among these, numerous indigenous plants, cherished both as culinary delights and medicinal treasures, harbor intrinsic properties that extend beyond mere sustenance. Their antimicrobial prowess and phytochemical composition underscore their significance, offering promise not just in nutrition but also in the quest for novel economic resources and therapeutic agents to combat resilient infectious pathogens (Sultan et al., 2023).

Despite advances in drug discovery, microbial infections—especially those resistant to treatment—remain a severe global health threat, particularly in tropical regions where they cause significant mortality (Bello et al., 2022). Rising antimicrobial resistance and the spread of multidrug-resistant strains have created a public health crisis, restricting treatment options (Balakrishnan et al., 2020). In response, exploring antimicrobial properties of certain plants has become essential. In many places, medicinal plants play a crucial role in healthcare, providing accessible and affordable remedies, especially in low-resource settings where they often form the foundation of primary healthcare (Ekor, 2014; Mokgehle et al., 2017; Kone et al., 2020; Noites et al., 2023).

Siphonochilus aethiopicus (Schweinf.) BL Burtt, known as African ginger, is a rhizomatous herb in the Zingiberaceae family, which includes around 52 genera and 1600 species of flowering plants. Native to equatorial and subtropical regions, it is commonly cultivated in tropical southern areas such as Malawi, Nigeria, South Africa, and Zambia (Atindehou et al., 2019; Seile et al., 2022). In Nigeria, *S. aethiopicus* is widely

used in traditional medicine to treat colds, hemorrhoids, and microbial infections and is also valued as a culinary spice. It is notably recognized for promoting women's fertility and facilitating erection (Krishnamoorthy et al., 2014; Noudogbessi et al., 2012). Research has identified various therapeutic properties of *S. aethiopicus*, including anti-cancer activity against MCF-7 cells (Noudogbessi et al., 2012), antiplasmodial activity (Igoli et al., 2021), antioxidant potential, phytochemical richness, and antibacterial effects (Jasson et al., 2023; Verrillo et al., 2021). Its essential oils, derived from leaves, rhizomes, and roots, contain compounds like monoterpenes and sesquiterpenes, effective against bacteria, fungi, trypanosomes, and tumor cells (Al-Tannak et al., 2022).

Monodora myristica, also known as African nutmeg, calabash nutmeg, or Jamaican nutmeg, is a multi-seed berry native to West Africa and belongs to the Annonaceae family (Sultan et al., 2023). Thriving in tropical forests, it grows in West Africa, Uganda, Kenya, and Tanzania, and has been introduced to Jamaica and the Caribbean. The tree has large, veined, deep green leaves and distinctive, aromatic flowers with hanging stalks, leading to woody fruits containing seeds within a fragrant pulp (Sindhusha et al., 2023). *Monodora myristica* has significant economic and medicinal value (Balakrishnan et al., 2020). Its seeds are traditionally used for treating ailments like headaches, pain, toothaches, hemorrhoids, and post-childbirth uterine hemorrhage (Noites et al., 2023). Economically, the seeds are prized; they are ground as a spice, especially in pepper soup, and serve medicinal and culinary roles in Africa and the Caribbean (Udeala et al., 1980; Igoli & Igoli, 2017). Recently, they have been used as popcorn flavoring. Research highlights the antioxidant properties of its essential oils (Owotokomo & Ekundayo, 2012; Das et al., 2020).

In the domain of nutrition, foods rich in phytochemicals provide not just essential antioxidant vitamins like vitamin C, vitamin E, and pro-vitamin A, but also a diverse array of other natural compounds with potent antioxidant capabilities (Sharififar et al., 2009). Extracts derived from spices have exhibited notable antioxidant activity, playing a crucial role in preventing undesirable flavor changes in snack foods and meat products (Bello & Bello, 2022).

Siphonochilus aethiopicus and *M. myristica* possess considerable medicinal significance and have undergone relatively extensive research to elucidate their pharmacological properties, including anti-asthmatic, antimalarial, antibacterial, antifungal, and anti-allergic effects. However, there remains a dearth of research focusing specifically on clinically relevant pathogenic bacterial and fungal strains, as investigated in this study. Thus, the primary objective of this research was to explore the phytochemical composition and antimicrobial potential of aqueous and methanolic extracts derived from *S. aethiopicus* and *M. myristica* against a selection of clinically significant bacterial and fungal strains.

Material and methods

Plant collection and authentication

The rhizomes of *S. aethiopicus* (Figure 1) and seeds of *M. myristica* (Figure 2) were collected in June 2023 during the morning hours from the Department of Forest Product Development and Utilisation of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria. The plant parts were authenticated by a specialist in Botanic.

Figure 1. A photograph of the rhizomes of *Siphonochilus aethiopicus*.

Figure 2. A photograph of the seeds of *Monodora myristica*.

Source of microorganisms

A total of 12 microbial species of clinical origin obtained from the University of Medical Sciences Teaching Hospital, Ondo State, Nigeria were investigated in this study. The bacterial species were *Escherichia coli*, *Enterococcus faecalis*, *Klebsiellla pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, while the fungal species were *Aspergillus flavus, Aspergillus glaucus, Candida albicans, Candida tropicalis, Trichophyton mentagrophytes* and *Trichophyton rubrum*. These organisms were subcultured to obtain the young actively growing cultures from the stocked cultures from previous studies (Kone et al., 2020; Godwin et al., 2021; Bello et al., 2022). The bacterial cultures were maintained on nutrient agar slants while the fungal spore suspension was kept on potato dextrose agar at 4°C until use.

Preparation of plant extracts

The rhizomes of *S. aethiopicus* were first washed thoroughly. The washed rhizomes were peeled, sliced and room-dried in the dark for three weeks. These were blended into powdery form using a domestic giant blender. A similar process was carried out on the seeds of *M. myristica.* The seeds were thoroughly washed, room-dried in the dark for three weeks, shelled and milled into powder. Then, 200 g of powdered forms of these plant parts were separately extracted with 800 mL of aqueous and methanol in different conical flasks. They were left for 72 hours in an orbital shaker and, thereafter, filtered using Whatman filter paper No 1. The filtrates were evaporated to dryness by means of a rotary evaporator attached to a vacuum pump. The percentage yield of each of the crude extract was measured for each solvent using the formula below:

The percentage yield crude extract = [dry weight (extract) / dry sample weight] x 100

Each extract obtained was separately weighed into airtight sample bottle and maintained in the refrigerator at 4°C until the experiments (Oni et al., 2021).

Phytochemical analysis

Phytochemical analyses were conducted employing standard laboratory techniques for both qualitative and quantitative assessments. The plant extracts underwent screening to detect alkaloids, saponins, phenols, flavonoids, tannins, phytate, terpenoids, and cyanogenic glycoside, following the methodologies outlined in earlier works by Evans (1989), Harbone (1998), and Sofowora (2008).

Qualitative phytochemical analysis

Alkaloids: The extract underwent testing by mixing with 2 mL of Wagner's reagent. The formation of a reddish-brown precipitate, as per the method outlined by Evans (1989), indicated the presence of alkaloids.

Cyanogenic glycosides - Fehling's test: A mixture of equal parts of Fehling's solutions I and II (5 mL) was added to approximately 3 mL of the extract and boiled for 5 minutes. The presence of a denser brick-red precipitate, following Evans' (1989) procedure, indicated the presence of glycoside.

Flavonoids: The alkaline reagent test was employed to assess the presence of flavonoids. The extracts were combined with 2 mL of a 2% NaOH solution. The development of an intense yellow color, turning colorless upon the addition of a few drops of diluted acid, confirmed the presence of flavonoids (Harbone, 1998).

Phenol analysis: Two grams (2 g) of the extract was immersed in 20 mL of methanol and then filtered. Testing was conducted by adding 1 mL of Folin-Concalteon with 1 mL of 20% $NaCO_3$ to 1 mL of the filtrate. The presence of phenol was indicated by the formation of a dark blue color, following Evans' (1989) method.

Phytate: One gram (1 g) of the extract was soaked in 2% hydrochloric acid for 3 hours, filtered, and then titrated against standard iron (III) chloride solution after the addition of ammonium thiocynate solution as an indicator. The persistence of a brownish-yellow color for 5 minutes, following Harbone's (1998) protocol, confirmed the presence of phytate.

Saponins: The foam test was conducted by adding 6 mL of water to 2 mL of the extract in a test tube and vigorously shaking. The formation of persistent foam confirmed the presence of saponins, following Harbone's (1998) methodology.

Terpenoids: The Salkowski test was employed to determine the presence of terpenoids. The sample extract solution was thoroughly mixed with 1 mL of chloroform, and the introduction of 3 mL of concentrated sulfuric acid resulted in a reddish-brown coloration at the interface, indicating the presence of terpenoids as per Sofowora (2008).

Tannins: Boiling 5 g of the extracts with 40 mL of water, followed by filtration and testing with ferric chloride, revealed the presence of tannins. A greenish-black precipitate, according to Sofowora (2008), confirmed the presence of tannins.

Quantification of phytochemicals in the extracts

Alkaloids: A beaker containing 5 g of the sample was filled with 200 mL of 10% acetic acid. After adding ethanol, the mixture stood for 4 minutes, underwent filtration, and the extract was concentrated in a water bath to one quarter of the original volume. Ammonium hydroxide solution was added dropwise until precipitation was complete. The resulting precipitate was washed with dilute ammonium hydroxide, filtered, dried, and weighed. The dried residue constituted the alkaloid, as per the method outlined by Evans (1989).

Cyanogenic glycosides: Approximately 2 mL of the sample dissolved in water was transferred into a conical flask containing chloroform, and the mixture was then filtered into another conical flask. Subsequently, 2 mL of pyridine and 29% sodium nitroprusside were added, followed by thorough shaking for 10 minutes. Further, 20% NaOH was introduced, and color development and absorbance were measured at 510 nm using a SpectrumLab70 spectrophotometer, following Evans' (1989) procedure.

Flavonoids: Dissolving about 0.25 g of the plant extract in 1 mL distilled water, 5% $NaNO_2$ solution, 150 µL of freshly prepared aluminum chloride ($AlCl_3$), and 1 M NaOH solutions were added. The mixture stood for 5 minutes, and absorbance was recorded at 510 nm on a spectrophotometer (SpectrumLab70), with results expressed as equivalents to quercetin, following Harbone's (1998) methodology.

Saponins: The saponin content was determined spectrophotometrically. Two grams of the sample were weighed into a beaker, and isobutyl alcohol (but-2-ol) was added. After filtration into a beaker containing 40% magnesium carbonate ($MgCO_3$) solution, approximately 1 mL of the solution was transferred into a volumetric flask. Then, 2 mL of iron (III) chloride ($FeCl_3$) solution was added, and the mixture stood for 30 minutes. Color development and absorbance were recorded at 380 nm on a SpectrumLab70 spectrophotometer, in accordance with Evans' (1989) method.

Total polyphenols: Using the Folin-Ciocalteu method, 125 µL of the plant extract was treated with distilled water and Folin-Ciocalteu's reagent. After standing for 6 minutes, 7% sodium carbonate solution was added, and the mixture stood for an additional 90 minutes. Absorbance was read at 760 nm on a SpectrumLab70 spectrophotometer, and the result was expressed as gallic acid equivalents, following Harbone's (1998) protocol.

Tannins: The total tannin content was evaluated following the established procedure by Keerthana *et al*. (2013). This involved diluting 0.5 mL of the sample extract with 80% ethanol. A 0.1 mL portion of the diluted sample was mixed with 2 mL of Folin-Ciocalteu reagent and allowed to stand for 7 minutes. Subsequently, 7.5 mL of 7% sodium carbonate solution was introduced, and the mixture stood for 2 hours. Absorbance was measured at 760 nm, and the tannin content was estimated using a tannic acid curve as the standard, as outlined by Harbone (1998).

Terpenoids: One hundred milligrams (100 mg) of the extract were soaked in 9 mL of ethanol for 24 hours. After filtration, the extract was subjected to extraction with 10 mL of petroleum ether using a separating funnel. The ether extract was separated into pre-weighed glass vials and dried completely. The ether was

evaporated, and the yield percentage of total terpenoid contents was measured, following the procedures outlined by Sofowora (2008).

Phytate: For each extract, 1 g was weighed into conical flasks, and a 100 mL portion of 2% HCl was added to digest the samples for 3 hours. The digested samples were filtered using filter paper, and 25 mL of the filtrates were measured into 250 mL conical flasks. A 5 mL volume of 0.3% NH_4SCN solution was introduced, and the resulting mixtures were titrated against 0.1 M ferrous chloride ($FeCl_3$) until a brownish-yellow color endpoint persisted for 5 minutes. The percentage phytate content was calculated as: titre value x 0.00195. Each determination process was conducted in triplicate, and the average titre value was obtained, following the protocols outlined by Sofowora (2008).

Antimicrobial assay

The antimicrobial efficacy of distinct concentrations (12.5 and 25 mg mL^{-1}) of aqueous and methanolic extracts from *S. aethiopicus* and *M. myristica* was individually assessed using the agar-well diffusion method. Cultures of the test organisms were cultivated on nutrient agar plates (for bacteria) and potato dextrose agar (PDA) plates (for fungi) following incubation for 18 hours at 37°C. Young actively growing bacterial cells and fungal spores were prepared to achieve a suspension equivalent to the McFarland standard, resulting in a final inoculum size of 0.5×10^8. For antimicrobial sensitivity testing, the bacterial suspension was inoculated on solidified Mueller Hinton agar (MHA) plates, and the fungal suspension was spread on PDA plates using the spread plate method. Wells with a diameter of six millimeters were created on the MHA and PDA plates, into which 0.5 mL of varying concentrations of aqueous and methanolic extracts from the plants were separately introduced. After standing for 30 minutes, the MHA plates were incubated at 37°C for 24 hours, while the PDA plates were incubated at 24°C for 48 hours. All experiments were conducted in triplicate under aseptic conditions. The appearance of clear zones of inhibition (mm) around the wells indicated the antimicrobial activities of the preparations. Average values were calculated and recorded accordingly (Bello et al., 2022). Negative controls for antibacterial and antifungal assays consisted of uninoculated MHA and PDA plates, respectively while the positive controls included MHA wells inoculated with ciprofloxacin (10 mg mL^{-1}) and PDA wells with fluconazole (100 mg mL^{-1}), respectively.

Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

The determination of minimum inhibitory concentrations (MIC) for the plant extracts utilized the broth dilution method. Seven concentrations (200, 100, 50, 25, 12.5, 6.25, and 3.125 mg/mL) of the extracts were examined. Test tubes were prepared with 9 mL of nutrient broth, and each received 1 mL of the standard inoculum (0.5×10^8) from the bacterial suspension. The tubes were individually inoculated with varying concentrations of plant extracts and incubated at 37°C for 24 hours, observing for bacterial growth. Similarly, for fungi, test tubes with 9 mL of potato dextrose broth were prepared, and 1 mL of the standard fungal inoculum (0.5×10^8) was inoculated into each broth. The tubes were individually treated with different concentrations of plant extracts and incubated at 25°C for 72 hours, monitoring for fungal growth, indicated by turbidity of the medium.

To determine the minimum bactericidal concentrations (MBC) of the extracts, a 20-µL sample from test tubes without bacterial growth was inoculated onto nutrient agar plates and incubated at 37°C for 24 hours. The MBC was considered the lowest extract concentration inhibiting the viability of the initial bacterial inoculum by at least 99.9% (Bello et al., 2022). Additionally, Minimum Fungicidal Concentrations (MFC) were determined by subculturing 2 µL from tubes showing no growth onto potato dextrose agar (PDA) plates and further incubating at 28°C for 72 hours. The lowest concentration without visible growth was defined as the MFC, indicating 99.5% killing of the original fungal inoculum. Fluconazole (Sigma) served as the positive control (1–3000 µg mL^{-1}), and DMSO (99.9%) served as the negative control for fungi. All experiments were conducted in duplicate and repeated three times for reproducibility.

Statistical analysis

The acquired data underwent statistical analysis utilizing the Statistical Package for Social Sciences (SPSS), IBM version 25. To assess variations in phytochemical constituents among plant extracts and the antimicrobial activities against test organisms, the Student's T-test was employed. Significance was attributed to values with $p < 0.05$.

Results

The qualitative screening of aqueous and methanolic extracts of fresh rhizomes of *S. aethiopicus* and seeds of *M. myristica* showed the presence of alkaloids, cryogenic glycosides, flavonoid, phenol, saponins, phytate, tannins, and terpenoids (Table 1). The phytochemical constituents were present in varying qualities and appeared to be present in larger qualities in methanolic extracts than the aqueous extracts. With the exemption of alkaloids, saponins, and dosage of total polyphenols, the quantitative phytochemical constituents of aqueous and methanolic extracts of *M. myristica* showed no significant difference ($p = 0.065$) (Table 2). Similarly, excluding terpenoids, the difference among the aqueous-extracted phytochemicals from *S. aethiopicus* and *M. myristica* was significant ($p = 0.039$). Furthermore, except from saponins and terpenoids, the methanolic extracts of the two plants exhibited statistical difference ($p = 0.043$). There was a statistical difference between the alkaloid, saponin, and total phenol contents of the aqueous and methanolic extracts of *M. myristica* ($p = 0.035$). Furthermore, the contents of alkaloids, cyogenic glycosides, total polyphenols, and total flavonoids in the extracts of *M. myristica* were significantly higher than those of *S. aethiopicus* ($p = 0.031$), while the tannin in both aqueous and methanolic extracts of *M. myristica* was significantly lower ($p = 0.049$) (Table 2).

Table 1. Qualitative screening of aqueous and methanolic extracts of rhizomes of *Siphonochilus aethiopicus* and seeds of *Monodora myristica*.

Phytochemicals	*Siphonochilus aethiopicus*		*Monodora myristica*	
	Aqueous	Methanol	Aqueous	Methanol
Alkaloids	+	++	++	++
Cyanogenic glycosides	++	++	+++	+++
Phytate	++	++	++	+++
Saponins	++	+++	++	++
Tannins	++	++	+	+
Terpenoids	+	++	++	+++
Total Flavonoids	++	++	++	+++
Total polyphenols	++	++	++	+++

Keys: - = Not present; + = Present in low quantity; ++ = Present in moderate quantity; +++ = Present in large quantity.

Table 2. Quantitative phytochemical constituents of aqueous and methanolic extracts of rhizomes of *Siphonochilus aethiopicus* and *Monodora myristica*.

Phytochemicals	*Siphonochilus aethiopicus*		*Monodora myristica*	
	Aqueous	Methanol	Aqueous	Methanol
Alkaloids (mg g^{-1})	105.74 ± 2.33a	107.31 ± 2.87a	226.23 ± 4.22b	235.75 ± 4.31c
Cyanogenic glycosides (mg g^{-1})	0.15 ± 0.00a	0.18 ± 0.01a	1.82 ± 0.04b	1.87 ± 0.05b
Phytate (mg g^{-1})	1.32 ± 0.09a	1.29 ± 0.10a	2.85 ± 0.33b	3.01 ± 0.27b
Saponin (mg g^{-1})	23.06 ± 1.62b	23.51 ± 0.99b	13.06 ± 1.11a	20.01 ± 2.36b
Tannins (mg g^{-1})	85.63 ± 2.21b	90.59 ± 2.27b	70.50 ± 1.48a	78.75 ± 1.99a
Terpenoids (mg g^{-1})	2.10 ± 0.21a	2.53 ± 0.29a	2.72 ± 0.19a	3.87 ± 2.23a
Total flavonoid (mgQE g^{-1})	30.60 ± 2.69a	31.32 ± 2.33a	72.00 ± 3.97b	73.82 ± 3.65b
Total polyphenols (mgGAE g^{-1})	129.55 ± 3.39a	131.29 ± 4.30a	269.20 ± 3.52b	279.28 ± 4.85c

Data show the mean values of triplicate experiments ± standard deviations. Data with different alphabetical superscripts along same row showed statistical differences at $\alpha = 0.05$.

The test organisms showed varying zones of inhibition to the aqueous and methanolic extracts of *S. aethiopicus* and *M. myristica.* However, the antibacterial activities exerted by these extracts showed no significant differences at 12.5 and 25 mg mL^{-1} concentrations considering the data along the same row ($p = 0.114$) (Table 3).

Table 3. Antibacterial activities of aqueous and methanolic extracts of *Siphonochilus aethiopicus* and *Monodora myristica*.

Bacteria	Ciprofloxacin (10 mg mL^{-1})	Zones of Inhibition in mm at different concentrations (percentage of control)							
		Siphonochilus aethiopicus				*Monodora myristica*			
		Aqueous (mg mL^{-1})		Methanol (mg mL^{-1})		Aqueous (mg mL^{-1})		Methanol (mg mL^{-1})	
		12.5	25	12.5	25	12.5	25	12.5	25
E. coli	25.0	12.0a ± 0.9	20.5b ± 2.1	14.0a ± 1.7	23.5b ± 2.0	16.5a ± 1.5	22.0b ± 1.9	20.5b ± 1.7	24.0b ± 2.5
E. faecalis	23.5	14.5a ± 1.2	22.5b ± 1.8	17.0a ± 2.0	24.0b ± 2.5	11.0a ± 0.7	23.0b ± 2.1	19.0b ± 1.9	25.5b ± 1.2
K. pneumoniae	23.0	7.0a ± 0.6	16.5b ± 1.2	15.0b ± 1.1	21.0c ± 1.8	9.5a ± 0.9	18.5b ± 1.5	19.5b ± 0.5	23.0c ± 1.5
P. aeruginosa	21.5	13.0a ± 0.7	22.5b ± 1.8	15.5a ± 0.7	24.0b ± 2.3	13.0a ± 0.8	20.0b ± 2.5	20.5b ± 1.75	25.5b ± 2.3
S. aureus	25.5	12.5a ± 0.8	20.0b ± 1.8	19.0b ± 1.0	26.5b ± 2.1	18.0a ± 0.9	21.5b ± 2.6	22.0b ± 1.2	28.0b ± 2.6
S. epidermidis	24.0	12.0a ± 1.2	18.5b ± 2.10	17.0b ± 1.7	25.0c ± 2.5	13.5a ± 1.1	20.0b ± 2.0	19.5b ± 1.6	25.5c ± 2.6

Data show the mean values of triplicate experiments ± standard deviations. Data with different alphabetical superscripts along same row showed statistical differences at $\alpha = 0.05$.

The antifungal activities of aqueous and methanolic extracts of *S. aethiopicus* and *M. myristica* also showed no significant difference (p = 0.191), although varying zones of inhibition were recorded (Table 4). There were no significant differences in the antifungal activities exerted upon *A. flavus*, *T. mentagrophytes* and *T. rubrum* by the aqueous extract at 12.5 and 25 mg mL^{-1} concentrations (p = 0.351) (Table 4). However, there were statistical differences (p = 0.041) between the antifungal activities of methanolic extracts of *S. aethiopicus*. Similarly, the antifungal activities of the methanolic extract of *M. myristica* showed significant difference (p = 0.042).

Table 4. Antifungal activities of aqueous and methanolic extracts of *Siphonochilus aethiopicus* and *Monodora myristica*.

Fungi	Fluconazole (100 mg mL^{-1})	*Siphonochilus aethiopicus*				*Monodora myristica*			
		Aqueous (mg mL^{-1})		Methanol (mg mL^{-1})		Aqueous (mg mL^{-1})		Methanol (mg mL^{-1})	
		12.5	25	12.5	25	12.5	25	12.5	25
A. flavus	27.0	14.0a ± 1.0	19.0a ± 1.7	17.0a ± 0.9	28.5b ± 2.1	15.5a ± 1.1	25.0b ± 2.6	21.5b ± 2.4	29.5b ± 3.1
A. parasiticus	30.0	12.0a ± 0.9	20.5b ± 2.0	20.0b ± 1.7	31.0c ± 1.8	16.5a ± 1.3	24.5b ± 2.3	21.0b ± 1.9	32.5c ± 2.3
C. albicans	28.5	15.0a ± 1.2	21.5b ± 2.0	19.0a ± 1.9	27.0b ± 2.3	14.0a ± 1.3	24.0b ± 1.6	17.0a ± 1.8	29.0b ± 2.4
C. tropicalis	25.0	13.5a ± 1.4	2.5b ± 1.9	22.0b ± 2.5	31.0c ± 2.7	17.0a ± 1.7	20.0b ± 2.0	20.0b ± 2.5	30.5c ± 2.6
T. mentagrophytes	28.0	15.0a ± 1.2	17.5a ± 1.6	19.5a ± 1.5	28.0b ± 1.6	13.5a ± 1.3	19.0a ± 2.1	19.5a ± 1.7	26.5b ± 2.5
T. rubrum	26.5	13.0a ± 1.1	18.5a ± 1.4	19.5a ± 1.5	30.0c ± 2.6	11.5a ± 1.4	23.5b ± 2.1	20.0a ± 1.3	28.0c ± 2.0

Data show the mean values of triplicate experiments ± standard deviations. Data with different alphabetical superscripts along same row showed statistical differences at α = 0.05.

The MIC and MBC of *S. aethiopicus* and *M. myristica* extracts on clinical bacterial isolates is shown in Table 5. The MIC of the aqueous extract of *S. aethiopicus* on bacterial species ranged from 3.125 to 12.5 mg mL^{-1} while its MBC ranged from 50 to 200 mg mL^{-1}. However, the MIC of the methanolic extract of *S. aethiopicus* ranged from 3.125 to 6.25 mg mL^{-1} while its MBC ranged from 12.5 to 100 mg mL^{-1}. Similarly, the MIC of the aqueous extract of *M. myristica* on bacterial species ranged from 3.125 to 12.5 mg mL^{-1} while its MBC ranged from 25 to 100 mg mL^{-1}. Furthermore, the MIC of the methanolic extract of *M. myristica* ranged from 3.125 to 12.5 mg mL^{-1} while its MBC ranged from 6.25 to 100 mg mL^{-1}. The MIC of the methanolic extracts of *M. mystirica* on the various clinical bacterial isolates, excluding *K. pneumoniae* showed no statistical difference from that of *S. aethiopicus* (p = 0.093). In similar vein, there was no statistical difference between the MICs of the aqueous extracts of the two plants (p = 0.102). However, the MBC of the methanolic extract of *M. mystirica* was signifantly lower than that of *S. aethiopicus* (p = 0.031) (Table 5).

Table 5. The MIC and MBC of *Siphonochilus aethiopicus* and *Monodora myristica* extracts on clinical bacterial isolates.

Bacteria	*Siphonochilus aethiopicus*				*Monodora myristica*			
	Aqueous (mg mL^{-1})		Methanol (mg mL^{-1})		Aqueous (mg mL^{-1})		Methanol (mg mL^{-1})	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	6.25^{a}	100^{c}	6.25^{a}	50^{b}	6.25^{a}	100^{c}	6.25^{a}	50^{b}
E. faecalis	3.125^{a}	50^{d}	3.125^{a}	25^{c}	3.125^{a}	25^{c}	3.125^{a}	6.25^{b}
K. pneumoniae	12.5b	200^{d}	3.125^{a}	100^{c}	12.5	100^{c}	12.5^{b}	100^{c}
P. aeruginosa	3.125^{a}	50^{c}	3.125^{a}	12.5^{b}	3.125^{a}	50^{c}	3.125^{a}	12.5^{b}
S. aureus	3.125^{a}	50^{c}	3.125^{a}	12.5^{b}	3.125^{a}	50^{c}	3.125^{a}	12.5^{b}
S. epidermidis	3.125^{a}	100^{c}	3.125^{a}	12.5^{b}	3.125^{a}	12.5^{b}	3.125^{a}	12.5^{b}

Data with different alphabetical superscripts along same row showed statistical differences at α = 0.05.

Table 6 shows the MIC and MFC of *S. aethiopicus* and *M. myristica* extracts on clinical fungal isolates. The MIC and MFC of the aqueous extract of *S. aethiopicus* against all fungal isolates were 12.5 and 200 mg mL^{-1}, respectively. The MIC of the methanolic extract of *S. aethiopicus* was 12.5 mg mL^{-1} while its MFC ranged from 12.5 to 50 mg mL^{-1}. In the same vein, the MIC of the aqueous extract of *M. myristica* ranged from 6.25 to 12.5 mg mL^{-1} while its MFC ranged from 100 to 200 mg mL^{-1}; the MIC and MFC of its methanolic extract were 3.125 and 12.5 mg mL^{-1}, respectively. The MIC of the methanolic extract of *M. mystirica* against the clinical fungal isolates was significantly lower than that of *S. aethiopicus* (p = 0.025).

Table 6. The MIC and MFC of *Siphonochilus aethiopicus* and *Monodora myristica* extracts on clinical fungal isolates.

Fungi	*Siphonochilus aethiopicus*				*Monodora myristica*			
	Aqueous (mg mL^{-1})		Methanol (mg mL^{-1})		Aqueous (mg mL^{-1})		Methanol (mg mL^{-1})	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
A. flavus	12.5^{b}	200^{d}	25^{c}	12.5^{b}	12.5^{b}	200^{d}	3.125^{a}	12.5b
A. parasiticus	12.5^{c}	200^{g}	25^{d}	50^{e}	6.25^{b}	100^{f}	3.125^{a}	12.5c
C. albicans	12.5^{c}	200^{g}	25^{d}	50^{e}	6.25^{b}	100^{f}	3.125^{a}	12.5c
C. tropicalis	12.5^{b}	200^{f}	25^{c}	50^{d}	12.5^{b}	100^{e}	3.125^{a}	12.5b
T. mentagrophytes	12.5^{c}	200^{e}	25^{d}	12.5^{c}	6.25^{b}	200^{e}	3.125^{a}	12.5c
T. rubrum	12.5	200	25	50	12.5	200	3.125a	12.5

Data with different alphabetical superscripts along same row showed statistical differences at $\alpha = 0.05$.

Discussion

Phytochemical activity

Traditional African medicine relies on numerous plants for treating bacterial and fungal ailments, a practice attributed to the secondary metabolites within these plants. While *S. aethiopicus* has been acknowledged for managing various diseases, information on the antimicrobial activities and phytochemical constituents of its aqueous and methanolic extracts is limited. This study identified alkaloids, cyanogenic glycosides, total polyphenols, saponins, tannins, terpenoids, and total flavonoids in both extracts of *S. aethiopicus*. Previous studies in Nigeria and South Africa identified alkaloids, flavonoids, and various terpenoid compounds, such as monoterpenes and sesquiterpenes, in volatile extracts of fresh rhizomes of *S. aethiopicus* (Igoli et al., 2014). The antifungal activity of *S. aethiopicus* aerial parts and rhizomes may be attributed to these antimicrobial compounds. Acetone extracts of *S. aethiopicus*, rich in flavonoids and phenolic acids, have demonstrated antimicrobial activity (Xego et al., 2017).

Terpenoids are renowned for their diverse activities, including antitumoral, antimicrobial, antiviral, anti-inflammatory, antiparasitic, and hyperglycemic properties (Paduch et al., 2007). Alkaloids, known for antioxidative, antimutagenic, anticarcinogenic, and antimicrobial activities, constitute a significant family of active compounds (Kaur, 2015). Flavonoids, recognized for their antifungal, antibacterial, and antioxidant characteristics, play a crucial role in plant defense (+adimurthy et al., 2023). Saponins, tannins, and anthraquinone were screened from *S. aethiopicus* by Noudogbessi et al. (2012) which aligns with our findings, although coumarins were absent in this study.

The aqueous and methanolic extracts of *M. myristica* similarly revealed the presence of alkaloids, cyanogenic glycosides, total polyphenols, saponins, tannins, terpenoids, and total flavonoids. Firempong et al. (2016) reported saponins, tannins, flavonoids, alkaloids, and general glycosides in ethanolic extracts of *M. myristica* plant parts. Nkwocha et al. (2018) reported substantial amounts of steroids and moderate quantities of saponins and terpenoids in *M. myristica*. Discrepancies in phytochemical quantities can be attributed to variations in extraction methods, solvents, plant parts, and geographical locations, as also noted by Firempong et al. (2016) and Oni et al. (2021).

The antimicrobial prowess of tannins arises from their capacity to deactivate microbial adhesions, enzymes, and cell envelope transport proteins, thereby impeding crucial microbial processes. Tannins play a role in thwarting the formation of iron, disrupting hydrogen bonding, or impeding specific interactions with vital proteins, such as enzymes within microbial cells (Jadimurthy et al., 2023). Flavonoids, on the other hand, exhibit the ability to form complexes with extracellular and soluble proteins, disrupting microbial membranes and hindering microbial growth (Oni et al., 2021). Alkaloids showcase their antimicrobial attributes by intercalating into microbial cell walls or DNA, inducing genetic modifications and structural instability (Yan et al., 2021).

The quantities of phytochemical constituents in the aqueous and methanolic extracts of *M. myristica* were significantly higher than those in *S. aethiopicus* ($p = 0.006$). Conversely, the concentrations of saponins and tannins were notably higher in *S. aethiopicus* than in *M. myristica* ($p = 0.04$). There existed a statistical disparity in the alkaloid, saponin, and total phenol contents between the aqueous and methanolic extracts of *M. myristica* ($p = 0.025$). Specifically, the contents of alkaloids, cyanogenic glycosides, total polyphenols, and total flavonoids in the aqueous and methanolic extracts of *M. myristica* were markedly higher than those in *S. aethiopicus* (Tables 1 and 2).

Antibacterial activity

The antimicrobial activities of the extracts revealed the antibacterial and antifungal potentials of *S. aethiopicus*. The aqueous and methanolic extracts of *S. aethiopicus* exerted varying degrees of antimicrobial activities on the bacterial species including *E. coli*, *Enterococcus faecalis*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, and *S. epidermidis*. These activities could be associated with the presence of certain phytochemicals such as flavonoids and terpenes which contain antibacterial properties (Jadimurthy et al., 2023). Although these compounds were found in both the aqueous and methanolic extracts of the *S. aethiopicus* in this study but methanolic extract was more active than the aqueous extract. This could be due to the presence of some other antibacterial compounds in the methanolic extract or as a result of molecular interactions among the various compounds in the methanolic extract. It could be also explained by the quantity of the compounds in both extracts. The ethyl acetate and alcoholic extracts of the various organs, such as leaves, rhizomes and roots of *S. aethiopicus* are found to be active on *E. coli, S. aureus, B. subtilis* and *K. pneumoniae* in South Africa (Coopoosamy et al., 2010).

The MIC of the aqueous extract of *S. aethiopicus* on bacterial species ranged from 3.125 to 12.5 mg mL^{-1} while its MBC ranged from 50 to 200 mg mL^{-1}. However, the MIC of the methanolic extract of *S. aethiopicus* ranged from 3.125 to 6.25 mg mL^{-1} while its MBC ranged from 12.5 to 100 mg mL^{-1}. A similar study showed a MIC value of ethyl acetate extract of rhizome of *S. aethiopicus* to be 5 mg mL^{-1} as previously by Coopoosamy et al. (2010). These results showed that aqueous and methanolic extracts of *S. aethiopicus* possess a broad-spectrum activity against a wide range of microorganisms including Gram-positive and Gram-negative bacteria, and some fungi. Atindehou et al. (2019) also asserted that ethyl acetate extract of the dry rhizome of *S. aethiopicus* showed antibacterial activities against selected bacterial and fungal strains. Enabulele et al. (2014) had earlier observed that the ethanolic extracts, at various concentrations, were more effective against the test organisms used in their study than the aqueous extracts.

Antifungal activity

The aqueous and methanolic extracts of *S. aethiopicus* and *M. myristica* showed antifungal activities against *A. flavus, A. glaucus, C. albicans, C. tropicalis, T. mentagrophytes* and *T. rubrum*. Sipahelut et al. (2019) investigated the antifungal and antibacterial properties of nutmeg fruit flesh oil, demonstrating its ability to inhibit the growth of food-spoiling fungi, food-spoiling bacteria, and pathogenic bacteria. Hoda et al. (2020) investigated the potential antifungal properties of hexane extract of *M. fragrans* against *A. fumigatus*. Their findings revealed that the extract inhibited melanin production by 76.09%, reduced ergosterol content by 83.63%, and decreased *A. fumigatus* cell hydrophobicity by 72.2% at a minimum effective concentration (MEC) of approximately 0.078 mg mL^{-1}. Additionally, Suthisamphat et al. (2020) demonstrated that the ethanolic extract of mace exhibited antimicrobial activity against six strains, including *H. pylori*, with MICs ranging from 125 to 250 µg mL^{-1}.

Firempong et al. (2016) assessed the antifungal activity of extracts from various parts of *M. myristica*. The ethanolic extracts of *M. myristica* contained a combination of triterpenoids, flavonoids, saponins, alkaloids, glycosides, steroids, and tannins. Among these extracts, the roots and seeds showed the most potent inhibition of *C. albicans* growth. Notably, the antifungal activities of most plant extracts were comparable to those of the standard drug, Clotrimazole. In this study, the antifungal activities exerted by the aqueous and methanolic extracts *S. aethiopicus* and *M. myristica* differed significantly ($p = 0.006$). This could not be dissociated from the differences in the phytochemical constituents of the plants and their parts investigated in this study. It had been earlier posited that certain plant parts exhibited more antifungal activity than others, even within the same species (Firempong et al., 2016). The high phytochemical compounds identified in these plants could have resulted in relatively high antifungal activities.

While variations in the zones of inhibition were observed, there was no statistically significant difference ($p = 0.14$) between the antifungal efficacy of the methanolic extracts from both plants at a concentration of 25 mg mL^{-1} and the benchmark antifungal drug, fluconazole. It is noteworthy that further refinement of the crude extracts holds the potential to yield activities on par with standard antibiotics or, in some cases, surpass them, mirroring observations in numerous isolated active compounds from similar sources. The dose-dependent nature of the antimicrobial activities of the plant extracts was evident, with increased concentrations correlating with enhanced effectiveness.

Conclusion

The study revealed that the aqueous and methanolic extracts of rhizomes of *S. aethiopicus* and seeds of *M. myristica* possess antibacterial and antifungal activities. The extracts of these plants are rich in various phytochemicals that could be purified, formulated, and standardized for the production of broad-spectrum antimicrobial agents. The phytochemical constituents and antimicrobial activities of the extracts of seeds of *M. myristica* are relatively higher than those of rhizomes of *S. aethiopicus* but the differences were not statistically significant. These findings augment the available data on the medicinal values and phytochemicals constituents of *S. aethiopicus* and *M. myristica.*

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