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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF AFRICAN NUTMEG (*MONODORA MYRISTICA*)

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ABSTRACT

Ethanolic and aqueous (hot and cold) extracts of dried seeds of Monodora myristica were tested for antibacterial and antifungal activities. Six clinical bacterial isolates (Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Citrobacter freudii) and two fungal isolates (Candida albicans, Aspergillus flavus) were used. Bacterial isolates were cultured and examined microscopically to confirm their identities through Gram reactions and biochemical tests, while fungal isolates were examined on Sabouraud dextrose agar to confirm their characteristics. Results were analyzed using a three-way ANOVA factorial experiment. The susceptibility of the test organisms to the extracts showed significant differences ($P < 0.05$). Ethanolic extract had the highest activity, followed by hot water extract, and then cold-water extract. Ethanolic extract was most effective against Enterococcus faecalis (24.7 ± 1.5 mm) and least effective against Escherichia coli (7.7 ± 1.0 mm). Hot water extract showed highest activity against Candida albicans (18.0 ± 1.5 mm) and lowest against Enterococcus faecalis (7.7 ± 1.0 mm). Cold water extract was most effective against Pseudomonas aeruginosa (14.3 ± 1.2 mm) and least effective against Citrobacter freudii and Enterococcus faecalis (both 9.7 ± 1.0 mm). Minimum inhibitory concentrations (MIC) ranged from ≤62.5 to 1000mg/ml for ethanolic and hot water extracts, and from ≥62.5 to 1000mg/ml for cold water extract. Minimum bactericidal/fungicidal concentrations (MBC/MFC) ranged from 125 to >2000mg/ml for ethanolic extract, ≤250 to >2000mg/ml for hot water extract, and ≤125 to >2000mg/ml for cold water extract. These findings suggest that Monodora myristica seeds exhibit broad-spectrum activity against bacterial and yeast pathogens.

KEYWORDS

Antibacterial, Antifungal, Antibiotic resistance, Natural products

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INTRODUCTION

According to Jawetz et al. (2010), an antimicrobial is a chemical that either destroys or stops the growth of microorganisms like bacteria, fungi, or protozoans. The indiscriminate use of antibiotics is now a worldwide concern (Luc, 2004). The past years have seen a rise in the prevalence of various resistances in human pathogenic organisms, primarily because of improper use of commercial antimicrobial medications that are typically used in the treatment of illnesses. This clarified why researchers look for novel antimicrobial compounds in a variety of places, including medicinal plants (Irivine, 2000). Numerous medications can be found in plants (Pam plona-Roger, 2004). It is estimated that about 25% of medications or their active components come from plant materials. Since the beginning of time, nature has provided medicinal agents (Agbo & Mboto, 2012).

Monodora myristica is one of the medicinal plants found in African countries, including Nigeria (Ekeanyanwu & Etienajirheywe, 2012). Documented biological and pharmacological activities include, neuromuscular, anti-diarrhoeal, anti-sickle cell, anti-inflammatory properties, as well as treatments for headache, cough, fever, and skin diseases (Kigigh & Charlie, 2012). Additionally, it has germicidal or antiseptic properties (Irvine, 2000), can control passive uterine haemorrhage in women immediately after childbirth (Barba et al., 2007; Kigigh & Charlie, 2012), and can be used as an insect repellent and snuff (Ekeanyanwu et al., 2010). The seeds, when ground into powder, can relieve constipation (Kigigh & Charlie, 2012), and the plant is also used to treat pneumonia, tuberculosis, and upper respiratory tract infections (Cimanga et al., 2002).

Since the introduction of antibiotics, there has been a significant increase in the resistance of various bacterial pathogens (Kaushik & Goyal, 2008). Many antibiotics currently in use are either too expensive, have undesirable side effects, or have lost their potency due to bacterial resistance (Alper, 1998). Consequently, scientists are focused on discovering natural compounds from medicinal plants to introduce new, more effective drugs (Kandiah et al., 2010, Abiodun et al., 2017). Several species of plants have been used for centuries as remedies for human diseases (Kaushik, 2000, Adeshina et al., 2010). Plants contain bioactive components that make them important in both traditional and modern healthcare systems, prompting scientists to identify native medicinal plants in indigenous pharmacopeia (Adehola & Oladimeji, 2005, Pereira et al., 2023).

Therefore, this study is aimed at determining the antibacterial and antifungal activities of the dried seeds of African Nutmeg (*Monodora myristica)* on some bacterial and fungal species.

MATERIALS AND METHODS

Sample Preparation and Extraction

The sample was prepared based on the method described by (Abii & Eligalam 2007, Aqueel et al., 2023). The dry seeds of *Monodora myristica* were cracked and dried at 25°C for three days. The dried sample was then pounded and reduced to a fine paste using a sterilized mortar and pestle.

Aqueous Extraction

Aqueous extraction of the plant materials was carried out using both cold and hot water. Exactly 100 g of the paste sample was weighed and soaked in 500 ml of distilled water in a 1L capacity beaker for 24 hours at room temperature, with occasional stirring (Adeyeye & Otokit, 1999, Bitwell et al., 2023). The extract was then filtered using Whatman No.1 filter paper to obtain a solution free from solids. Another 100 g of the paste sample was soaked in 500 ml of hot water in a 1L capacity beaker and filtered in the same manner. Both filtrates were evaporated to dryness in a water bath at 100°C (Adehola & Oladimeji, 2005, Abha et al., 2015) to obtain a solid mass, which was weighed and stored properly in a tightly closed bottle in the refrigerator.

Ethanolic Extraction

For ethanolic extraction, 100 g of the paste sample was weighed and soaked in 500 ml of 95% ethanol in a 1L capacity flask for 24 hours. The mixture was then filtered using Whatman No.1 filter paper and evaporated to dryness in a water bath at 100°C (Adehola & Oladimeji, 2005, Bartnik & Facey, 2024) to remove the ethanol. The dried extract was weighed and stored in a tightly closed bottle in the refrigerator.

Determination of extract yield

The percentage yield of each extraction was determined gravimetrically (Brglez-moizer et al., 2016), using the formula below.

$$\text{Extract yield (\%)} = \frac{W_2 - W_1}{W_s} \text{X} \frac{100}{1}$$

Where:

W_1 = Weight of empty beaker

W_2 = Weight of beaker + extract after evaporation of the filtrate

Ws = Weight of the sample

Collection of Test Bacteria

Test bacterial pathogens were sourced from the University of Calabar Teaching Hospital (UCTH). The organisms were identified and confirmed using standard protocols for cultural and morphological identification, as well as biochemical characterization of isolates (Cheesbrough, 2002). The selection of clinical organisms was based on the classes of organisms commonly encountered in microbiological laboratories in Calabar.

Preparation and Confirmation Tests for Bacterial Isolates

a) **Macroscopic Examination**

Bacterial isolates from blood and urine clinical specimens were cultured on appropriate selective, enriched, and routine media such as Eosin Methylene Blue (EMB) agar, MacConkey agar (MCA), Mannitol Salt agar, Blood agar, Bile Esculin Agar (BEA), and Nutrient agar. They were incubated for 24 hours. After incubation, cultures were examined macroscopically for bacterial colonial characteristics such as pigmentation (diffusing or non-diffusing), nature of growth (pure or mixed), amount of growth [none, slight (+), moderate (++), heavy (+++)], opacity (transparent or translucent), shapes, form (pin or rhizoid), elevations (flat, raised, or convex), nature of colonies (dry, moist, or mucoid), and edges of colonies (entire or lobate) (Etok et al., 2004).

Mixed cultures were sub-cultured on nutrient agar and incubated for 24 hours at 37°C to obtain pure cultures. Criteria were based on culture morphology and colour characteristics. Pure

cultures were preserved on nutrient agar slants in the refrigerator until required for microscopic and biochemical characterization (Etok et al., 2004).

b) **Microscopic Examination**

Pure cultures of bacterial isolates were examined microscopically by observing cultures under a microscope with a x100 objective using oil immersion after Gram staining for characteristics such as Gram reactions, cell arrangement, and shape of isolates (Etok et al., 2004).

c) **Biochemical Characterization**

Various biochemical tests, namely catalase, coagulase, citrate, indole, ornithine decarboxylase, methyl-red, oxidase, Voges-Proskauer,k and Triple Sugar Iron (TSI) tests, were carried out to further identify and confirm the isolates (Cheesbrough, 2002).

Preparation and Confirmation of Test Fungal Isolates

Fungal isolates were examined microscopically after sub-culturing onto Sabouraud dextrose agar for colonial characteristics such as colour, aerial hyphae, and odour (for yeast cells). Microscopic examination of pure fungal cultures was carried out by staining with lactophenol cotton blue and observing under the microscope with x10 and x40 objective lenses for characterization (Cheesbrough, 2002).

Maintenance of Pure Cultures

Pure cultures of the test organisms, both bacterial and fungal species, were preserved and maintained in the refrigerator on nutrient and Sabouraud dextrose agar slants, respectively. These were sub-cultured twice every month to check for viability and purity by plating onto appropriate media and Gram staining (El-Mahmood et al., 2008).

Preparation of Sensitivity Discs

Sensitivity discs were prepared according to the methods described by Agbo and Mboto (2012), and Ekwenge and Okorie (2010). Discs of 6mm diameter were made from Whatman No. 3 filter

paper. Batches of 100 discs were transferred into McCartney bottles, slightly sealed, and sterilized by autoclaving at 121°C for 15 minutes.

Sterility Testing

The sterility of the extracts was tested before use for antimicrobial evaluation by plating them on blood agar, Sabouraud dextrose agar, and Nutrient agar plates and incubating with CO_2 (growth of anaerobic organisms), at 20^0C, 25^0C, 35^0C and 37^0C respectively for 14 days, then observed for visible colonies. Each plate was then subcultured to confirm the presence of microorganisms. This was done following the USP <71> Sterility Tests.

Preparation of Media

All media used for the research, including nutrient agar, Sabouraud dextrose agar, Triple Sugar Iron agar, nutrient broth, blood agar, and Muller-Hinton agar, were prepared according to the manufacturer's instructions.

Sterilization of Materials

All materials, including glassware and canisters, were properly washed with detergent and rinsed with water, wrapped in aluminium foil, and sterilized in a hot air oven at 170°C for 1 hour. Media were sterilized by autoclaving at 121°C for 15 minutes.

Preparation of Stock Concentration of Extracts

A stock solution of 500mg/ml of the extracts was prepared by dissolving 1g of each extract in 2ml of sterile distilled water in different test tubes and labelled appropriately (Bukar et al., 2010). The stock solutions of the extracts were reconstituted to varying working concentrations prior to impregnation into discs. A double-fold serial dilution of each extract was carried out from the stock solution (Gafer et al., 2010). Exactly 2ml of distilled water was transferred into four test tubes and sterilized by autoclaving at 121°C for 15 minutes. These test tubes, including the stock concentrations, were placed in test tube racks. Exactly 1ml of the extract solution (stock solution) was transferred into the first tube next to the stock concentration. This continued serially (serial

dilution) up to the four tubes for each extract, yielding varying working concentrations of 500mg/ml, 250mg/ml, 125mg/ml, and 62.25mg/ml, respectively, from each extract (Agbo and Mboto, 2012). The sterile discs were then counted into the mixture; 10 discs to 1ml of extract solutions. More discs were added until the extract solution was absorbed. The approximate concentration of the extract absorbed by each disc was calculated using the formula described by Ekwenge and Okorie (2010).

$$\text{Concentration per discs} = \frac{CE}{N}$$

Where:

CE = Concentration of reconstituted extract

N = Number of discs used

Preparation and standardization of Inocula

Colonies of the test bacterial and fungal isolates from their respective stock cultures were transferred into prepared nutrient broth and Sabouraud dextrose broth. These cultures were incubated at 37°C for 24 hours and 48 hours, respectively (Agbo and Mboto, 2012). The standardization of inocula was carried out as described by Cheesbrough (2002). The overnight cultures were appropriately diluted by gradually adding freshly prepared nutrient and Sabouraud dextrose broth. The turbidity of the inocula was standardized by comparing it with a 0.5% Barium chloride solution (McFarland standard) as described by Cheesbrough (2002).

Antimicrobial Sensitivity Screening of Extracts

Disc Sensitivity Screening

The antimicrobial sensitivity testing of the extracts was performed using the disc diffusion method on Mueller-Hinton agar (Doughari, 2006) for bacteria and Sabouraud dextrose agar for fungi (Awofisayo et al., 2010). The media were prepared, sterilized, and 15 ml of the media were poured into Petri dishes and allowed to set. The agar plates were then seeded with 0.1 ml aliquots of each

standardized test organism in triplicates and spread carefully using a sterile L-shaped glass rod to ensure even distribution. The plates were allowed to dry for 15 minutes at room temperature.

Discs impregnated with varying working concentrations of extracts were placed on each agar plate seeded with the different test organisms, maintaining a distance of 5cm between each disc. Standard antibiotic discs and distilled water were similarly used as positive and negative controls. The plates were left on the bench for 20 minutes for proper diffusion before incubation at 37°C for 24 hours (Bukars et al., 2010). The zones of inhibition formed on the media after incubation were measured with a transparent ruler and expressed in millimetres (Duru & Mbata, 2010) to determine the antimicrobial effectiveness of the different concentrations of the extracts (Doughari, 2006).

Minimum Inhibitory Concentrations

The broth dilution method, as described by Awofisayo et al. (2010), was used to determine the minimum inhibitory concentration (MIC) of the extracts against test organisms. A twofold serial dilution of the extracts at 250 mg/ml in nutrient broth and Sabouraud dextrose broth was carried out to obtain varying concentrations of the extracts: 250 mg/ml, 125 mg/ml, 62.25ml, 31.1 mg/ml, and 15.6 mg/ml. Exactly 0.2 ml aliquots of the standardized suspension of the test organisms were inoculated into each test tube and incubated at 37°C for 24 hours. After incubation, the MIC was read as the lowest concentration of the extracts that inhibited the growth of the test organisms, using turbidity as the criterion.

Minimum Bactericidal/Fungicidal Concentrations (MBC/MFC)

MBC/MFC was determined by first selecting the tubes that showed no growth during the MIC determination. A loopful from each tube was then sub-cultured onto extract-free agar plates and incubated at 37°C for 24 hours (Duru and Mbata, 2010). The lowest concentration in the MIC test tube at which no visible growth was observed on the sub-cultured plates was noted as the Minimum Bactericidal/Fungicidal Concentration (MBC/MFC).

RESULTS

Table 1 Morphological and biochemical characteristics of bacteria isolates employed in the study

Isolates codes	Gram´s reaction	Shape	Cat	Coa	Cit	Mot	Ind	Ori	MR	VP	Oxi	Glu	Lac	Suc	Gas	H_2S	Probable organisms
1	-	Short rod	ND	ND	-	+	+	+	+	-	-	+	+	+	+	+	*Escherichia coli*
2	+	Cocci in cluster	+	+	+	-	-	+	+	-	-	+	+	+	-	-	*Staphylococcus aureus*
3	+	Cocci in chain	-	-	+	-	-	+	+	-	-	+	-	-	+	-	*Enterococcus faecalis*
4	-	Short rods	ND	ND	+	-	-	-	-	+	-	+	+	+	+	-	*Klebsiella pneumonia*
5	-	Short rods	ND	ND	+	+	+	-	-	+	+	+	-	-	+	-	*Pseudomonas aeruginosa*
6	-	Short rods	ND	ND	+	+	-	+	-	-	+	+	+	+	+	+	*Citrobacter freudii*

Key: Cat = Catalase, Coa = Coagulase, Cit = Citrate, Mot = Motility, Ind = Indole, Ori = Orinthine, MR = Methyl red, VP = Voges Proskauer, Oxi = Oxidase, Glu = Glucose, Lac = Lactose, Suc = Sucrose, ND = Not determined, + = positive test, - = negative test.

The identification system used to confirm the organisms were Biochemical test, Microscopy, and Cultivation while the identification manual used were the Cowan & Steel Manual for the identification of medical bacteria and the Bergey's Manual of determinative bacteriology (Cowan & Steel, 1974, Bergey et. al., 1923).

Table 2 Morphological characteristics of fungal isolates employed in the study

IsolateCodes	Color of colonie	Odor	Germ tube	Color of aerial hypha	Color of substrate hyphae	Nature of hyphae	Shape and kind of spore	Presence of special structure	Appearance of sporangio-phore	Xtics of head	Orgs
1	Creamy white	Yeasty	+	NA	NA	NA	NA	NA	NA	NA	*Candida albicans*
2	Brown	ND	ND	Dark brown	Dirty brown	Septate	Circular condia	Foot cell present	Long erect	Multinucl-ate	*Aspergillus flavus*

Key: NA = Not Applicable
ND = Not Determined

The identification system used to confirm the organisms was biochemical test, microscopy, and cultivation, while the identification manual used was the Cowan and Steel Manual for the

identification of medical bacteria and the Bergey's Manual of Determinative Bacteriology (Cowan & Steel, 1974; Bergey et al., 1923).

Antimicrobial Activity of Extracts

Antibacterial activity

The results of the antibacterial screening revealed that the ethanolic and aqueous (hot and cold water) extracts possessed antibacterial activity and inhibited test bacteria to varying degrees. The ethanolic extract inhibited the Gram-negative bacteria; *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, and Citrobacter freudii* with a maximum diameter of the zones of inhibition of 7.7mm, 15.7mm, 12.3mm, and 10.0mm respectively at a concentration of 500mg/ml (Table 3) while that of Gram-positive bacteria; *Staphylococcus aureus and Enterococcus faecalis* showed a maximum zone of inhibition of 12.0mm and 24.7mm respectively at a concentration of 500mg/ml (Table 3). The hot water extract inhibited the Gram-negative bacteria *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa,* and *Cirobacter freudii* with a maximum diameter of the zones of inhibition of 9.3mm, 8.3mm, 10.3mm, and 11.0mm respectively at concentrations of 500mg/ml (Table 4). Similarly, for Gram-positive, Staphylococcus aureus, and Enterococcus faecalis, the maximum inhibition zones obtained were 8.3mm and 7.7mm, respectively, at concentrations of 500mg/ml (Table 4). The cold-water extract showed a maximum diameter of the zones of inhibition of 9.8mm, 14.3mm, and 9.7mm, respectively for Gram negative bacteria: *Escherichia coli, Pseudomonas aeruginosa, and Citrobacter freudii* at a concentration of 500mg/ml (Table 5) and a maximum inhibition zone of 9.7mm for *Enterococcus faecalis* (Table 5).

Antifungal activity

The results of the of the susceptibility screening showed that the hot water and ethanolic extracts exhibited varying antifungal activity against *Candida albicans* but showed no activity against *Aspergillus flavus*. The cold-water extract showed no activity (ineffective) against both *Candida albicans* and *Aspergillus flavus*. The ethanolic and hot water extracts showed a maximum inhibition zone of 8.2mm and 18.0mm respectively at concentrations of 500mg/ml (Table 3 and 4).

Effects of extract concentration on antimicrobial activity of test plant (*Monodora myristica*)

There was a significant difference (P <0.05) in the various extracts used for the study. The extracts formed factor B for the statistical analysis (ANOVA factorial experiment). Four varying concentrations of the test plant extracts, 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml formed factor A for the statistical analysis. Out of the four varying concentrations of the extract, 500mg/ml concentration gave the best results of all the test organisms.

The concentration effect of the ethanolic extract of the test plant on *Escherichia coli* gave a zone of inhibition ranging from 7.7 to 5.3 mm while that of *Staphylococcus aureus* from 12.0 to 3.3 mm. *Enterococcus faecalis* from 24.7 to 3.6 mm, *Klebsiella pneumonia* from 15.7 to 6.5 mm, *Pseudomonas aeruginosa* from 12.3 to 5.0 mm, *Citrobacter freudii* from 10.0 to 6.3 mm and *Candida albicans* from 8.2 to 0.0 mm. For *Aspergillus flavus*, there was no significant difference between the effect of ethanolic extract concentrations for 500mg/ml, 125mg/ml, 125mg/ml and 62.5mg/ml.

The hot water extract gave the zones of inhibition against *Escherichia coli* ranging from 9.3 to 5.0 mm, *Staphylococcus aureus* from 8.3 to 0.0 mm, *Enterococcus faecalis* from 7.7 to 4.0 mm, *Klebsiella pneumonia* from 8.3 to 0.0 mm and *Candida albicans* from 18.0 to 4.3 mm for 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml. As for *Aspergillus flavus*, there was no significant difference between the effects of hot water extract concentrations because there was no zone of inhibition for the varying concentrations.

The cold-water extract gave a mean ± standard deviation zones of inhibition against *Escherichia coli* from 9.8 to 6.3 mm, *Enterococcus faecalis* from 9.7 to 0.0 mm, *Pseudomonas aeruginosa* from 14.3 to 6.7 mm and *Citrobacter freudii* from 9.7 to 0.0mm for 500mg/ml, 250mg/ml, 125mg/ml, and 62.5mg/ml. For *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Candida albicans* and *Aspergillus flavus*, there was no significant difference between the effects of cold-water extract concentrations because there was no zone of inhibition for the varying concentrations (Table 3-5).

Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC)

The MIC and MBC/MFC results showed another antimicrobial activity of the extracts (Table 6). The organisms' MIC and MBC/MFC data are also variable concentrations and extract-dependent.

The minimum inhibitory concentration (MIC) of ethanolic extract for the different test organisms ranges between <62.5 - > 1000mg/ml that of hot water extract was also between <62.5 - > 1000mg/ml. At the same time, that of water was between 62.5 - > 1000mg/ml (Table 6).

The minimum bactericidal and fungicidal concentration (MBC/MFC) activity of the different extracts against the different organisms range between 12.5 - > 2000mg/ml for hot water extract and 125 - > 2000mg/ml for cold water (Table 6).

Table 3 Effects of ethanolic extract concentration on antimicrobial activity of the test plant (*Monodora myristica)*

S/N	Organisms	Concentration (mg/ml)			
		62.5	125	250	500
1	*Escherichia coli*	5.3 ± 0.6	6.0 ± 0.2	6.1 ± 1.0	7.7 ± 1.0
2	*Staphylococcus aureus*	3.3 ± 0.2	4.7 ± 0.6	6.7 ± 0.0	12.0 ± 0.0
3	*Enterococcus faecalis*	3.6 ± 1.0	6.7 ± 1.5	8.3 ± 0.6	24.7 ± 1.5
4	*Klebsiella pneumonia*	6.5 ± 0.3	9.3 ± 0.1	11.0 ± 0.0	15.7 ± 1.2
5	*Pseudomonas aeruginosa*	5.0 ± 0.3	5.3 ± 1.5	7.0 ± 1.2	12.3 ± 0.6
6	*Citrobacter freudii*	6.3 ± 0.3	7.0 ± 1.0	8.3 ± 1.5	10.0 ± 0.0
7	*Candidas albicans*	NA	3.5 ± 1.2	6.0 ± 0.5	8.2 ± 1.6
8	*Aspergillus flavus*	NA	NA	NA	NA

Key: Values (results) were presented in mean ± standard deviation. The values were presented (measured in millimetres)

. NA = No activity

Table 4 Effects of hot water extract concentration on antibacterial activity of test plant (*Monodora myristica*)

S/N	Organisms	Concentration (mg/ml)			
		62.5	125	250	500
1	*Escherichia coli*	5.0 ± 0.0	6.1 ± 1.2	6.7 ± 0.5	9.3 ± 1.8
2	*Staphylococcus aureus*	NA	3.6 ± 1.5	6.0 ± 0.0	8.3 ± 2.2
3	*Enterococcus faecalis*	4.0 ± 1.5	6.3 ± 0.5	6.7 ± 1.2	7.7 ± 1.0
4	*Klebsiella pneumonia*	NA	3.6 ± 1.5	5.7 ± 1.0	8.3 ± 1.0
5	*Pseudomonas aeruginosa*	4.0 ± 1.5	5.1 ± 0.5	7.7 ± 1.5	10.3 ± 1.2
6	*Citrobacter freudii*	NA	5.3 ± 1.2	6.7 ± 1.5	11.0 ± 0.0
7	*Candidas albicans*	4.3 ± 0.1	5.7 ± 1.2	6.7 ± 0.6	18.0 ± 1.5
8	*Candidas albicans*	NA	NA	NA	NA

Key: Values (results) were presented in mean ± standard deviation; the values were presented (measured in millimetres). NA- No Activity

Table 5 Effects of cold-water extract concentration on antimicrobial activity of the test plant (*Monodora myristica*)

S/N	Organisms	Concentration (mg/ml)			
		62.5	125	250	500
1	*Escherichia coli*	6.3 ± 1.5	7.3 ± 0.6	9.0 ± 0.0	9.8 ± 1.8
2	*Staphylococcus aureus*	NA	NA	NA	NA
3	*Enterococcus faecalis*	NA	5.0 ± 1.3	9.0 ± 0.0	9.7 ± 1.5
4	*Klebsiella pneumonia*	NA	NA	NA	NA
5	*Pseudomonas aeruginosa*	6.7 ± 1.2	8.7 ± 1.5	9.7 ± 1.0	14.3 ± 1.2
6	*Citrobacter freudii*	NA	5.0 ± 0.0	8.0 ± 0.0	9.7 ± 1.0
7	*Candidas albicans*	NA	NA	NA	NA
8	*Candidas albicans*	NA	NA	NA	NA

Key: Values (results) were presented in mean ± standard deviation; the values were presented (measured in millimetres). NA- No Activity

Table 6: Summary of the MIC and MBC of extracts to test organisms.

S/N	Organisms	Ethanolic extract (mg/ml)		Hot water extract (mg/ml)		Cold water extract (mg/ml)	
		MIC	MBC	MIC	MBC	MIC	MBC
1	*Escherichia coli*	≤62.5	>500	≤62.5	≤500	>62.5	≤125
2	*Staphylococcus aureus*	62.5	500	≤125	500	>500	>1000
3	*Enterococcus faecalis*	≤62.5	≥250	62.5	500	≤125	≥250
4	*Klebsiella pneumonia*	≤62.5	125	≤125	500	>500	>1000
5	*Pseudomonas aeruginosa*	≤62.5	≤500	≤62.5	≤500	≥62.5	≥125
6	*Citrobacter freudii*	≤62.5	≥125	≤125	250	125	≤250
7	*Candidas albicans*	125	≥500	≤62.5	≤250	>500	>1000
8	*Aspergillus flavus*	>1000	>2000	>1000	>2000	>1000	>2000

DISCUSSION

In this study, extracts of *Monodora myristica* obtained using different solvents exhibited varying degrees of antimicrobial activity against both bacteria and fungi. The results confirmed that extracts obtained with a polar solvent (ethanol) showed larger inhibition zones resulting in better activity than those obtained with a non-polar solvent (water). The hot-water extracts showed better activity than the cold-water extracts. This can be explained by the fact that most of the secondary metabolites identified in the extracts, known to have antimicrobial properties, are also polar components and might have been extracted by the polar solvent (Ajaiyeoba, 2002; Aqueel et al., 2023). The results also suggest the tested plant seeds have antibacterial and antifungal actions.

The ethanolic extract (polar solvent) showed inhibition zones ranging from 3.3 to 24.7 mm, followed by hot water with inhibition zones ranging from 3.6 to 18.0 mm, and cold-water extracts ranging from 5.0 to 14.3 mm. Therefore, both ethanol and hot water effectively extracted the plant constituents. This supports the use of hot water and ethanol to extract plant materials in herbal medicinal preparations. There were no significant differences ($P < 0.05$) in the ethanolic, hot water, and cold-water extracts against Aspergillus flavus.

The hot water, cold water, and ethanolic extracts displayed reasonable activity against the organisms tested, thus exhibiting a broad spectrum of activity against bacteria and yeast. This finding aligns with earlier studies by Udoh et al. (2004) and Kigigh & Charlie (2012), who indicated that African nutmeg exerted antimicrobial and antifungal activities against Staphylococcus aureus, Escherichia coli, Candida albicans, and Bacillus subtilis.

The promising results obtained against some Enterobacteriaceae bacteria with the extracts appear to justify the use of the seeds of this plant in herbal or traditional medicines. Furthermore, the results obtained against Staphylococcus aureus, Pseudomonas aeruginosa, and Enterococcus faecalis, which are implicated in wounds, burns, and sores, also justify its use by traditional medical practitioners in the treatment of such cases (Kigigh & Charlie, 2012). This suggests that its potency could be exploited in managing diseases caused by these human pathogens.

The concentrations of the various extracts had a varying effect on the organisms tested, indicating that the concentration is directly proportional to the zones of inhibition, i.e., the higher the concentration, the larger the zone of inhibition.

CONCLUSION

The results of this study show that ethanolic, hot water and cold-water extracts of the dry seeds of *Monodora myristica* possess antibacterial and antifungal activities. The ethanolic solvent showed the most antimicrobial activity on the bacterial isolates. However, the demonstration of antimicrobial activity by hot and cold-water extracts provides a scientific basis for using plant seeds in straditional disease treatment. This may be beneficial to the pharmaceutical industry. It can, therefore, be concluded that *Monodora myristica* dry seeds can significantly contribute to the health management of humans.

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