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Research Article

Screening of Phytochemicals, Antimicrobial and Antioxidant Activity of *Monodora Myristica*

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Abstract: Antibiotic resistance has become a serious global concern. It is important to identify new sources of natural antioxidants and antimicrobials. The present study describes for the first time the antioxidant, and antibacterial activities of various extracts of *Monodora myristica* a traditional medicinal plant. Each prepared concentration of the different extracts was tested for its antimicrobial activity against Staphylococcus aureus (S. aureus), Bacillus subtilis (B. subtilis), *Pseudomonas aeruginosa* and Escherichia coli (E. coli) on nutrient agar plates using disc diffusion method. At 50mg/ml showed inhibition of 13 and 15 mm against E. coli only for dichloromethane and methanol extracts respectively. The standard ciprofloxacin at 1mg/ml showed inhibition to all four tested pathogens, while antioxidant activity was analyzed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. A mixture of phytochemicals like phenols, alkaloids, flavonoids, glycosides were found in dichloromethane and methanolic extract of *Monodora myristica* which also showed remarkable potential with antioxidant and antimicrobial activities. The current study provides initial data that justify the use of *Monodora myristica* folkloric medicine. Our results showed that *Monodora myristica* has powerful antibacterial bioactivity against E. coli and antioxidant activity. Further investigations are needed to identify and characterize these constituents.

Keywords: Oral, Mouth, Maxillofacial, Surgery, Surgeon, Teamwork, Appliances, Devices, Apparatus, Design, unique, Bone, Multidisciplinary, Distraction, Alveolus, Alveolar Distraction.

INTRODUCTION

Antibiotic resistance has become a serious global concern, and the discovery of novel antimicrobial herbal constituents may provide valuable solutions to overcome the problem [1]. New resistance mechanisms are emerging and spreading globally, threatening the ability to treat common infectious diseases, resulting in prolonged illness, disability, and death [2]. Indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases has led to an alarming increase in development of multiple drug resistance, thus necessitating the need for development of alternative antimicrobial drugs from medicinal plants for the treatment of infectious diseases [3]. Antimicrobial compounds have drawn attention of many pharmacologists and are now becoming one of major areas of research.

Many plants have medicinal value. The medicinal value of these plants lies in phytochemical constituents that cause definite pharmacological action on the human body. It is well known that plants with medicinal properties are rich source of these antimicrobial compounds [4]. The properties of many plants remain unrevealed. One such is *Monodora myristica* is a species in the family Annonaceae. It contains approximately 35 species, distributed throughout tropical Africa. Two of the species, *Monodora myristica* and *Monodora tenuifolia* (M. tenuifolia) are widely used as spices.In traditional medicine practice, *Monodora myristica* is widely used to relieve toothache, dysentery,

diarrhea, dermatitis, headache, and as vermifungal [5].

An imbalance between free radicals and antioxidants leads to oxidative stress in the human body which may result in several chronic and fatal diseases and even antibiotic resistance. In response, humans produce antioxidants which neutralize these free radicals, but due to the decreasing levels of these antioxidants as a result of environmental factors and other reasons, this may cause an increase in oxidative stress which can lead to cell damage and death. Supplementation containing phytochemicals is required at this stage, to prevent the damage which is caused by these free radicals from natural or synthetic sources. The investigation of phytochemicals from *monodora myristica* is crucial.

The present investigation was intended to screen and to evaluate the phytochemicals and antimicrobial effects of the methanol and dichloromethane fractions of *Monodora myristica*.

MATERIALS AND METHOD

Collection and Identification of Plant material

The leaves of the plant *Monodora myristica* were collected from the wild where they naturally grow in Yenagoa Local Government Area Bayelsa state. The leaves were identified and authenticated in the Department of Pharmacognosy and Herbal Medicine, Niger Delta University and samples deposited in the herbarium.

Plant preparation

The leaves of *Monodora myristica were* washed with tap water and then rinsed with distilled water and dried under shade for 7 days. The dried leaves were dried in oven at 60°C for 2 hours. The dried leaves were grounded to powder using a blender into coarse powder particles.

Extraction of Plant material

Extraction with methanol

200 grams of the powdered leaves were extracted with 1.2 L of methanol. Cold maceration was carried out for 72 hours with frequent agitation. The mixture was filtered through Whatmann No 1 filter paper. The filtrate was concentrated using rotary evaporator into a semisolid and stored at 4 ^{0}C for further analysis.

Extraction with Dichloromethane

150 grams of the powdered leaves were extracted with 700 ml of dichloromethane for 72 hours with frequent agitation. The mixture was through Whatmann No 1. The filtrate was concentrated using rotary evaporator and the concentrate stored at 4 ^{o}C for further analysis.

Phytochemical screening

Testing for the presence of phytochemicals was carried out using the methods of Trease and Evans [6].

Test for Tannins Distilled water (1 ml) was added to each extract (0.5 g) and stirred and then filtered. Ferric chloride solution was added to the filtrate. A blue-black green or blue-green precipitate was taken as evidence for the presence of tannins.

Test for Saponins To each extract (0.5 g) was added distilled water (10 ml) in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The froth was mixed with 3 drops of olive oil and shaken vigorously after which it was observed. The persistent frothing was taken as evidence for presence of saponins.

Test for Alkaloids 0.5 g of each extract was stirred with 1% aqueous hydrochloric acid (3 ml) on a steam bath and filtered. 1 ml filtrate of the resultant solution was then treated with a few drops of Mayer's reagent. Precipitation with Mayer's reagent was taken as preliminary evidence for the presence of alkaloids.

Test for Terpenoids To 0.5 g of each extract was added 2 ml of chloroform and concentrated sulphuric acid (3 ml) was carefully added and the formation of reddish brown coloration at the interface indicates the presence of terpenoids.

Test for Flavonoids a portion of each extract was heated with Ethyl acetate (10 ml) over a steam bath for 3 minutes. The resulting mixture was then filtered and 4 ml of the filtrate was then shaken with 1ml of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids.

Test for cardiac glycosides 0.5 g of each extract was dissolved in glacial acetic acid (1 ml) containing one drop of Ferric chloride solution. Concentrated sulphuric acid (1 ml) was added gently down the side of the test tube, a brown ring formation at the inter-phase indicates the presence of deoxysugar which is a characteristic of cardenolides.

Test for Anthraquinone 0.5 g of each extract was taken into a dry test tube and 5 ml of chloroform was added and shaken with equal volume of 100% ammonia solution. Pink, violet or red color in the ammoniacal layer (lower layer) indicates the presence of free anthraquinones.

Test for steroids 0.1 g of each extract was dissolved in 2 ml of chloroform and sulphuric acid added carefully to form a lower layer and reddish brown *coloration* at the interface indicates the presence of steroids.

Quantitative analysis

Estimation of alkaloid content

The total alkaloid content was estimated using the method described by Unuofin *et al.* [11]. 5 g of the pulverized *monodora myristica* was immersed in 200 mL of 10% acetic acid in ethanol. The mixture was allowed to stand for 4hr at room temperature. It was subsequently filtered and the filtrate was concentrated using a water bath at 55 ^{o}C to a quarter of its original volume. Concentrated ammonium hydroxide was added in single drops until completion of the precipitation process. The solution was then washed with dilute ammonium hydroxide and filtered again. The residue obtained was first dried and then weighed. The alkaloid content was calculated using the equation:

%Alkaloid = Weight of precipitate / Weight of original sample ×100

Saponin Determination

The method used was that of Onyesife *et al*. [7]. The sample *monodora myristica* was ground and 20 g was put into a conical flask and 100 cm3 of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 h with continuous stirring at about 55 ^{o}C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The concentrate was transferred into a 250 ml seperatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight; the saponin content was calculated. % Saponins contents = Weight of residue /Weight of sample ×100.

Estimation of tannin content

The total tannin content was estimated using the Folin - Ciocalteu method of Vijay and Bhambar [12]. 7.5 ml of distilled water was added to a tube containing 0.1ml of *monodora myristica*. 0.5 ml of Folin-Ciocalteu phenol reagent and 1 ml of 35 % Na_2CO_3 solution was then added. The whole solution was made up to 10 ml with distilled water. The mixture was vortexed and kept at room temperature for 30 min. The absorbance was read at 725 nm using a spectrophotometer. The total tannin content was expressed as mg/g GAE equivalent.

DPPH radical scavenging assay

DPPH radical scavenging activity of *monodora myristica* extracts were determined according to the method described by Wintola and Afolayan [8] with some modifications. A preparation of 1 ml of 0.135 mM DPPH in methanol was mixed with 1ml of various concentrations (1 – 7 mg/ml) of the plant extracts and vitamin C. The mixture was left in the dark at room temperature for 30 min after being vortexed. The absorbance of the mixture was then measured spectrophotometrically at 517 nm. Vitamin C was used as standard. The DPPH radical scavenging activity was calculated from the equation: DPPH radical scavenging activity = Abs control - Abs sample/ Abs control ×100 where Abs control was the absorbance of DPPH radical + methanol; Abs sample was the absorbance of DPPH radical + sample extract or standard (Vitamin C).

Test microorganisms

For antimicrobial studies, Staphylococcus aureus (S. aureus), Bacillus subtilis (B. subtilis), *Pseudomonas aeruginosa* (P.aeruginosa) and Escherichia coli (E. coli) were procured from the Medical Microbiology and Parasitology Department, Niger Delta University, Bayelsa State and stored at -20 °C for further studies.

Antibacterial activity assay

The antibacterial potential test was carried out using the agar disc diffusion method [9]. Negative controls were prepared by using the same solvents employed to dissolve the samples. Inhibition zones were measured and compared with the standard reference antibiotic ciprofloxacin. Each extract was subjected to serial dilution by using dimethyl sulphoxide (DMSO) as a solvent to give 50 mg/ml, 10 mg/ml, 2 mg/ml, and 1 mg/ml solutions of *monodora myristica*. The concentration of ciprofloxacin standard used for this study was at 1 mg/ml. Each prepared concentration of the different extracts was tested for its antimicrobial activity against Staphylococcus aureus (S. aureus), Bacillus subtilis (B. subtilis), *Pseudomonas aeruginosa* and Escherichia coli (E. coli) on nutrient agar plates using disc diffusion method. Whatman No. 1 sterile filter paper discs (6 mm diameter) were impregnated with methanol and dichloromethane extracts of *monodora myristica* and placed on the inoculated agar. All the plates were incubated at 37 ^{0}C for 24 h. Evaluation of antibacterial activity was measured showing the diameter of the zones of inhibition against the tested bacteria. Each method in this experiment was replicated three times.

RESULTS

Table-3.1: Results of phytochemical screening of *Monodora myristica* (Gaertn) Dunal

Phytochemicals	Positive indicator	Methanol	DCM
Tannins	Greenish-black	++	-
Saponins	Frothing	-	+++
Alkaloid	Cream color	+	+
Terpenoids	Reddish brown	++	+
Flavonoids	Yellow	-	+
Cardiac glycoside	Brown /violet ring	+++	+++
Anthraquinone	Pink	++	++
Steroids	Blue-green	+	+

KEY: - = not present + = present in little quantity ++ = present in high quantity +++ = present in large quantity
DCM = Dichloromethane

Table-3.2: Results of quantitative phytochemical of *Monodora myristica* (Gaertn) Dunal

Phytochemicals	Quantitative value	
	Dichloromethane	Methanol
Alkaloids	17.04 %	13.89 %
Tannins	4.3 mg/GAE	0.3 mg/GAE
Saponins	12.5 %	37.4 %

Table-3.3: Showing zone of inhibition (mm) of Dichloromethane extract

Conc. (mg/ml)	*B. subtilis*	*E. coli*	*P.aerugi nosa*	*S. aureus*
50	-	13	-	-
20	-	-	-	-
10	-	-	-	-
2	-	-	-	-
Ciproflox acin (1 mg/ml)	14	16	12	19

Table-3.4: Showing zone of inhibition (mm) of methanolic extract

Conc. (mg/ml)	*B. subtilis*	*E. coli*	*P.aerugi nosa*	*S. aureus*
50	-	15	-	-
20	-	-	-	-
10	-	-	-	-
2	-	-	-	-
Ciproflox acin (1 mg/ml)	19	16	13	20

Table-3. 5: Showing % scavenging activity of the *Monodora myristica* extracts

Conc. (mg/ml)	% Inhibition		
	Ascorbic acid	Methanol	Dichloromethane
1	20	21	18
3	34	39	28
5	46	47	44
7	52	53	50

DISCUSSION

The qualitative phytoconstituent tests confirmed the presence of alkaloids, tannins, saponin, terpenoid, flavonoid, anthraquinone, cardiac glycoside, steroid and phenol in the extracts of dichloromethane and methanolic exracts of *Monodora myristica*. Total tannins content in terms of gallic acid equivalent showed 4.3 mg/GAE and 0.3 mg/GAE for dichloromethane and methanolic extracts of *Monodora myristica* respectively. Also the

quantitative determination of alkaloid showed 17.04 % for dichloromethane and 13.89 % for methanolic extracts of *Monodora myristica*. The saponin quantitative content also showed 12.5 % and 37.4 % for dichloromethane and methanolic extracts of *Monodora myristica* respectively as showed in table 3.2. In case of antioxidant activity, methanolic extract of the samples showed effective scavengers of DPPH at 7 mg/ml 53 % of the radical DPPH was scavenged and at same concentration methanolic extract and Ascorbic acid scavenged 50 % and 52 % respectively. These results are in support of Singh and Sharma [10].

The different crude extracts from *Monodora myristica* exhibited antibacterial potential against Staphylococcus aureus (S. aureus), Bacillus subtilis (B. subtilis), *Pseudomonas aeruginosa* and Escherichia coli (E. coli) on nutrient agar plates using disc diffusion method at four concentrations of 50 mg/ml, 20 mg/ml, 10 mg/ml and 2 mg/ml with dimethyl sulphoxide (DMSO). Only the highest concentration of 50 mg/ml exhibited exhibited 13 1nd 15 mm for dichloromethane ane methanol against E. coli respectively as shown on tables 3.3 and 3.4. the standard ciprofloxacin at 1 mg/ml exhibited inhibitory effect against all clinical microbes as shown on tables 3.3 and 3.4.

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