



Antimicrobial Evaluation of crude Extracts, Fractions and Oil from *Monodora myristica* Seeds (Gaertn.) Dunal

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ABSTRACT

Monodora myristica is a tropical tree of the Annonacea family of flowering plants. *M. myristica* is popularly known as ehuru seeds by the Ibos. It is used as antimicrobial and anti-malarial remedies in Ibo-Nigeria folkloric medicine. This study was designed to ascertain the antimicrobial properties of extracts, fractions and oils from *M. myristica* seed. The anti-microbial screening was done using agar cup diffusion method against the organisms. The result of the antimicrobial screening shows that the extracts, fractions and oils were very sensitive against the organisms used. After 24 h incubation, the zone of inhibition of the oil against *Escherichia coli* (18 mm), *Staphylococcus aureus* (14 mm), *Pseudomonas aeruginosa* (18 mm) and *Candida albicans* (18 mm). The result compares favourably with the standard antibiotic, ciprofloxacin. The n-butanol fraction has the highest activity against *Candida albicans* (24 mm) very close to that of the standard nystatin (28 mm). In conclusion, the present findings support the ethnomedicinal uses of *M. myristica* seed as an antimicrobial agent.

KEYWORDS: *Monodora myristica*, antimicrobial, ciprofloxacin

INTRODUCTION

Ehuru seeds are used for preparing special hot soups for nursing mothers for easy control of uterine hemorrhage [1-3]. It is also believed that adding this spice in their soups helps the nursing mother's milk to start flowing normally immediately after childbirth. Ehuru seeds can also be used for treating stomach ache [1-3]. The stem bark of African nutmeg can be used for treating hemorrhoids [3, 4]. The pulp of the ehuru seeds contain essential oil such as; dipentene, pinene and camphene. These types of oil can be used industrially for manufacturing perfumes, soaps and washing detergents [5]. African nutmeg is used to make an ointment that can be used for treating rheumatism. The oil can also be

used as cooking [5]. According to researchers, *M. myristica* can be used for treating arthritis [6, 7]. It has been revealed that ehuru seeds contain cholesterol lowering ability thus can be used for treating individuals with high cholesterol level [8,9]. Because of the important nature of this plant, this work is aimed at determining the antimicrobial properties of the extract, fraction and oils from the seed.

MATERIALS AND METHODS

Seed Collection

The seed of the plant was purchased from ogige market in Nsukka Local Government Area of Enugu State and was identified by a botanist, John Ahiaba from Kogi state University Anyigba

and authenticated by a botanist, Mr Alfred Ozioko, of the International Centre for Ethnomedicine and Drug Development (InterCEED) Nsukka, Enugu State. The voucher specimen was deposited at the InterCEED with number 3045-12

Microorganism Collection

Twenty four hour (24 hours) cultures of six (6) human pathogenic bacteria and fungi made up of both Gram positive (*S. aureus* and *B. subtilis*), Gram negative (*P. aeruginosa*, *S. typhi* and *E. coli*) bacteria and fungi (*C. albicans*) were used for the in-vitro antimicrobial assay. All microorganisms were obtained from maintained stock in the laboratory Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

Extract Preparation

The seed of the *M. myristica* was sun dried for the period of two weeks, it was then de-husked and ground using electronic grinding machine.

Extraction of *M. myristica* Seed for Antimicrobial Test

Portions of ground powder (5 g) were weighed and extracted using exhaustive cold maceration with 95 % aqueous methanol as solvent. The extract was dried and weighed. The obtained extract was tested for antimicrobial potential.

Defatting *M. myristica* seed

Exactly 50 g of the powder of *M. myristica* was weighed and defatted using a Soxhlet extractor with petroleum ether (60-70 °C grade) as solvent. The process was repeated in batch until all ground powder was completely defatted. The total mass of the powdered seed defatted was 900 g.

Extraction of *M. myristica* seed

A 5 g of the marc obtained from defatting of *M. myristica* seed was weighed and poured into small corked bottle. A 10 ml of 95 % methanol was measured and poured inside the bottle and then covered. This was macerated for a period of

24 hours. This procedure was repeated 4 times until the methanol extract became colorless. The aim was for exhaustive extraction. The extract was dried under fan and weighed. The obtained extract was evaluated for antimicrobial potential.

Extraction of the marc of *M. myristica* seed

The marc obtained from *M. myristica* seed was spread under the fan for the petroleum ether to evaporate completely. This was allowed for a period of 3 days and it was weighed using an electronic balance. The mass of the marc weighed was 600 g. A 2000 ml of 95 % of methanol was poured inside a 300 g quantity of the marc in a 2500 ml conical flask and covered properly. After 24 hours, the sample was clarified using cotton wool and filter paper. The extract was then carefully dried under the fan for a period of 3 days. The mass of the extract was weighed using an electronic weighing balance.

Fractionation of the extract from the marc of *M. myristica*

The obtained extract (44 g) was partitioned into the following solvents. They are n-hexane, dichloromethane, ethyl acetate, n-butanol and water. After the dispersion of 22 g of the extract into 50 ml of water, the respective solvents were used to carry out the partitioning.

Antimicrobial evaluation of the extracts, fraction and oil from *M. myristica* seed

The standard solution of the extracts, fractions and oils used against the microorganisms were prepared according to Ijeh *et al* [10]. The Antimicrobial test of the extracts, fractions and oils from *M. myristica* seed against the organisms was carried out using the agar cup diffusion method described by Ebi and Ofoefule [11]. Standard concentrations of ciprofloxacin and nystatin were taken as positive standard antibacterial and anti-fungal solution respectively. The solvents used in dissolving samples served as the negative controls. A 0.1 g of each of the extract fraction and oils from *M. myristica* seeds was weighed using an electronic balance and dissolved in 1 ml of dimethyl sulfoxide. This standard solution was tested against the

following microorganisms; *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *S. typhi*, *C. albicans*. The method used is called Agar Cup Diffusion Method. An agar plate “seeded” with the different organism is prepared by introducing 0.1 ml of the broth culture of the test organisms into 20 ml of sterile nutrient agar or SDA at 45°C. For bacteria and fungi respectively .It was mixed and poured into sterile Petri dish and allowed to solidify. 0.1 g of the plant extract was weighed and dissolved in 1ml of DMSO to form 100 mg/ml concentration of the different extracts. The seeded agar plates

were divided into sections and holes bored on each with the aid of a sterile cork-borer. Plant extracts (0.1 ml) of dissolved were introduced into the different cups. The petri dishes were allowed to stand for 1 hour to allow for pre-diffusion of plant extracts into the agar and incubated for 24 hours at 30°C for bacteria and 25°C for fungi. The extract producing clear zones of inhibition around the cups are considered to be active against the organism. The inhibitory zone diameter was determined in three replicates.

RESULTS AND DISCUSSION

The percentage yields of 22.7 % and 7.3 % for the oil and extract respectively from *M. myristica* indicates a significant presence of oil and phytochemicals in the plant seed. Thus, it has been used as an alternative source of oil for the pharmaceutical industry [12]. The extract from cold maceration of *M. myristica* showed significant activity against *E. coli*, *P. aeruginosa* and *C. albicans* and therefore has both antifungal and antibacterial activity. This agrees with earlier findings by Adam *et al* [13] and Vukovic *et al* [14] and strongly supports the traditional use of this plant as an antimicrobial agent. From Table 2,

The oil from *M. myristica* exhibited good activity against *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans* thereby affirming the antibacterial and anti-fungal activity activities of the oil. This agrees with earlier finding by Fleischer [15]. Furthermore, the extract from the marc of *M. myristica* (after removing the oil) also had appreciable activities against *E. coli*, *S. typhi* and *C. albicans* thus establishing its antibacterial and antifungal activity. This agrees with earlier finding by Corbo *et al* [16]. As stated above, the volume of oil obtained from as 900 g quantity of *M. myristica* was 350 ml representing an appreciable yield (22.7 %) and therefore is of economic value.

Table: Yield from extracts, fractions and oils of *M. myristica* seed

The table below (Table 1) shows the yield and the percentage yield of the extracts, fractions and oils from *M. myristica* seed

Sample	Quantity(g)	Yield(g)	% yield
<i>M.myristica</i> oil	900	240	22.66
Extract from <i>M.myristica</i> marc	600	44.1	7.35
n-hexane fraction	22	2.4	10.9
dichloromethane fraction	22	2.5	10.9
ethylacetate fraction	22	2.3	11.36
n-butanol fraction	22	2.5	11.36
Aqueous fraction	22	2.6	11.81

Table 2: Antimicrobial activity of oil and extract from marc of *M. myristica* seed

The table (Table 2) below shows the antimicrobial activities of the oil and extracts from *M. myristica* seed

Sample	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aureginosa</i>	<i>S. typhi</i>	<i>C. albicans</i>
Soxhlet extract (oil)	+	13	18	18	+	18
Extract from marc	+	+	18	12	14	16
Methanol extract (cold)	+	+	15	20	+	14
Ciprofloxacin	52	32	48	30	36	-
Nystatin	-	-	-	-	-	30

Keys: + = organism grows (no activity), Numerical value = activity present
 IZD = Inhibitory zone diameter (mm), Concentration =100 mg/ml
B. subtilis =Bacillus subtilis, *S. aureus* = Staphylococcus aureus,
E. coli =Escherichia coli, *P. aeruginosa* =Pseudomonas aeruginosa
S. typhi =Salmonella typhi, *C. albicans*=Candida albicans, - =Test not done

Antimicrobial activities of the fractions

The table below (Table 3) shows the antimicrobial activities of the fractions from *M. myristica* seed

Table 3: Antimicrobial activities of the fractions from *M. myristica*

FRACTIONS	<i>B.substilis</i>	<i>S.aureus</i>	<i>E. coli</i>	<i>P.aureginosa</i>	<i>S.typhi</i>	<i>C.albicans</i>
n-Hexane	+	+	+	+	+	22
dichloromethane	16	+	+	16	+	15
ethyl acetate	+	13	+	+	+	12
n-Butanol	18	18	+	+	+	24
Aqueous	14	+	+	16	+	17
ciprofloxacin	52	30	48	30	36	-
Nystatin	-	-	-	-	-	28

Keys: + = organism grows (no activity), Numerical value = activity present
 IZD = Inhibitory zone diameter (mm), Concentration =100 mg/ml
B. subtilis =Bacillus subtilis, *S. aureus* = Staphylococcus aureus,
E. coli =Escherichia coli, *P. aeruginosa* =Pseudomonas aeruginosa
S. typhi =Salmonella typhi, *C. albicans*=Candida albicans, - =Test not done

This report agrees with earlier finding by Bello *et al* [17]. From Table 3, the n-hexane fraction exhibited a very high activity against *C. albicans* implying a very high antifungal activity.

Research has shown that oil rich in phenolic compounds possesses high levels of antimicrobial activity [18, 19]. Dichloromethane fraction showed activity against *B. subtilis*, *P. aeruginosa* and *C. albicans* and therefore have both antibacterial and anti-fungal activity. This agrees with earlier findings by Adam *et al* [13]. The ethyl acetate fraction showed low activity against *S. aureus* and *C. albicans* therefore has both weak antibacterial and antifungal activity. The lower antimicrobial activity could be due to the presence of minor active constituent which may have a synergistic effect [20]. The n-butanol fraction exhibited the highest activity against *C. albicans*. One of the major mechanism of antifungal property is to diffuse into cell membrane and cause them to expand thereby increasing their fluidity or disordering membrane embedded enzymes [21]. These fractions also had activities against *B. subtilis* and *S. aureus*, and also have antibacterial activity. This agrees with earlier finding by Mshana *et al* [22]. The aqueous fraction has a mild activity against *B. subtilis*, *P. aeruginosa* and *C. albicans*; have both anti-fungal and bacterial activity, this agrees with earlier finding by Adam *et al* [13].

CONCLUSION

Taken together, the oil from *M. myristica* and the extract with their fractions exhibited interesting antimicrobial activities against bacteria and fungi. Consequently, oil and extract from *M. myristica* can be developed into cheaper and safer alternative antimicrobial chemotherapy.

CONFLICT OF INTEREST

Authors declared no conflict of interests in the conduct and reporting of this study

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