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RESEARCH ARTICLE

ASSESSMENT OF ANTIMICROBIAL ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF *Monodora myristica* (EHURU) SEEDS

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ABSTRACT

Objective: To assess the antimicrobial activity of *Monodora myristica* seeds on four selected human pathogens, *Escherichia coli, Staphylococcus aureus, Salmonella typhi*, and *Pseudomonas aeruginosa*. **Methods:** Disc diffusion technique was used for *in vitro* antibacterial screening. **Results:** The most susceptible bacterium were *E. coli* (17mm) while the most resistant bacterium was *P. aeruginosa*. The minimum inhibitory concentration result showed that the seed extracts of *M. myristica* was bacteriostatic. *M. myristica* extracts when compared with some conventional antibiotics had less sensitivity action against the test organisms. **Conclusion:** Our findings show that the seed extracts of *M. myristica* possess some antimicrobial activities which can be employed in the development of novel therapeutic agents against the test organisms.

Key words: Antimicrobial activity, Disk diffusion, *Monodora myristica,* Ehuru seeds.

INTRODUCTION

Monodora myristica is used as spice in the preparation of foods and medicines. *Monodora myristica* commonly known as Jamaican or African nutmeg, Ehuru in Igbo or Ariwo in Yoruba belonging to the Anonaceae family is one of the most important trees of the evergreen forest of West Africa. It is most prevalent in the southern region of Nigeria. Almost every part of the tree has economic importance [1]. The seed when ground to powder is a popular condiment used to prepare pepper soup as a stimulant to relieve constipation and to control passive uterine haemorrhage in women immediately after child birth [2].

The increasing reports of resistance to current antibiotics employed in treatment of infections have led to the reconsideration of using traditional medicine for the treatment of these multiple antibiotic resistant organisms. [3-4]. The potential of plants as source for new drugs is still largely unexplored. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [5]. Antimicrobials of plant origin have enormous therapeutic potentials [6-7]. These plants can also serve as preservatives [8].

Hence, in this study it was intended to assess the antimicrobial activity of *Monodora myristica* seeds on four selected human pathogens *Escherichia coli, Staphylococcus aureus, Salmonella typhi, and Pseuomonas aeruginosa.*

MATERIALS AND METHODS

Collection and Identification of plant material

The plant samples, *Monodora myristica* were purchased at Umuahia Main market in Abia State. They were immediately taken to the Department of Forestry, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State for proper identification.

Preparation and extraction of plant material

The seeds of *M. myristica* were dried at room temperature for 5days and ground into powder using a sterile grinder. The extraction was done by the soaking method using two solvents, distilled water and ethanol. The powdered samples (200g) were extracted using 200 ml of distilled water and 200ml 95% v/v ethanol respectively in different 500ml Erlenmeyer flasks. The filtrate was concentrated by using a rotary evaporator at 40^{o}C under reduced pressure. The extracts were then collected in fresh sterile universal bottles and kept at 4^{o}C until ready for use.

Collection and Maintenance of Test Organisms

The test organisms used were *Escherichia coli, Staphylococcus aureus*, *Pseudomona aeruginosa* and *Salmonella typhi.* They were obtained from the stock cultures of the Microbiology Laboratory of Federal Medical Center, Umuahia, Abia State, Nigeria. Their identity were reconfirmed and stored at 4^{o}C in nutrient agar slant.

Reconstitution of the extracts

One gram each of the different extracts was reconstituted with 2 ml each of dimethyl sulfoxide (DMSO) to achieve a concentration of 500 mg/ml each and serially diluted. The test tubes were labeled against the content concentrations.

Determination of Antimicrobial Activity of the extracts.

The sensitivity of selected organisms to the seeds of *M. myristica* was determined using the disc diffusion method as described by Ladipo *et al.* [9]. Few colonies of test organisms were picked and suspended in sterile saline and adjusted to same turbidity to Macfarland turbidity standard tube No. 0.5 and incubated at 37^{0}C for 24hours. The plates were observed for the zone of clearance around the discs. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the disc including the disc diameter (6mm).

Determination of the MIC and MBC

The minimum inhibitory concentrations (MIC) of the extracts were determined by dilution to various concentrations according to the macro broth dilution technique [10Nweze and Onyisi, 2010]. Standardized inocula of each organism to be tested was added to series of sterile tubes of nutrient broth containing two fold dilution of the extract and incubated at 37°C for 24 hours. The MIC was read as the least concentration that inhibited the growth of the test organisms. The minimum bactericidal concentration (MBC) was determined by sub-culturing the test dilution onto fresh drug-free solid medium and incubating further for 18 to 24 hours. The highest dilution that yielded no single cell colony on the solid medium was taken as the minimum bactericidal concentration.

RESULTS

The aqueous and ethanolic extracts from seeds of *M. myristica* and antibiotics showed varied levels of activity on some test isolates while showing no activity on others. The greatest activity of the aqueous extract was observed against the *E. coli,* followed by *Salmonella typhi*. and *S. aureus*; while *P. aeruginosa* did not lead to any activity (Table 1). Table 3 shows

the comparism between the activity of some conventional antibiotics and seeds of *M. myristica* against the test organisms

Table 1: Antibacterial sensitivity assay of aqueous extract of *Monodora myristica seeds*.

Diameter of zone of inhibition (mm)

Test Organisms	Different extract concentrations			MIC(µg/ml)
	2000µg	**1000µg**	500µg	
Salmonella typhi.	14	13.5	-	1000
S. aureus	12.5	12	-	1000
E. coli	17	14	-	1000
P. aeruginosa	-	-	-	

Key: - = no activity.

Table 2: Antibacterial sensitivity assay of ethanolic extract of *Monodora myristica seeds*.Diameter of zone of Inhibition (mm)

Test Organisms	Different extract concentrations			MIC(µg/ml)
	2000µg	**1000µg**	500µg	
Salmonella typhi.	-	-	-	
S. aureus	-	-	-	
E. coli	-	-	-	
P. aeruginosa	-	-	-	

Key: - = no activity

Table 3: Comparison of aqueous extracts of *Monodora myristica* and some conventional antibiotics.

Diameter of zone of inhibition (mm)

Test organisms	**500µg**	**1000µg**	2000µg	Gentamycin	Tetracycline	Ciprofloxacin
Salmonella typhi.	-	13.5	14.0	16.5	12.0	23.0
S. aureus	-	12.0	12.5	26.0	25.0	21.5
E. coli	-	14.0	17.0	13.6	12.0	21.3
P. aeruginosa	-	-	-	21.0	14.5	23.5

Key: - = no activity.

DISCUSSION

Bacterial resistance is presently a global problem and challenge to public health. Therefore search for antibacterial agents are on-going and extended to the field of medicinal plants [11 Mitra, 2014].

Results of the investigation are presented in tables 1 to 3. The results showed significant antimicrobial activity against the organism tested. The aqueous extracts of *Monodora myristica* showed sensitivity effect at concentrations of 2000µg, 1000µg and 500µg respectively against *Salmonella typhi*, *S. aureus* and *E.coli* and no sensitivity against *P. aeruginosa.* The most susceptible bacteria were *E. coli* (17mm) followed by *S. typhi* (14mm) and *S. aureus* (12.5mm) while the most resistant bacterium was *P. aeruginosa.*

The ethanol extract showed no sensitivity effect at all. However, failure of some of the extracts to exert antibacterial effect on the test organisms is not enough to conclude that the plants does not contain substances that can exert antibacterial activity against the test organisms because potency of the extracts depend on the method used to obtained the extract [12]. Research has shown that the age of plant when harvested and the season of plant determine the amount of the active constituents since the active ingredients of plants can vary in quality and quantity from season to season.

The Minimum inhibitory concentration result showed that the seed extracts of *M. myristica* was only able to inhibit the growth of the test organisms but did not exert a killing effect on them. This suggests that the aqueous extract of *M. myristica* was bacteriostatic but not bactericidal.

The result of the comparism of the activity of the plant extracts with conventional antibiotics against the test organisms showed that Gentamycin (16.5mm) and Ciprofloxacin(23mm) showed more sensitivity against *Salmonella typhi* than *M. myristica* seed extract (14mm) while Tetracycline (12mm) showed lesser sensitivity. For *Staph aureus,* the conventional antibiotics had higher sensitivity action than *M. myristica* seed extracts. On *E.coli* the plant seed extract showed higher sensitivity (17mm) than Gentamycin (13.6mm) and Tetracycline (12mm) and lesser sensitivity than Ciprofloxacin (21.3mm). Emeruwa, [13] in his work reported that constitutional antibiotics may show more sensitivity against the test organisms than the plant extracts.

CONCLUSION

Many evidences gathered in earlier studies have confirmed *M. myristica* to be bioactive. Therefore, its seed extracts could be seen as good source for useful drugs that possess great potentials as antimicrobial agents against selected pathogens and thus can be used as alternative medicine in the treatment of infections caused by these strains of bacteria.

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