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Antibacterial Activity of Ethanol Extract of *Monodora myristica* Seed

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Abstract

The study evaluated the antibacterial activity of ethanol seed extract of *Monodora myristica* (African nutmeg). Extraction was done by soxhlet method with 75% ethanol, test organisms were clinical isolates of *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli*, Broth culture and Mueller-hinton agar were prepared, Lorkes method was employed in carrying out acute toxicity, antibacterial action and Minimum Inhibitory Concentration (MIC) were determined by agar well diffusion method, and agar dilution respectively. Standard antibacterial agents used were gentamicin, amoxicillin and chloramphenicol. Phytochemical screening revealed the presence of saponin, tannin, flavonoids, steroids, terpenoids, cardiac glycoside, protein and carbohydrate. Acute toxicity testing showed no mortality up to 5000 mg/kg in mice. The seed extract had antibacterial against *B. subtilis* and *P. aeruginosa*. Minimal inhibitory concentration of seed extract demonstrated resistance to *Staph. aureus* and *E. coli* at high concentration (MIC) (400 mg/ml) whilst *P. aureuginosa* and *B. subtilis* showed no growth at varying concentration at certain doses, notably 400 mg/ml and 200 mg/ml, even at lesser doses with *B. subtilis*. When compared with standard antibacterials cell/L (viz; gentamycin, chloramphenicol, amoxicillin) the activity of the standard ratio (10 ug/ml) of gentamicin was higher than that of the seed extracts on different concentration against *S. aureus*, against the plant extract agent with the post-hoc tukey test (0.00, p = 0.008). The conventional chloramphenicol produced statistically insignificant values ($p > 0.05$) on *P. aeruginosa* and *B. subtilis*. The assessment of activity of seed extract on test organism against standard amoxicillin revealed that there were great differentiation in favor of standard amoxicillin (P < 0.05). These findings suggest that *M. myristica* seed extract possesses moderate antibacterial potential.

Keywords

Monodora myristica, Antibacterial, Nutmeg, LD_{50}, Phytochemicals

1. Introduction

Our environment is replete with harmful microbes, and as such plants and animals are susceptible to the various diseases the organism cause [1]. Scientists are working assiduously to contain, prevent and treat these debilitating diseases. Historically, natural medicinal plants have been used to treat such diseases either with their active ingredients or through the whole plants [2]. Plants act as important sources of medicines fibre, food, shelter and other source in day to day human beings uses. With the help of animals and the human being, roots, stems, leaves flowers, fruit and seed are fed on [3]. Plant are priceless components in human diet that gives the body minerals salts, vitamins and certain hormone precursors not only protein and carbohydrate, but also with nutritive and caloric values that make them essential in diets [4].

There are agricultural examples of some of these plants that contain seed of *Monodora myristica* known as African nutmeg, African nutmeg is a perennial edible vegetable plant species of the family Annonacea, is a berry growing wild in the evergreen forest of the West Africans [5]. Their seeds are also economic and medically useful [6]. The diversity of plant phytochemicals suggests that medicinal plant when developed can be a natural antibacterial against the prevalent bacterial infections [7]. African and continental foods in Nigeria has the seed, and embedded on a white smelling pulp, they are mostly used as a condiment in food which contain (*Myristica fragrans*), which naturally comes after it is pounded, they can also be applied as an aromatic stimulating additive into medicine and snuff, when grind finely they can be used to act as a stimulus or in the treatment of constipation, the powders can be sprayed over boils particularly those that are induced by guinea worm [8]. Medicinal plant typically has a set of compound that may serve as the natural antibacterial agent against the common bacterial infection [7].

Little is available about the nutritive composition, anti-nutritive composition and physiochemical properties of African Nutmeg. The production and large-scale manufacturing of chemically synthesized drugs over the last century transformed the health care in most highlighted regions in the globe. Nevertheless, in developing nations, even the treatment of the vast majority of the population is based on traditional practitioners and herbal medicine. The population in Africa is 90 percent and that in India 70 percent willing to utilise the traditional medicines in addressing their health care needs. Traditional medicine contributes a majority of the health care in China which is about 40 percent and over 90 percent of all hospitals in China have departments that deal with traditional medicine [9]. It is estimated that today, 50 percent of the western drugs use plant material or have models which are made with the usage of plant material [10].

The percentage of hard to treat infectious diseases, primarily the associated infection of the enteric bacteria has increased in the recent past, because of the development and the sustained transmission of the drug resistant mechanisms. Unlike synthetic, plants derived medicines are quite safe and provide strong therapeutics advantages and cost effective treatment [11]. The information available on the research of the African nutmeg is therefore in need of argumentation [5]. In addition, all these require determination of the antibacterial ability of *Monodora myristica* on human pathogen due to emergence of high resistance to synthetic antibiotics. Despite the extensive ethnomedicinal use of *M. myristica* as a condiment and remedy, there is limited experimental data on its antibacterial efficacy, this is with respect to the potential comparative effects of the ethanol extract with conventional antibiotics, other plant extracts have not been extensively explored, although some studies have observed synergistic action with other natural products like honey and Aloe vera gel. This study is with respect to the potential comparative effects of the ethanol extract with conventional antibiotics aimed to evaluate the phytochemical composition and antibacterial potential of ethanol seed extracts of *M. myristica* against selected bacterial pathogens.

2. Method

2.1. Collection and Identification of Plant Material

Monodora myristica (ehuru) was purchased from Onitsha main market and identified by a plant taxonomist Mrs Anthonia, Emezie at Pharmacognosy department, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu.

2.2. Ethical Approval

Ethical approval was obtained from Nnamdi Azikiwe University Teaching Hospital Ethics Committee (NAUTHEC), Nnewi, Anambra State.

2.3. Sample Preparation

The pods of *M. myristica* were opened up and the grain weighing 750 g was collected, air dried for some days, crushed with the mechanical/electrical grinder, and sieved to obtain a fine specimen of the sample of about 719 g.

2.4. Extraction of Pulverized Seed of *Monodora myristica*

Using soxhlet extraction method, some 719 g of the ground material was extracted with 75% ethanol over a period of time of 72 hours to generate the ethanolic extract. Concentration was done using HH-S water bath and approximately 292.30 g of ethanol extract was obtained and stored in a 50 ml conical flask laced with an aluminium foil and stored a refrigerator of 4˚C.

2.5. Phytochemical Screening

The phyto screening and quantitative analysis of the extract were conducted to reveal the constituents of the extract by using standard phytochemical method as indicated by Evans and Sofowora [12] [13].

2.6. Test Organisms

The antimicrobial tests was done using clinical isolates of *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. All the organisms were obtained in Pharmaceutical Microbiology and Biotechnology laboratory at Nnamdi Azikiwe University, Awka, Nigeria. All the microbial isolates were grown in their respective selective media and cultured in the axenic culture.

2.7. Culture Media and Reagent

Culture media used were nutrient broth, and Mueller-Hinton Agar (Oxoid Limited, England).

The formation of culture media was done in keeping with the strategy of the manufacturer. After the autoclaving, it was allowed to cool to a temperature of 50˚C and added immediately to a flat bottom petri dish in a horizontal plane. Surgical tools called Mc Farland 0.5 turbidity prepared standard, sodium hypochlorite solution, 0.5.

Ingredient	Gram/1L
Agar	15.00
Peptone	5.00
sodium chloride	5.00
Beef extract	1.50
Yeast Extract	1.50
PH	7.4 ± 0.2 at 25˚C
Distilled water	1 ltr

Ingredient	Quantity (g/L)
Peptic digest of animal tissue	5.00
Sodium chloride	5.00
Beef extract	1.50
Yeast extract	1.50
pH	7.4 ± 0.2 at 25˚C
Distilled water	1 ltr

2.8. Preparation of Media and Reagent

Broth Nutrient broth (13 g/L) was prepared according to the specification given by the manufacturer. Nutrient agar (28 g/L) was prepared in the same way. Mueller-Hinton agar (38 g/L) was prepared as well.

2.9. Nutrient Agar Preparation

The weight of nutrient agar powder was 8.4 grams which was dissolved in distilled water 300 ml that had been mixed with that and then the solutions, 20 ml apiece were put into McCartney bottles and steamed in autoclave at a temperature of 121˚C for 15 minutes.

2.10. Nutrient Broth Preparation

The nutrient broth powder weighing 3.9 gram was dissolved in 300ml of distilled water and mixed and then resorted into 20ml of every solution was collected in McCartney bottles that were subjected to autoclave at 121˚C 15 minutes.

2.11. Mueller Hinton Preparation

The weight of Mueller Hinton powder (8.4) was weighed, dissolved in 300 ml of distilled water and subsequently homogenised prior to addition of the test solution, 20 ml, in different McCartney bottles and be sterilised by autoclave using 121˚C at most before adding 20 ml of the extracts [14].

2.12. Collection of Test Organisms

Cultures of test organism (bacteria) which consisted of gram positive (*S. aureus* and *B. subtilis*) and gram negative (*P. aeruginosa* and *E. coli*) were taken in-vitro and then used in the antimicrobial test. Clinical isolates: Ensured that all the lab isolates belonged to the laboratory of Pharma advisor microbiology and biotechnology, faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka. They were incubated in 48 hours at 4˚C in refrigerator.

2.13. Experimental Animal

12 mice of mixed sex weighing 21 - 28 g were purchased from the department of Pharmaceutical Science animal house and were kept in the cage of animal house Pharmacology department, Faculty of Pharmaceutical sciences, Nnamdi Azikiwe University, Agulu. They were then used for the acute toxicity study of seed extract of *M. myristica*. The animals were provided with vital feed that was palletized commercial rat feed (palletized feed) along with the tap water.

2.14. Acute Toxicity Test

It was the Lorke method [15] for carrying out acute Toxicity of *M. myristica* seed. The experiment was conducted in two steps. During the first phase of the research 9 mice were randomised into three different groups of three mice each and treated to receive 10, 100 and 1000 mg/kg. body weight of the *M. myristica* extracts by suprolipidious technique that is oral lavage using sterile syringe. The signs of toxicity were noted in the mice which included salivation, entire body stretching, weakness, drowsy, restrained breathing, coma and death in the initial two hours, periodically after another six hours and eventually after twenty four hours. The second phase of the study would represent the second group of 3 mice randomised into three groups with the result of the first phase determining the administered body weight (kg) of the *M. myristica* seed extract 1600, 2900 and 5000 mg/kg each. These were monitored to find out indications of toxicity and mortality as in phase one and the number of deaths registered.

$$\frac{\text{Dose mg/kg} \times \text{weight (kg)}}{\text{Stock (mg/ml)}} = \text{dose volume (ml)}$$

2.15. Antibiotic Susceptibility Testing (AST)

The aim of this paper was to examine the ethanol extract of *Monodora myristica* (seed) in terms of its antibacterial systems. The extract of *Monodora myristica* was obtained as a seed extract and bacteria that were tested (*S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli*) were also performed against the bacterial using agar well diffusion assay. This phytochemical screening showed Saponin, Glycoside and Protein. The highest dose of 5000 mg/kg of seed extract was not lethal to the mice, there was no death in them by the use of Lorke method of determination of oral LD_{50} in rat. It worked only with *B. subtilis* and *P. aeruginosa*, had the minimum inhibitory concentration of 50 mg/ml product and 100 mg/ml product of the seed extract. Going further, the extract of the seed was found to be inactive to *E.coli* and *S.aureus*. Presence of the standard (gentamicin 30 ug/ mL) has shown no reaction with the seed extracts, where, however, presence of standard (gentamicin concentration of 10 ug/ml) was very high when compared to the seed extract against various concentration against *S. aureus*, *P. aeruginosa*, an activity close to that of the seed extracts was present against *E. coli* ($p = 0.008$). Conventional chloramphenicol was statistically not significant in *P. aeruginosa* and *B. subtilis* on ($p > 0.05$). Comparison of the effect of the seed extract on the test organism and the standard Amoxicillin revealed that the differences between the two were great in favor of the standard Amoxicillin ($p < 0.05$). The research was shown to be successful with the consequences that ethanol possessed antibacterial properties.

2.16. Determination of Antibacterial Activity of Ethanol Extract of *M. myristica*

Diffusion Agar well method; 800mg of the extract was weighed and dissolved into 2 ml of DMSO and final resultant concentration was termed as 400 mg/ml which at 45˚C was deemed as stock and then 2 times serial dilution was performed each on a test tube with 2 ml of DMSO up to 200 mg/ml, and down to 50 mg/ml, 25 mg/ml and 12.5 mg/ml [16] at 37˚C, the antimicrobial was done in the extract by quantifying the zone of hindrance round any wells (except the well diameter) with aid of sterile syringe and pre-diffusion using 25 to 30 minutes and incubating them, the seed extracts were investigated on all bacteria. The bacterial against positive control was 10 ug/ml of gentamicin, Amoxicillin 10 ug/ml, and Chloramphenicol 30 ug/ml. The diameter of the inhibition radii against the test organisms has assumed its concentration strength and was determined and inserted into further analysis. Any deliberation of all the tests was carried out in duplicate of the bacteria pathogens as well as the antimicrobial performance in terms of mean diameter clear zone (mm) at plant extracts.

2.17. Determination of Minimum Inhibitory Concentration (MIC)

The MIC of it was determined by agar dilution method. The extract of 4000 mg/ml was drawn and 1-fold serial dilution in the test tube coated with 2 ml of each of the segmented concentration to acquire the end results of 2000 mg/ml, 1000 mg/ml, 500 mg/ml, 250 mg/ml and 125 mg/ml. To speed up the study it was done in serial dilution of 1ml of the respective concentrations of the extract solution and 19 ml of molten Mueller Hinton agar were used to dilute the 1 ml of extract solution, giving the resultant concentrations of 400 mg/ml, 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml. Upon continuous dilution from 4000 mg/ml after adding the extract solution of 19 ml of molten Mueller Hinton agar and 1 ml of nutmeg extract making 20 ml as a diluent reduces the concentration from 4000 mg/ml, 2000 mg/ml, 1000 mg/ml, 500 mg/ml, 250 mg/ml and 125 mg/ml to 400 mg/ml, 200 mg/ml, 100 mg/ml 50 mg/ml, 25 mg/ml and 12.5, Which had been stored in the sterile Petri-dish and allowed to solidify. Streaking was carried out followed by incubation of colonies on the agar within 24 hours at 37˚C. The plates were then checked and a result of (+) or (−) was recorded on respective plates as presence and absence of growth respectively.

2.18. Statistical Analysis

The results were represented by use of mean and standard deviation. To conduct the statistical analysis, one way analysis of variance (ANOVA) was run and conducted in SPSS (version 20) statistical programme. The obtained results were considered to be significant at P 0.05 or less.

3. Results

3.1. Phytochemical Screening of *Monodora myristica* Seed Extract

As observed in the phytochemical screening (Qualitative), the seed extracts of *M. myristica* presented alkaloids, saponin, flavonoid, tannins, steroid and terpenoid (Table 1). The traces with low intensity of the presence exhibited the presence of Flavonoids, saponins, and Terpenoids over the other phytoconstituents demonstrated in the ethanol extract.

3.2. Result of Toxicity Study of *M. myristica* Ethanol Seed Extract

Table 2 indicated that the plant extract was not toxic to the rat even at considerable dosage of 5000 mg/kg. The model utilised by Lorke [15] was utilized and the experiment was conducted in two stages with varying number of rats. During the phase one, no signs of toxicity were recorded at 10 mg/kg, 100 mg/kg and 1000 mg/kg. Phase two toxicity; two of the three mice each dose 1600, 2900 and 5000 mg/kg, of the drug have not been recorded.

Table 1. Phytochemical screening of *monodora myristica* (ehuru) seed extracts.

Phytochemical Analysis of Ethanol Extract (Qualitative).		
S/N	**Parameters**	**Ethanol**
1	Alkaloid	+
2	Saponin	+
3	Tannin	+
4	Flavonoids	+
5	Steroids	+
6	Terpenoids	+
7	Cardiac glycoside	+
8	Protein	+
9	Carbohydrate	+

KEY: + = Trace/mildly present, − = Absent.

Table 2. Tabular result toxicity study of *M. myristica* ethanol seed extract.

Phase	Dose	No. of Deaths
	10 mg/kg	0/3
I	100 mg/kg	0/3
	1000 mg/kg	0/3
II	1600 mg/kg	0/3
	2900 mg/kg	0/1
	5000 mg/kg	0/1

Note: Phase 1 = 3 is the number of mice; Phase 2 = 1 is the number of mice; $LD_{50} > 5000$ mg/kg.

3.3. Statistical Analysis Comparing Ethanol Extract of *M. myristica* with Antibiotic (Gentamicin) Using Post Hoc Test

Table 3 compared mean IZDs of the various concentrations of the extracts on the organisms and found this to be significantly different ($p = 0.000$). The IZDs of 400, 200, and 100 mg of concentration on *P. aeruginosa* and *B. subtilis* were also staticstically significant ($p = 0.053$) yet were also significantly different compared to that of *S. aureus* and *E. coli* ($p = 0.000$). *B. subtilis* had the only significant activity with the extract at 50 mg concentration ($p = 0.000$).

Table 4 applied the Post Hoc Tukey test to establish the differences in the mean of IZDs obtained using gentamicin at 10 ug/mL of concentration and plant extracts at a different concentration form. The activity using normal gentamicin was very high as compared to extract in different concentrations in the case of *S. aureus*, *E. coli*, *P. aeruginosa* and *B. subtilis*.

($P < 0.05$). See Table 3.

Table 3. Comparative potency evaluation of plant extracts against Control (Gentamicin) on Test Organisms.

Test group	*S. aureus* (Mean ± SD IZD mm)	*E. coli* (Mean ± SD IZD mm)	*P. aeruginosa* (Mean ± SD IZD mm)	*B. subtilis* (Mean ± SD IZD mm)	F	p
400 mg	0.00 ± 0.00	0.00 ± 0.00	5.50 ± 0.71	7.50 ± 0.71	118.00	0.00
200 mg	0.00 ± 0.00	0.00 ± 0.00	4.00 ± 0.00	6.50 ± 0.71	163.67	0.00
100 mg	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 0.00	3.50 ± 0.71	46.33	0.01
50 mg	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 0.00	-	-
25.5 mg	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	-	-
12.5 mg	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	-	-
Gentamicin (10 µg/ml)	20.00 ± 4.24	21.00 ± 2.82	20.00 ± 4.24	21.00 ± 5.66	-	-

F = ANOVA value. P < 0.05.

Table 4. Post-hoc (Tukey) multiple comparisons of extracts against control (Gentamycin) against test organisms.

	Gentamycin			
Extract concentration	***S. aureus***	***E. coli***	***P. aeruginosa***	***B. subtilis***
400 mg/ml*	0.000	0.000	0.001	0.005
200 mg*	0.000	0.000	0.000	0.003
100 mg*	0.000	0.000	0.000	0.001
50 mg*	0.000	0.000	0.000	0.001
400 mg*	-	-	0.957	0.999
200 mg*	-	-	0.415	0.570
100 mg*	-	-	0.100	0.280
50*	-	-	0.863	0.802
200 mg*	-	-	0.297	0.458
100 mg				
50 mg				

3.4. Chloramphenicol

As seen in Table 5 and Table 6, the various concentrations of the plant extract failed to effectively show any activity on *S. aureus* and *E. coli*, however the concentrations were active on *P. aeruginosa* and *B. subtilis*. In *S. aureus* with the standard (30 mg of Chloramphenicol). Another significant activity in comparison with those of the extracts was seen on *E. coli* ($p = 0.008$). As it was noted, the standard was statistically non-significant ($p > 0.05$) on *P. aeruginosa* and *B. subtilis*.

Table 5. Comparative potency evaluation of plant extracts against control (Chloramphenicol) on test organisms.

Test group	*S. aureus* (Mean ± SD IZD mm)	*E. coli* (Mean ± SD IZD mm)	*P. aeruginosa* (Mean ± SD IZD mm)	*B. subtilis* (Mean ± SD IZD mm)	F	p
400 mg/ml	0.00 ± 0.00	0.00 ± 0.00	5.50 ± 0.71	7.50 ± 0.71	118.00	0.00
200 mg/ml	0.00 ± 0.00	0.00 ± 0.00	4.00 ± 0.00	6.50 ± 0.71	163.67	0.00

Continued

100 mg/ml	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 0.00	3.50 ± 0.71	46.33	0.01
50 mg/ml	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 0.00	-	-
25.5 mg/ml	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	-	-
Chloramphenicol (30 μg)	0.00 ± 0.00	3.00 ± 1.41	0.00 ± 0.00	5.00 ± 1.41	-	-

F = ANOVA value. $p < 0.05$.

Table 6. Post hoc Tukey multiple comparison of plant extract against control (chloramphenicol) on test organisms.

	Extract comparison			
Chloramphenicol	***S. aureus***	***E. coli***	***P. aeruginosa***	***B. subtilis***
	400 mg*	0.008	0.894	0.083
	200 mg*	0.008	0.997	0.429
	100 mg*	0.008	0.997	0.429
	50 mg*	0.008	0.998	0.036
400 mg	200 mg*	-	0.997	0.781
	100 mg*	-	0.561	0.008
	50 mg*	-	0.000	0.000
200 mg	100 mg*	-	0.894	0.036
	50 mg*	-	0.001	0.004

In the same way, Table 7 and Table 8 below showed the action of a normal Amoxicillin (10 μg/mL) against the diverse organisms used in a study. Along with the extracts, no activity was demonstrated in *S. aureus* and *E. coli*, but activities and was demonstrated in *P. aeruginosa* and *B. subtilis* at concentrations of 100 mg and 50 mg respectively. The comparison between the IZDs of the standard and the test organism showed that significant difference ranged against the standard ($p < 0.05$).

Table 7. Comparative potency evaluation of plant extracts against control (Amoxicillin) on test organisms.

Test group	*S. aureus* (Mean ± SD IZD mm)	*E. coli* (Mean ± SD IZD mm)	*P. aeruginosa* (Mean ± SD IZD mm)	*B. subtilis* (Mean ± SD IZD mm)	F	p
400 mg	0.00 ± 0.00	0.00 ± 0.00	5.50 ± 0.50	7.50 ± 0.71	118.00	0.00
200 mg	0.00 ± 0.00	0.00 ± 0.00	4.00 ± 0.00	6.50 ± 0.71	163.67	0.00
100 mg	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 0.00	3.50 ± 0.71	46.33	0.00
50 mg	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 0.00	-	-
25 mg	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	-	-
12.5 mg	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	-	-
Amoxicillin (10 μg/ml)	17.00 ± 4.00	10.50 ± 2.50	14.00 ± 2.00	19.00 ± 1.41	-	-

F = ANOVA value. $p< 0.05$.

Table 8. Post-hoc (Tukey) multiple comparisons of extracts against antibiotic control (Amoxicillin) on test organisms.

	Extract concentration				
	Compared groups	***S. aureus***	***E. coli***	***P. aeruginosa***	***B. subtilis***
	400 mg*	0.000	0.000	0.001	0.000
	200 mg*	0.000	0.000	0.000	0.000
	100 mg*	0.000	0.000	0.000	0.000
400 mg	200 mg*	-	-	0.806	0.781
	100 mg*	-	-	0.128	0.008
	50 mg*	-	-	0.000	0.001
200 mg	100 mg*	-	-	0.577	0.004

3.5. Minimum Inhibitory Concentration of the Seed Extract of *Monodora myristica*

As indicated in Table 9 below, the extract was significantly active, MIC was established of the plant extract on the chosen pathogenic bacteria. Table 9 showed sensitivity to *staph. aureus* and *E. coli* at high concentration *i.e.* 400 mg/ml to a diameter of 8 mm whilst *P. aureuginosa* and *B. subtilis* showed no growth at varying concentration *i.e.* 50 mg/ml to 400 mg/ml to diameter of 8 mm.

Table 9. Minimum inhibitory concentration of the ehuru extract against test organisms.

	Extract concentration (mg/ml)					
Test organism	**400**	**200**	**100**	**50**	**25**	**12.5 (mg/ml)**
Staph. aureus	+	+	+	+	+	+
E. coli	+	+	+	+	+	+
P. aeruginosa	−	−	+	+	−	+
B. subtilis	−	−	−	−	+	+

Key: − = no growth; + = growth.

4. Discussion

The current research sought to determine the antibacterial activity of *M. myristica* seed. Ethnomedical research in the field of antibacterial properties of nutmeg has been reported [17]. During the research, the outcome indicated that the extract of *M. myristica* had several phytochemicals. This is in accordance with the results obtained in this paper, these phytochemical metabolites present in the plant extract are phenols present in the cell of the plant, and are highly potent inhibitors of majority of the hydrolytic enzymes which are proteolytic enzymes subcategories as macerating enzymes linked to plant pathogen [18]. This is correlated to the fact as well, as to the reason why the plant extract possesses the antibacterial properties as well.

Acute tests are applied in determining the adverse effects of a single dose exposure of the chemical over a short period of time, it provide toxic effects likely to be manifested by a short exposure to the chemical. It has traditionally been the most significant when it comes to the procedure of evaluating and determination of the toxicity of substance. It is a pre-exploratory study of toxicity, it provides the information on the expected health risks that will be encountered when one is exposed to acute exposure to drugs [19] [20]. The increase in ethanol extract of *M. myristica* tested in the male Albino mice in one oral dose 10 mg/kg to 5000 mg/kg revealed that the plant extract was not a toxic, the result of the acute toxicity revealed that there was no toxicity up to the highest dose of 5000 mg/kg. The above report claimed that *M. myristica* seed extract is safe within twenty four hours, Kemi [21] affirms that there is no reported toxicity of any extract of the plant in the plasma and liver of mice on ethanol extract of the plant given to mice.

Plant-based antibacterial have massive treatment prospects because they have minimal side effects unlike synthetic antibacterial. Plants could be useful in treatment of various conditions and the use of herbs is gaining more patient compliance as they do not have common side effects of allopathic drugs [22]. The antimicrobial effect of active compounds of the plant extract varies depending on the active compounds, and they are typically antimicrobial with the negative effect as they are measured and marked in the form of minimum inhibitory concentration. The findings thus showed that Nutmeg (ehuru) exhibited more activity against Gram-positive *B. subtilis* and Gram-negative *P. aeruginosa*, however less against Gram-positive *S. aureus* and Gram-negative *E. coli*. The same values are applicable as a guide when it comes to treatment of certain infections. A probable reason for the selective activity of nutmeg extract against certain bacteria can be ascribed to differences in bacterial cell envelope, composition, specific resistance mechanisms, and variations in the extract's active phytochemical components and their mechanisms of action. In essence, the varied effects are not simply about Gram-positive versus Gram-negative status but rather the unique interplay between the diverse active compounds in nutmeg and the specific structural and physiological defenses of each individual bacterial species and strain.

The antimicrobial efficacy of the extract is associated with the activities that were undertaken by Olusimbo *et al.* [23]. The validation of *M. myristica* as an antibacterial plant using stabilising Nigerian plant. Moreover, IZD (Inhibition Zone Diameter) is also different across plants and across concentrations. In this way, some gram-positive and also a few gram-negative bacteria in both concentrations were sensitive to ethanol extract of *M. myristica*, and similarly, this finding was consistent with the study reported by Olusimbo *et al.* [23]. In addition, the inferences are congruent to the inferences made by the group of researchers. The article published by Yuniasih *et al.* [17], stated that they have indicated that the antibacterial effect of the nutmeg oil can inhibit the growth of *E. coli*, *S aureus*, as well as *P. auregi* bacteria. Moreover, Narasimhan and Dhake [24] X-rayed the antimicrobial properties of nutmeg by establishing the minimum inhibitory concentration of all the constituents of nutmeg with the targeted Gram positive and Gram negative organisms, since the statistics of all the antimicrobial activities of the varied constituents were satisfactory as doubled. The antimicrobial activity of nutmeg in *Escherichia coli* O157 strain has also been reported. The team of researchers also explained with evidence that the strain of *E. coli* O157 become highly susceptible to opinene which is a human element that is found in nutmeg, this therefore gushes out to mean that, *β*-pinene contributes to the antimicrobial action by the bacterium on this specific strain. The chemicals present in the nutmeg is capable of contributing to the overall antibacterial effect of the nutmeg. These findings assigns the presence of a variety of bioactive nutmeg phenols such as: trimyristin, myristic, lignans, b-pinene, and plant phenolics that are probable causes of its antimicrobial effect in bacteria [25].

From the findings, the standard antibacterial agents included; Gentamicin 10 µg/mL, Amoxicillin 10 µg/ml, Chloramphenicol 30 µg/ml served as the positive control against bacterial proved effective than the extract of *Monodora M*, this could be because standard antibacterial agents are often superior to extracts like *Monodora myristica* in experimental studies primarily due to standardization, consistent potency, known mechanisms of action, and stability/purity. Whiliki *et al*. [26] reported thus, the extract concentrations were less than those observed with the reference antibacterial drug (ciprofloxacin) and antifungal agent (fluconazole), which is indicative that the reference drugs had a better antimicrobial action. The result from this study therefore, lends credence to the approved conventional use of standard antibacterial, comparison of the different standard antibacterial agents to the extract's effectiveness indicates that the purity and lower active compounds in the extract, moreover, the extract is still in its crude state which requires a lot of purification in order to isolate the active compounds [27].

Another study conducted by Kalemba and Kunicka [28] by evidence showed that MIC and minimum bactericidal concentration (MBC) of the herbal compounds under broth dilution procedures above serial dilution of herbal compound indicated that the MIC of the sage as an essential oil was reported to be that of 100 - 1000, 2 g/ml that was obtained after incubation duration of the herbal product under incubation. Additionally, Lagha *et al*. [29] have found that nutmeg was active as an antibacterial agent in animals as against clinical isolates for this study, the team of researchers reported that the extract of nutmeg displayed antimicrobial action by acting against all the microbes that were examined and its activity did not change on exposure to a high temperature (80.0˚C ± 2.0˚C) and pH conditions as used (3.0, 6.8 and 11). Moreover, they also determined that the MIC of 0.16 - 0.63 extract had a potent antibacterial action with the growth of microorganisms in raw beef through derivation of 21 days of chilled or frozen beef. These early findings using the extract of *Monodora myristical* are some indications that there is still much to be conducted in identifying the antibacterial vehicles of this plant and fully classifying their dispensation.

The study limitations were that, just ethanol solvent was used for the extraction, the yield and concentration of bioactive compounds in the extract can vary significantly based on the extraction method used, the part of the plant used, climatic factors, soil conditions, and the maturity stage of the seed at harvest. This makes standardization difficult, as such other solvents like aqueous, methanol, essential oil, seed can be collected from other location along with fractionation can be done, further research is needed to isolate these active components to maximize their therapeutic potential in drug formulation.

5. Conclusion

According to our findings, *M. myristica* (ehuru) has been shown to possess antibacterial activity against *P. aeruginosa* and *B. subtilis* at concentration of 50 mg/ml and 100 mg/ml respectively. It thus provides evidence that the seed extract of *Monodora myristica* has antibacterial action and thus its employment in traditional medicinal practises of controlling infections is empirically acceptable.

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Conflicts of Interest

None of the authors claimed a conflicting interest.

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