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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL EFFECT OF SEEDS OF *Monodora myristica* ON SELECTED MICROORGANISMS

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ABSTRACT

The increase in the prevalence of effects of many synthetic antimicrobial agents and incidence of multiple drug resistant microorganisms has spurred scientists on the research for plant-based antimicrobial of therapeutic potentials. The *Monodora myristica* has been used in Nigeria traditionally as condiments and has been harnessed as a therapeutic agent in the treatment of skin infection and dysentery. Aqueous, n-hexane and ethanolic extracts of the seeds was screened for antimicrobial activity against pathogenic microorganisms implicated in causing vagina infections. This finding showed that the efficacy of the extracts was concentration dependent. Aqueous, ethanolic and N-hexane extract showed varying degree of inhibition with different magnitude against the test isolates. Ethanolic extract showed highest activities against *Candida albican*, followed by *Staphylococcus aureus* and *Klebsiella pneumoniae* with 21.5mm, 19.5 mm each respectively. The same patterns of of inhibition was also observed in N-hexane extract. The antimicrobial effect of the two extracts were statistically not significant. The extracts had minimum concentration that ranged between 150mg/ml and 200mg/ml. The ethanolic extract showed more inhibitory effects compare to the aqueous and n- hexane extracts. The ethanolic extract found to contain highest quantity of phytochemicals while aqueous extract has lowest amount of the phytochemicals. It is therefore can be affirmed that *Monodora myristica* seed extracts could be exploited as therapeutic agents to drug resistant microorganisms.

Keywords: *Monodora myristica*, Therapeutic agents, Drug resistant microorganisms

Introduction

From time immemorial, plants have been utilized worldwide for the treatment of infectious diseases. Nearly 80% of the World's populace relies on plant-based medicine for the care of their health (Singh and Bindhu, 2019). The plant parts or its products meet societal health need stems from the fact that indiscriminate use of commercial antimicrobial drugs commonly utilized in the treatment of infectious diseases has led to the

development of multiple drug resistance (Osugwu and Onwuegbuchulam, 2015), the use of plants is safe and cost effective in traditional as well as in modern medicine (Treasure *et al.*, 2020). Most of these native plants possess bioactive compounds that exhibit physiological activities microorganisms and are also used as precursors for the production of useful drugs. Thus, the importance of these plant products in medicine is as a result of the

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presence of bioactive compounds such as phenolics, alkaloids, flavonoids, tannins, resins, steroids, and other secondary metabolites which they contain and are capable of producing definite physiological action in the body (Bishnu *et al.*, 2009). Phytochemicals carry out essential medicinal roles in the body. There is urgent need for discovery of new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas *et al.*, 2003; Olusimbo *et al.*, 2011).

Hence, this study was aimed at assessing the antimicrobial effects of *Monodora myristica* seeds on selected human pathogens implicated in causing several infections.

Materials and Methods

Collection and Identification of Plant Material

Fresh seeds of *Monodora myristica* were collected from Oja-Tuntun market Ilorin. The seeds were authenticated as UILH/001/1255 at the Department of Plant Biology, University of Ilorin Kwara State.

Extraction of *Monodora myristica* Seeds

Fifty grams of *Monodora myristica* powder was dissolved in 250 ml sterile distilled water and mixed thoroughly before placing on a mechanical shaker for agitation for 72 hours at 200 rpm. The mixture was filtered using Whatman No. 1 filter paper into beakers, to obtain the filtrates; while 50 grams of the blended seed in 250ml of 70% ethanol and n-hexane in separate flasks, and was extracted using soxlet extractor. The extracts were concentrated using the rotary evaporator (Cole Parmer IKA RV 10 digital) at 50°C. The concentrates were lyophilized using a freeze-drier (model; U. therm international (H.K) limited FD-12-MR) at -4° C for few days and stored at 4° C until needed (Osuagwu and Eme, 2013).

Sterility Test of the Plant Extracts

This was done by weighing 0.2 grams/ 0.2 ml of each extracts and dissolved 9.8ml of sterile distilled water was added. Each of the aqueous, ethanol and n-hexane extract's sterility was confirmed by inoculating a drop of each of the extracts on sterile Mueller Hinton agar plates each and incubated at 37 °C for 24 hours.

Collection and Maintenance of Microbial Isolates

The microbial isolate used for this study were obtained from the Department of Microbiology, University of Ilorin. These include *Staphylococcus aureus* ATCC 6538, *Streptococcus* spp, *Enterococcus faecalis* ATCC 10231, *Klebsiella pneumoniae*, *Escherichia coli* ST2747, *Escherichia coli* ATCC 35218 and *Candida albicans* ATCC 334. The isolates were stock on nutrient agar and potato dextrose agar slant for bacteria and fungi respectively, and kept at 4° C. All the microbial isolates were sub-cultured for purification prior to biochemical identification.

Antimicrobial Susceptibility Testing

Agar diffusion method was adopted for this assay. The antimicrobial activity of the extracts against the test organisms was determined as described by Zakariyah *et al.* (2017) with slight modifications. A loopful of the standardized bacterial cell suspension (10⁸cfu/ml) was streaked evenly on each solidified Mueller Hinton agar plate. The seed extracts were reconstituted in 3% Di-methyl Sulfoxide (DMSO) to obtain the working concentrations of 50, 100, 150, and 200 mg/ml (Performance, 2006). 200 µl of each extract at different concentrations was inoculated aseptically into five wells (6 mm in diameter) earlier bored with sterile cork borer in each plate. The negative control was 200 µl of 3% DMSO; the plates were allowed to stand for 30 minutes for pre-diffusion of the extracts. The plates were incubated upright at 37° C for 24 hours for bacteria and 25° C for 48 hours for *Candida*

albicans. The mean diameter of zones of inhibition was recorded in millimeters (mm).

Standard Antibiotic Testing

The antibiotic used were Gentamycin 40 mg/ml against bacteria, and 25 µg of fluconazole against fungi, 200 µl of each antibiotics at different concentrations was inoculated into four wells (6 mm diameter) bored with a sterile cork borer in each plate. 200 µl of sterile distilled water was used as a negative control; the plates were allowed to stand for 30 minutes. The plates in duplicates were incubated at 37⁰ C and 25⁰ C for 24 hours and 48 hours for bacteria and fungi respectively. The mean diameter of zones of inhibition was recorded in millimeters (mm) (Performance, 2006).

Antibiotic Disc Testing

The antibiotic disc were ciprofloxacin 5 µg, nitrofurantoin 300 µg against bacteria, and 25µg of fluconazole against fungi, The plates in duplicates were incubated at 37⁰ C and 25⁰ C for 24 hours and 48 hours for bacteria and fungi respectively. The mean diameter of zones of inhibition was recorded in millimeters (mm) (Bamidele *et al.*, 2013).

Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Cidal Concentration (MCC)

The minimum inhibitory concentrations (MIC) of the extracts were determined by dilution to various concentrations according to the (Nweze 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.

The seed extracts that demonstrated significant antimicrobial activity were subjected to MIC and MCC assay, using the agar dilution method. The concentration of extracts that produced the least zone of inhibition in the antimicrobial susceptibility test was further diluted for up to two new concentrations. Standardized inocula of each organism to be tested were inoculated on sterile MHA plates. Wells were made using a 4mm sterile cork borer on the MHA plates into which the different concentrations of extracts were added aseptically. The plates were incubated and the MIC was read as the least concentration that inhibited the growth of the test organisms (Ukaegbu-Obi *et al.*, 2015; Zakariyah *et al.*, 2017).

Minimum cidal concentration was determined using tubes containing 0.2 ml of different concentrations of the extracts, 9.7ml of Mueller Hinton Broth and 0.1 ml of standardized inocula of each test organisms. The mixtures were incubated for 18-24 hours. After incubation, 0.1 ml of the broth was inoculated on MHA plates and incubated for 18-24 hours. The highest dilution that yielded no single cell colony on the solid medium was taken as the minimum bactericidal concentration.

Phytochemical Screening of Seed Extracts

Qualitative Phytochemical Screening

The aqueous, ethanolic and n-hexane extracts *Monodora myristica* seeds were tested for the presence of phytochemicals such as, alkaloids, tannins, phenolics, glycosides, terpenoids, saponins, flavonoids, steroids and phlobatannins using the standard procedures (Ogukwe *et al.*, 2004; Kar, 2007)

Statistical Analysis

All the results obtained were analyzed using simple statistics of one-way ANOVA (SPSS 20). The data were expressed as mean ± SD, difference between the means was considered significant at 95% Confidence Interval

Results

Table 1 depicts that the aqueous extract of *Monodora myristica* seeds were insignificant at concentrations of 50, 100, and 150 mg/ml except 200 mg/ml, where it was mildly significant on *S. aureus*, *Streptococcus* spp, *E. faecalis*, *Klebsiella pneumoniae*, and *Candida albicans*.

Table 2 shows that the ethanol extracts of *Monodora myristica* seeds were highly significant on all the organisms at highest concentration of 200 mg/ml, except for *Escherichia coli* ST2747, which was slightly significant. As the concentrations of the extract decreases, the significances of the zones of inhibition decreases.

Table 3 portrays the n-hexane extract of *Monodora myristica* seeds were also significant but not as high as the ethanolic extract.

Table 4 compares the antimicrobial activity of the extracts against the standard antibiotics which was more effective than the plant extracts.

Table 5 shows that the aqueous extract possessed minimum inhibitory concentration against most of the test organisms at 200 mg/ml while ethanolic and n-hexane extracts showed minimum inhibitory concentration against most of the test organisms at 150 mg/ml.

Qualitative analyses of phytochemicals in aqueous, ethanolic and n-hexane extracts of *Monodora myristica* seeds showed the presence of alkaloids, phenolics, steroids and tannins in all extracts while terpenoids and flavonoids were absent in all but glycosides was present in only ethanolic extracts (Table 6).

Figure 1 shows that the ethanolic extract has the most significant level of phytochemical components amongst the three solvents used for the extraction of *Monodora myristica* seeds except in steroids with high concentration in n-hexane while the aqueous extracts had the lowest concentration in all.

Table 1: Effect of Aqueous Extract of *Monodora myristica* Seeds on the Test Organisms Using Agar-well Diffusion Method

Organisms	Concentrations / Zone of Inhibition (mm)			
	200 mg/ml	150 mg/ml	100mg/ml	50mg/ml
<i>Staphylococcus aureus</i> ATCC 6538	13.5±0.50 ^a	11.0±1.00 ^b	9.5±0.5 ^{bc}	6.0±1.0 ^c
<i>Streptococcus</i> spp	12.5±0.50 ^a	10.0±0.0 ^b	8.5±0.50 ^c	6.0±0.00 ^d
<i>Enterococcus faecalis</i> ATCC 10231	12.5±0.45 ^a	11.5±0.45 ^b	8.5±0.50 ^c	6.5±0.45 ^d
<i>Klebsiella pneumoniae</i>	13.5±0.50 ^a	11.0±0.00 ^b	10.5±0.50 ^{bc}	10.5±0.50 ^c
<i>Escherichia coli</i> ATCC 35218	9.0±0.00 ^a	8.0±0.00 ^a	6.0±0.00 ^b	6.0±0.00 ^b
<i>Escherichia coli</i> ST2747 (MDRs)	6.0±0.00 ^a	6.0±0.00 ^a	6.0±0.00 ^c	6.0±0.00 ^a
<i>Candida albicans</i> ATCC 334	12.5±0.50 ^a	12.0±0.50 ^a	11.5±0.50 ^b	9.5±0.50 ^c

Mean values with different superscripts in the same column are significant different. Mean values were separated using Duncan Multiple Range Test (DMRT) (N=3)

Table 2: Effect of Ethanolic Extract of *Monodora myristica* Seeds Extract on the Test Organisms Using Agar-well Diffusion Method

Organisms	Concentrations / Zone of Inhibition (mm)			
	200 mg/ml	150 mg/ml	100mg/ml	50mg/ml
<i>Staphylococcus aureus</i> ATCC 6538	19.0±0.50 ^a	16.0±1.00 ^b	11.5±0.50 ^c	9.0±1.00 ^d
<i>Streptococcus</i> spp	18.5±0.50 ^a	16.0±0.00 ^b	12.5±0.50 ^{bc}	9.0±0.00 ^c
<i>Enterococcus faecalis</i> ATCC10231	17.5±0.50 ^a	15.5±0.45 ^b	13.5±0.50 ^{bc}	10.5±0.45 ^c
<i>Klebsiella pneumoniae</i>	19.5±0.50 ^a	16.5±0.50 ^b	14.5±0.50 ^{bc}	11.5±0.50 ^c
<i>Escherichia coli</i> ATCC 35218	17.5±0.50 ^a	16.0±0.00 ^a	9.0±0.00 ^c	6.0±0.00 ^c
<i>Escherichia coli</i> ST2747 (MDRs)	14.5±0.50 ^a	12.0±0.00 ^{ab}	8.5±0.00 ^c	7.0±0.00 ^c
<i>Candida albicans</i> ATCC 334	21.5±0.50 ^a	16.5±0.50 ^b	11.5±0.50 ^c	9.5±0.50 ^c

Mean values with different superscripts in the same column are significant different. Mean values were separated using Duncan Multiple Range Test (DMRT) (N=3)

Table 3: Effect of N-hexane Extract of *Monodora myristica* Seeds Extract on the Test Organisms Using Agar-well Diffusion Method

Organisms	Concentrations / Zone of Inhibition (mm)			
	200 mg/ml	150 mg/ml	100mg/ml	50mg/ml
<i>Staphylococcus aureus</i> ATCC 6538	18.0±0.50 ^a	16.5±1.00 ^a	11.5±0.50 ^b	9.0±1.00 ^c
<i>Streptococcus</i> spp	18.5±0.50 ^a	14.5±0.00 ^b	12.5±0.50 ^{bc}	9.0±0.00 ^c
<i>Enterococcus faecalis</i> ATCC 10231	16.5±0.50 ^a	16.5±0.45 ^b	13.5±0.50 ^{bc}	10.5±0.45 ^c
<i>Klebsiella pneumoniae</i>	18.5±0.50 ^a	16.5±0.50 ^b	14.5±0.50 ^{bc}	10.5±0.50 ^c
<i>Escherichia coli</i> ATCC 35218	17.0±0.00 ^a	16.5±0.00 ^a	9.0±0.00 ^c	6.0±0.00 ^c
<i>Escherichia coli</i> ST2747 (MDRs)	12.5±0.50 ^a	11.5±0.05 ^a	8.5±0.00 ^c	7.0±0.00 ^c
<i>Candida albicans</i> ATCC 334	20.5±0.50 ^a	14.5±0.50 ^b	11.5±0.50 ^c	7.5±0.50 ^c

Mean values with different superscripts in the same column are significant different. Mean values were separated using Duncan Multiple Range Test (DMRT) (N=3)

Table 4: Comparison of antimicrobial sensitivity of the extracts with antibiotics using agar-well and disc diffusion method

Organisms	Concentrations/ Zone of Inhibition (mm)							
	Aqueous extract 200 mg/ml		Ethanol extract 200 mg/ml		N-hexane extract 200 mg/ml		Gentamycin 40mg/ml	Ciprofloxacin 5µg
	A	D	A	D	A	D	A	D
<i>S. aureus</i> ATCC 6538	13.5±0.50 ^a	13.0±0.00 ^a	19.5±0.50 ^a	18.5±0.50 ^a	18.0±0.50 ^a	18.5±0.50 ^a	24.5±0.50 ^a	27.5±0.50 ^b
<i>Streptococcus</i> spp.	12.5±0.50 ^a	12.5±0.50 ^a	18.5±0.50 ^a	17.5±0.50 ^a	18.5±0.50 ^a	16.5±0.50 ^a	26.5±0.45 ^{ab}	26.0±0.00 ^a
<i>Enterococcus</i> <i>faecalis</i> ATCC 10231	12.5±0.45 ^a	12.5±0.00 ^a	16.5±0.50 ^a	17.0±0.00 ^a	16.5±0.50 ^a	17.0±0.00 ^a	24.5±0.15 ^{ab}	28.5±0.50 ^{ab}
<i>Klebsiella</i> <i>pneumoniae</i>	13.0±0.50 ^a	13.5±0.50 ^a	19.5±0.50 ^a	18.0±0.50 ^a	18.5±0.50 ^a	18.5±0.50 ^a	24.0±0.00 ^a	26.0±1.00 ^{ab}
<i>Escherichia</i> <i>coli</i> ATCC 35218	9.0±0.00 ^a	9.5±0.50 ^a	17.5±0.50 ^a	17.5±0.50 ^a	17.0±0.00 ^a	17.5±0.50 ^a	20.5±1.00 ^a	27.0±0.5 ^b
<i>Escherichia</i> <i>coli</i> ST2747 (MDRs)	6.0±0.00 ^a	6.0±0.00 ^a	12.5±0.50 ^a	11.0±0.00 ^a	12.5±0.50 ^a	12.0±0.00 ^a	26.0±0.00 ^a	25.5±0.5 ^a
<i>Candida</i> <i>albicans</i> ATCC 334	12.5±0.50 ^a	11.0±0.00 ^a	21.5±0.50 ^a	19.5±0.00 ^a	20.5±0.50 ^a	19.0±0.00 ^a	27.0±0.00 ^a	29.5±0.50 ^{ab}

Mean values with different superscripts in the same column are significant different. Mean values were separated using Duncan Multiple Range Test (DMRT) (N=3) Key: A- Agar diffusion, D- Disk diffusion, MDRs- Multi-Drug Resistant strain

Table 5: Minimum Inhibitory Concentration (MIC) and Minimum Cidal Concentration (MCC) of the Extracts

Organisms	Concentrations (mg/ml)					
	Aqueous Extract		Ethanollic Extract		N-Hexane Extract	
	MIC	MCC	MIC	MCC	MIC	MCC
<i>Staphylococcus aureus</i> ATCC 6538	150	200	150	200	150	200
<i>Streptococcus</i> spp	200	-	150	200	150	200
<i>Enterococcus faecalis</i> ATCC 10231	200	-	150	200	150	200
<i>Klebsiella pneumoniae</i>	150	200	150	200	150	200
<i>Escherichia coli</i> ATCC 35218	-	-	150	200	200	-
<i>Escherichia coli</i> ST2747 (MDRs)	-	-	200	-	200	-
<i>Candida albicans</i> ATCC 334	200	-	150	200	150	200

Key: MDRs- Multi-Drug Resistant strain

Table 6: Qualitative Analyses of *Monodora myristica* Seeds Extracts

Phytochemical Components	Extracts		
	Aqueous	Ethanol	N-Hexane
Alkaloids	++	+++	+++
Tannins	+	++	+
Phenolics	++	+++	++
Glycosides	-	+	-
Terpenoids	-	-	-
Saponins	-	++	-
Flavonoids	-	-	-
Steroids	+	++	+++
Phlobatannins	-	+++	++

Key: +++ = Present in an appreciable amount ++ = Present in a moderate amount + = Present in a trace amount or minute amount - = Completely absent

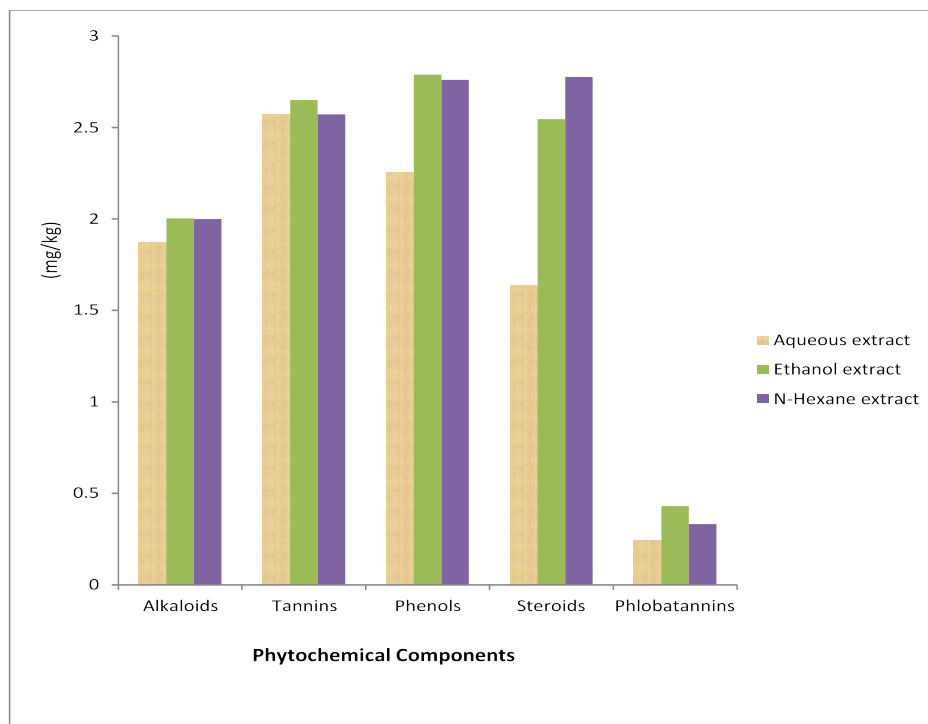


Figure 1: Quantitative Analyses of *Monodora myristica* Seeds Extracts

Discussion

The emergence of multiple drug resistant microorganisms is presently a global problem and a major cause of failure of the treatment of diseases (Awad and Aboud, 2015).

This study investigated the effects of aqueous, ethanolic, and n-hexane extract of *Monodora myristica* on some selected pathogens; *Staphylococcus aureus*, *Streptococcus* spp, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *E. coli* ST2747 (MDRs), *E. coli* ATCC S35218 and *Candida albicans*, found to be associated with various infections with significant risk of morbidity and mortality.

The efficacy of the aqueous extract of *Monodora myristica* seeds against test organisms was low which corroborated the work of Enabulele *et al.* (2014) this may be attributed to the absence and low phytochemical compounds as against the

ethanolic and N-hexane extract. At the concentration of 200 mg/ml in (Table 2), it was observed that the aqueous extract of *Monodora myristica* seeds was mildly effective against *Staphylococcus aureus* and *Klebsiella pneumoniae* statistically insignificant ($P < 0.05$) as compared with other test organisms which affirmed the work of Osuagwu (2013). Ethanolic extract was found to be most effective against all the test organisms at the 200 mg/ml as shown in Table 2. The n-hexane extract of *Monodora myristica* seeds was effective as ethanolic extract at the same concentrations, except against *Escherichia coli* ST2747 (MDRs), where it was mildly significant with 12.5 mm.

Antimicrobial susceptibility testing showed that the organisms had higher susceptibility only on all the test organisms at 200 mg/ml and 150 mg/ml concentrations of the extracts. In antibiotics sensitivity testing, all bacteria showed high

susceptibility to Gentamycin except for *E. coli* which showed moderate susceptibility, while for Ciprofloxacin; it was highly susceptible. *Candida albicans* showed high susceptibility to Fluconazole and Nystatin. Although none of the extract was effective as standard antibiotics but the ethanolic extract was highly effective against *Staphylococcus aureus*, *Streptococcus* spp, *Enterococcus faecalis*, and *Candida albicans* except *E. coli* ST2747 (MDRs). *E. coli* ATCC S35218 was moderately susceptible to the ethanolic and n-hexane extracts.

The presence of alkaloids, phenols, saponins, steroids and tannins in *Monodora myristica* seeds has been reported by Osuagwu, (2013). Also, high concentrations of phenols and phlobatannins with trace amount of tannins in ethanolic extract of *Monodora myristica* seeds might have accounted for its antimicrobial and astringent properties (Ijeh, 2004; Enabulele *et al.*, 2014).

The ethanolic extract has the most significant level of phytochemical components amongst the three solvents used for the extraction of *Monodora myristica* seeds, this depict that ethanol has the capacity to extract more phytochemical components than other solvents used in this study. The presence of these phytochemicals components in the seeds of *Monodora myristica*, confer it for its medicinal value (NNMDA, 2008). This study reveals that the more the phytochemical components the more its antimicrobial activity, which in line with (Ajayi *et al.*, 2008).

Conclusions

This study reveals that *Monodora myristica* seeds extract at the highest concentration of 200 mg/ml, showed varying degree of antimicrobial against test microorganisms associated with vagina infection. The phytochemical components of the seed extract found to be responsible for its antimicrobial activities. The n-hexane extracted pure oil with limited phytochemical components

and it rendered moderate effectiveness a, while aqueous extract contains the lowest phytochemical components, and was the least effective against all pathogens, which is due oily nature of the seed; a non-polar solvent will be preferred for the extraction of *Monodora myristica* seeds, therefore, this study affirmed the efficacy of *Monodora myristica* seed extract against bacterial pathogens associated with vaginal infections.

Conflict of Interest:

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