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ANTIMICROBIAL AND CHEMICAL COMPOSITION OF ESSENTIAL OIL OF MONODORA MYRISTICA SEEDS ON SOME CLINICAL BACTERIA ISOLATES

Musbau, S[1]., Asiru, R.A.[2] and Muhammad, Y.[2]

[1]Department of Microbiology, Yobe State University, Damaturu, Nigeria
[2]Department of Microbiology, Bayero University Kano Nigeria
[2]Department of Microbiology, Bayero University Kano Nigeria

[1]Correspondence author: mustee@gmail.com;

ABSTRACT

Monodora myristica seeds were obtained from Damaturu main market in Yobe State, ground into fine powder and stored in an air tight plastic container at room temperature until when used. Essential oils were extracted using Soxhlet extraction method and determinations of active component were carried out using gas chromatography-mass spectroscopy (GC-MS) technique. Microbial isolates were obtained from clinical samples obtained from hospital in Yobe state. Identification and characterization of isolates were carried out following standard microbiological procedures. The antimicrobial activity was determined using agar well diffusion method. Data obtained were subjected to a one-way analysis of variance. Significant means were separated using Duncan Multiple Range Test at 95% confidence level. The result showed that M. myristica oil contained eleven components which were mainly Indole-3-ethanol, acetate with the highest peak (6.22). Results of the antibacterial activity of the oil extracts (hexane, chloroform and ethanolic) reveal that all the extracts at different concentrations were active against the test isolates namely Proteus vulgaris, Pseudomonas aeruginosa, Escherichia coli, Shigella boydii and Klebsiella oxytoca. The minimum inhibitory concentration (MIC) values for all the three oil extracts ranged between 0.5 and 1.0%, while the minimum bacterial concentration (MBC) values for all the three oil extraction ranged between 1.0 and above 1.5%. The expressed antimicrobial activity is a demonstration of the efficacy of the oil against clinical isolates.

Keywords: Essential oils, antimicrobial activity, *Monodora myristica* and agar well diffusion method.

INTRODUCTION

Antibiotics played an important role in decreasing morbidity due to infectious diseases. Infectious diseases remain the leading cause of death worldwide and resistance to antibiotics by bacteria increasing worldwide probably due to the wide spread use of these antibiotics. Lord Jim O'Neill and his team published a high pro-file review report in 2014 which estimated that antimicrobial resistance (AMR) could cause 10 million deaths a year by 2050 (De-KM *et al.*, 2016). Due to increase in drug resistance, couple with the multiplicity of side effects of the antibiotic and the emergence of diseases for which no treatment yet exists, makes the search for the new antimicrobial agents a highly relevant and important subject for research. The number of resistant pathogenic bacteria grows at an alarming rate worldwide and the search for novel anti-microbial agents from medicinal plants to combat such pathogens has become crucial for avoiding the emergence of untreatable bacterial infections (Bandow, 2003; Pfaller *et al.*, 1998). For centuries, plants have been used in the traditional treatment of microbial infections. The plant *Monodoramyristyca* belong to the custard apple family of flowering plants called Annonaccea. It is widely distributed from Africa to Asia, Central to the South America and Australia (Omobuwajo *et al.*, 2013). It's native to west, central and east African extending from Sierra Leon to Uganda, Kenya, Kongo and Angola (Keay, 2000). It grows well in the ever green forest zone of West Africa and most prevalent in the Southern part of Nigeria (Adegoke *et al.*, 2013). In former times, its seeds were widely sold as an inexpensive nutmeg substitute. However, the most economically essential parts are the seeds are embedded in the white sweet-smelling pulp of the sub-spherical fruit. After harvesting, between Aprils to September every year a series of unit operations (fermentation, washing, drying and cracking) are carried out. The kernel is obtained by cracking the nuts, which is easier done by heating. The kernel, when ground to powder, is a popular condiment use to prepare pepper soup as a stimulant to relieve constipation and to control passive uterine hemorrhage in women immediately after childbirth; it also has diuretic properties and is used for mild fever (Okafor, 2018). This study was designed to investigate the antimicrobial and chemical composition of the oil extract of the seeds of *Monodora myristica*. Result of the study is expected to contribute to available data on the medicinal value of the plant seed.

MATERIALS AND METHODS

Source of *Monodora myristica* Seeds

Monodora myristica seeds were source from Damaturu main market. Sample of the seeds were sent to herbarium section of the department of plant Biology, Bayero University Kano for identification with allotment of accession numbers BUKHAN 254.

Preparation of Sample

The seeds were sun dried for seven days, after which they were pounded into powdered using mortar and then sieved through a 2.0 mm filter and subsequently stored in an air tight sterile container until it was used.

Extraction of oil from the seeds

Monodora myristica oil was extracted using Soxhlet extraction process as described by (**John,** 2012). Five Hundred Milliters (500ml) of chloroform, ethanol and hexane' were poured in to a round bottom flask which is equipped with a Soxhlet apparatus and condenser. Six pieces of anti-bumping granules were added and 50g of the powdered sample were inserted in the center of the extractor. The extraction was carried out at 61.2^0C, 78.4^0C and 69.0^0C for chloroform, ethanol and hexane respectively. When the solvent boil, the vapor rose through the vertical tube in to the condenser at the top. The liquid condenser drip in to the filter paper thimble in the center which contains the solid sample that was extracted. The extracts sip through pores of the thimble and fill the siphon table, where it flows back down in to the round bottom flask quick fit. These were allowed to continue for 3- 4 hours. It was removed from the tube, dry in the oven, cool in the desiccators and then weigh again so as to determine the amount extracted. Further extraction was repeated at interval. At the end of extraction process the resulting filtrate was left in an evaporating dish so that the solvent evaporate.

Samples of Bacteria

Clinical bacterial isolates from samples of urine, blood, sputum, catheter tips, stool, urogenital, and abscesses were collected from pathology department of General Hospital Potiskum in Yobe state. Isolates were identified using several biochemical procedures as described by Cheesbrough (2017). The species of bacteria identified were, *E. coli*, *Shigella boydii*, Klebsiella oxytoca, *Proteus vulgaris* and *Pseudomonas aeruginosa*. The cultures of bacteria were maintained on nutrient agar slants at 4^0C.

Evaluation of antimicrobial activity

The agar diffusion methods as described by Mostafa, *et al.* (2018) and (Osadebe and Ukwueze, 2004) were adopted for the study. Using McFarland standard (1.5×10^8 CFU/ml) a suspension of the test organism was inoculated on Mueller- Hinton agar (MHA) (Oxoid, UK). A sterile glass rod was used to spread the organism on the petri dish. Three holes, each measuring 6.0 mm in diameter were made in each of the solid agar plates using a sterile Cork borer (6 mm). A stock solution of the extract were prepared by dissolving 2ml of the extract into 8ml of Dimethyl sulfoxide (DMSO) to obtain a concentration of 2000mg. the stock solution were further diluted to obtain different concentrations of 20%,40% 60%, 80%, and 100%. Using a Pasteur pipette, different concentration of plant extracts were transferred into the holes made on petridish. Positive control (Amikacin 30 mg) and DMSO were use as positive and negative/solvent control respectively; two plates were used for each concentration of the extracts. The plant extracts were thereafter allowed to stand for one hour for pre-diffusion of the extracts (Esimone, *et al.*, 1998) and were subsequently incubated at 37°C for 24 h. After incubation, plates were observed for formation of a clear zone around the hole which corresponds to the antimicrobial activity of the tested compounds. The zone of inhibition (ZOI) was observed and measured in mm.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The antibacterial effect of the oil extract was evaluated by the method of microdilution (Santos, 2012) as

recommended by The National Committee for Clinical Laboratory Standards ((CLSI), 2021). A stock solution of 15mg/ml of extracts was prepared using an aqueous solution of 20% DMSO (v/v). 200 µl of this dilution was transferred in to the microplate containing 200µl of Muller-Hinton broth. Then, serial dilutions were performed resulting in concentrations of 15, 10 and 5 mg/ml. The inoculum containing 5 x 10^5 cfu/ ml (0.5 in McFarland scale) was added to each well. A well was reserved in microplate for sterility control of the broth (containing Müller-Hinton broth) and Amikacin was use as positive control (30mg/ml), The microplates were incubated under conditions of aerobically for 18-24 h at 37°C when 10µl of 2, 3, 5-triphenyl-tetrazolium (CTT) 2% were added to each well to detect the colour change of the CTT (colorless) to red, reflecting the bacterial metabolism active. The MIC was defined as the lowest concentration of the extracts that visibly inhibited the bacterial growth.

The MBC was determine by withdrawing 10µl of aliquots from each well containing the extracts and transferred to Petri dishes containing Muller-Hinton agar. The plates were incubated for 24 h at 37 °C. The appearance of bacterial colony for a given concentration indicates it was able to kill 99.9% or more bacterial inoculum used. The process were performed in triplicate i.e. 5µg/ml, 10µg/ml and 15µg/ml.

RESULTS AND DISCUSSION

Chemical composition of essential oil of *Monodora myristica*

The detected active phyto-constituents components, molecular formula, molecular weight, retention time and the relative percentages of the essential oil of *M. myristica*, were shown in Table 1. Eleven components were detected. This value was lower than that obtained by Owokotemo and Ekundayo (2012) who identified 22 compounds in the seed oil and 20 compounds in the stem bark oil of *M. myristica.* The major hydrocarbons constituents were Indole-3-ethanol, acetate (6.22%), Bicyclo[3.1.0]hexan-3-ol (4.69%), (2S,4R)-p-Mentha-[1(7) (4.02%), 9,12-Octadecadienoic acid (3.64%), Octadecanoic acid (2.29%), Naphthalene (1.41%), and Quinoline (0.62%). Several studies regarding the constituents of the essential oils of *M. myristica* essential oils obtained in Nigeria have exhibited a few differences. Owolabi *et al.* (2009) reported geranial (40.1%), neral (29.74%) and myrcene (11.3%) as the major components of the oil while Onyenekwe *et al.* (1993) reported alpha-phellandrane (50.4%), alpha-pinene (5.5%), myrcene (4.35%) and germacrene-D-4-ol (9.0%) as the most abundant compounds of essential oil of *M. myristica*. The differences in occurrence and concentration of compound may be probably due to the origin of the plant material (Lawrence *et al*., 1988), genetic factors, culture and environmental conditions (Charles and Simon, 1990). Some of the compounds identified in this study have anti-microbial properties.

Table 1: Active phytoconstituents detected in *Monodora myristica* (Nutmeg) using GCMS

S/No	Name	Molecular formula	Molecular weight	Retention time(min)	Peak Area (%)
1	Bicyclo[3.1.0]hexan-3-ol,	C10H16O	152	4.427	4.69
2	Indole-3-ethanol, acetate (ester)	C12H13NO2	203	4.787	6.22
3	Quinoline	C11H11N	157	4.797	0.62
4	Phenol	C10H14O	150	5.039	0.55
5	Naphthalene	C15H24	204	6.178	1.41
6	tau.-Cadinol	C15H26O	222	6.824	0.37
7	p-Menthane	C10H18O	154	26.496	0.49
8	9,12-Octadecadienoic acid	C18H32O2	280	10.624	3.64
9	Octadecanoic acid	C18H36O2	284	10.692	2.29
10	2-(3-Hydroxybutyl)cyclooctanone	C12H22O2	198	19.597	0.22
11	(2S,4R)-p-Mentha-[1(7)	C10H16O2	168	19.596	4.02

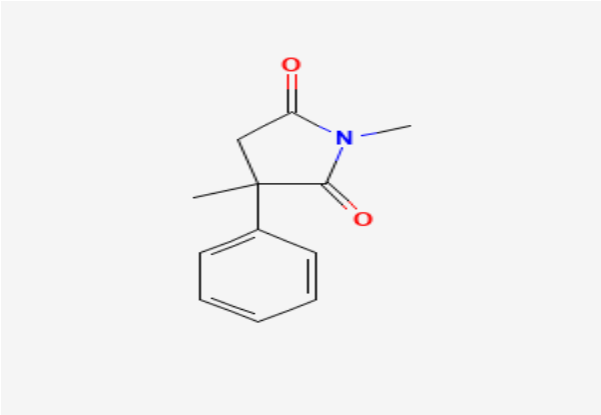

Figure 1: Molecular structure of Indole-3-ethanol, acetate (ester) ($C_{12}H_{13N}O_2$) with the highest peak (6.22) identified *Monodora myristica* (Nutmeg)

Antimicrobial activity of *Monodora myristica* essential oil

Results of the antibacterial activity of the seed extracts of *Monodora myristica* against clinical isolates namely *E. coli*, *Shigella boydii*, Klebsiella oxytoca, *Proteus vulgaris* and *Pseudomonas aeruginosa* at different concentration are presented in table 2. The result showed that the oil extracts are effective though at varying degrees, to all the test bacteria. This result agrees with the findings of Enabulele and Ehiagbonare (2011) who reported susceptibility of bacteria to plant extracts on the basis of zones of inhibition varies according to strains and species. It is observed that at various concentrations, all the solvent used for the extraction were active against the test bacteria. Various researchers have reported

alcohol-based extracts to be more effective than water based extracts (Ellof, 1998; Adegoke and Adebayo, 2009) while others have also reported contrary results (Kela, and Kufeji, 1995); El-Mahmood, 2009). The essential oil inhibited the growth of the bacterial strains used in this study at different rates depending on the concentration and the type of tested bacteria. These results are in agreement with Mohamed *et al*. (2016) and Mardafkan *et al*. (2015),who concluded that Gram positive and Gram negative bacteria were both susceptible to the oil. The expressed antimicrobial activity was concentration dependent as higher concentrations of the respective oils corresponding to maximal antimicrobial activity. This trend is consistent with earlier reports by Idu *et al.* (2014) and Karigar *et al*. (2010) which revealed concentration dependent antimicrobial activity of seed oil extracts of *Khaya senegalensis* and *Leucaena leucocephala*, respectively. The result from this study therefore, support the use of these solvents in traditional medicine as oil extracts from all solvents were found to be highly effective against the test bacteria. In comparing the percentages of effectiveness of the various oil extracts at different concentrations with the control antibiotic (amikacin) indicates that. at the different concentrations they were used, most of the oil extract were effective more than the control even with the fact that they are in their crude state.

Table 2: Mean zones of inhibition of *Monodora myristica* seed oil against bacteria

Bacteria	Zones of Inhibition in mm at different concentrations (percentage of control) with standard error of mean															
	20% Hexane	20% Chloroform	20% Ethanol	40% Hexane	40% Chloroform	40% Ethanol	60% Hexane	60% Chloroform	60% Ethanol	80% Hexane	80% Chloroform	80% Ethanol	100% Hexane	100% Chloroform	100% Ethanol	Control Amikacine 30mg
E. coli	3.33^{a} ±3.77	4.30^{a} ±3.17	3.33^{a} ±5.77	3.30^{a} ±9.71	4.68^{a} ±9.21	4.33^{a} ±7.51	5.20^{a} ±8.36	7.20^{a} ±8.30	5.00^{a} ±8.66	5.50^{a} ±1.39	7.50^{a} ±1.39	6.00^{a} ±7.39	7.00^{a} ±0.00	8.20^{a} ±0.00	7.33^{a} ±1.70	5.33 ±1.90
P. vulgaris	6.00^{b} ±0.00	6.21^{b} ±0.00	7.00^{b} ±0.00	6.33^{b} ±2.79	6.63^{b} ±2.89	8.33^{b} ±2.89	8.65^{b} ±2.41	6.95^{b} ±2.40	10.67^{b} ±2.31	12.20^{b} ±1.83	9.20^{b} ±1.87	14.00^{b} ±1.73	16.00^{b} ±0.00	13.00^{b} ±0.00	17.0^{b} ±0.00	12.33 ±1.06
P. aeruginosa	2.00^{c} ±0.00	4.00^{c} ±0.00	1.67^{c} ±2.89	3.40^{c} ±5.00	5.20^{c} ±7.00	3.00^{c} ±5.20	4.90^{c} ±6.83	7.90^{c} ±6.53	4.00^{c} ±6.93	5.00^{c} ±0.00	10.20^{c} ±0.00	5.00^{c} ±8.66	5.43^{c} ±4.24	14.43^{c} ±3.24	5.33^{c} ±9.24	5.33 ±1.45
S. boydii	1.57^{d} ±2.89	2.57^{d} ±2.89	1.67^{c} ±2.09	2.63^{d} ±4.00	3.63^{d} ±14.00	2.33^{d} ±4.04	6.00^{d} ±5.00	6.00^{d} ±5.02	5.00^{d} ±5.00	7.68^{c} ±6.00	7.28^{d} ±6.00	7.67^{d} ±6.06	10.00^{d} ±9.54	10.70^{d} ±9.54	11.0^{d} ±9.64	10.33 ±1.00
K. oxytoca	5.67^{e} ±4.04	5.00^{e} ±0.04	8.67^{d} ±2.89	5.90^{e} ±5.00	5.90^{e} ±0.00	9.33^{e} ±4.51	8.00^{e} ±2.65	8.38^{e} ±2.63	12.00^{e} ±4.00	11.23^{e} ±2.52	11.20^{e} ±2.54	15.67^{e} ±3.51	14.93^{e} ±2.99	14.53^{e} ±2.97	20.3^{e} ±2.89	13.46 ±1.32
p-value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the essential oils against bacterial isolates

The minimum inhibitory concentration and minimum bactericidal concentration of the essential oils against bacterial isolates are shown in Table 3. The MIC and MBC of *M. myristica* varied for all the three oil extracts ranged between 0.5 and 1.0%, while the minimum bacterial concentration (MBC) values for all the three oil extraction ranged between 1.0 and above 1.5%. MIC and MBC are values normally used as predictive indices of the efficacy of antimicrobial agents. The differences in the effectiveness of the oil extracts could be due to the crude nature of plant extracts which may contain some impure substances that may be inert and do not have antibacterial activity.

Table 3: MIC and MBC of *Monodora myristica* (Nutmeg) oil against bacteria isolates

	Ethanol Extraction		Hexane Extraction		Chloroform Extraction	
Organisms	MIC%	MBC%	MIC%	MBC%	MIC%	MBC%
Escherichia. Coli	0.5	X	1.0	1.5	0.5	1.0
Proteus vulgaris	0.5	X	0.5	X	0.5	1.5
Pseudomonas aeruginosa	1.0	1.5	1.0	1.5	1.0	1.5
Shigella boydii	1.0	X	1.0	X	1.0	X
Klebsiella oxytoca	1.0	1.5	0.5	1.5	0.5	1.5

KEY: MIC = minimum inhibitory concentration, MBC= minimum bactericidal concentration, X = MBC above 1.5%

CONCLUSION

In this study, *M. myristica* essential oil contained eleven components which were mainly hydrocarbons constituents consisting of Indole-3-ethanol, acetate (6.22%), Bicyclo[3.1.0] hexan-3-ol (4.69%), (2S,4R)-p-Mentha-[1(7) (4.02%), 9,12-Octadecadienoic acid (3.64%), Octadecanoic acid (2.29%), Naphthalene (1.41%), and Quinoline (0.62%).The oil showed a higher antimicrobial activity than the positive control (amikacin), The study revealed that *Monodora myristica*, seeds contain some important bioactive components with pronounced antibacterial activities.

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