Trop J Nat Prod Res, January 2018; 2(1):38-41 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Brine Shrimps Toxicity, Bactericidal and Antifungal activity of *Monodora myristica* (Gaertn) Essential Oils

Ignatius A. Owokotomo

Department of Chemistry, School of Sciences, Federal University of Technology, P.M.B 704 Akure, Nigeria

ARTICLE INFO

Article history:
Received 31 July 2017
Revised 24 December 2017
Accepted 02 January 2018
Published online 07 January 2018

ABSTRACT

Essential oils from the seeds and stem bark of *Monodora myristica* were obtained through hydro-distillation using Clevenger-type apparatus. The toxicities of the volatile oils were assayed using the brine shrimps' toxicity assay and the LC_{50} (median lethal concentration) was calculated using Finney's probit analysis. The antimicrobial assay was carried out using the agar diffusion method. Ten microbes consisting of five bacteria and five fungi were used in this study. The bacteria were three species of Gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis, Salmonella typhi*) and two Gram-negative bacteria (*Escherichia coli*, and *Pseudomonas aeruginosa*) and the fungi were *Candida albicans, Candida krusei, Candida glabrates, Aspergillus niger* and *Penicillum nostatum*. *Monodora myristica* seeds and stem-bark displayed an equal level of toxicity (LC_{50} = 0.1 µg/mL) in brine shrimps lethality assay. In comparison to standard antibiotics (Gentamicin) and antifungal agent (Tioconazole), the activity of *Monodora myristica* essential oils for growth inhibition of the test microorganisms was low with the zone of inhibition of 18.0 ± 2.0 mm recorded against *Bacillus subtilis*, the most susceptible organism to the essential oil.

Keywords: Essential oils, Brine shrimps, inhibition zone, antimicrobial activity, *Monodora myristica*.

Introduction

Plants are one of the most important sources of medicines. In fact, most proprietary drugs contain active compounds once derived from plants.[1], including morphine from *Papaver somniferum* and Atropine from *Atropa belladonna* .[2]

Most of the people living in rural African settlements rely on medicinal plant preparations for the treatment of diseases.[3] Likewise, in advance societies, there has been in recent time, relative increase in the demand for natural products which have culminated in a renewal of interest in essential oil research.[4] Also, due to current high cases of microbial resistance to available drugs for the treatment of many diseases, there is the increasing need to find new solutions. Essential oils are believed to be the most promising natural antimicrobials because they do not cause microbial resistance due to the diversity of mechanisms of action.[5] It should be noted also, that because essential oils are composed of moderately potent compounds in combinations, they might exhibit fewer side-effects than individual potent compounds.[6]

Monodora Myristica (Gaertn.) Dunal (Annonaceae) also known as 'Ariwo' in South Western Nigeria is used both as spices and in traditional medicine.[7,8] It is highly valued in many African countries because of its aromatic flavour.[9,10]

There is paucity of literature on the Brine shrimps toxicity and bioactivity of essential oils from *Monodora myristica* from Nigeria, Thus, in this research, the brine shrimps toxicity, bactericidal and antifungal activity of the essential oils from the seeds and stem-bark of *Monodora myristica* grown in Nigeria were investigated.

Materials and Methods

Collection of Plant Materials

The seeds and stem-bark of *Monodora myristica* were collected in a farm at Iwaro-Oka, Ondo State, South Western Nigeria on the 10th day of July 2011 and were identified at the Forestry Research Institute of Nigeria, Ibadan. Voucher number, *FHI109023*.

Extraction of Essential Oils and Analyses

The plant parts were washed and then subjected to hydro-distillation separately for 3 - 5 h using an all-glass Clevenger-type apparatus. The chromatograms were auto-integrated by Shem-Station and the constituents were identified by comparison of the GC-MS data with (NIST02) library spectra and data from literature.[11]

Hatching of Artemia salina (Brine shrimps)

An aquarium of plastic container with two compartments was used. Holes were created into the divider to allow water circulation and to enable hatched shrimps to swim to the side exposed to light. The container (Aquarium) was filled with fresh sea water prior to the introduction of two spatula-full of brine shrimps to one side which was covered with a booklet in order to produce a dark environment which was favoured for hatching, while the other side was left exposed to light. The aquarium was then left for two days until the hatched brine shrimps swim across the divider to the side exposed to light.

*Corresponding author. E mail: adekunleowokotomo@gmail.com
Tel: +234 8142352610

Citation: Owokotomo AI. Brine Shrimps Toxicity, Bactericidal and Antifungal activity of *Monodora myristica* (Gaertn) Essential Oils. Trop J Nat Prod Res. 2018; 2(1):38-41. doi.org/10.26538/tjnpr/v2i1.8

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Brine Shrimps Toxicity assay
Toxicity of the essential oils was conducted using a modified brine shrimps (*Artemia salina)* lethality assay described by Krishnaraju *et al.* (2005).[12]

Preparation of Sample for Toxicity Test
Stock solution was prepared by emulsifying 20.0 mg of the essential oils separately in 0.3 mL of dimethylsulphoxide (DMSO) and the volume was made up with 1.7 mL of fresh seawater to equal 1000.0 ppm concentration. After this, serial dilution was done to obtain two additional concentrations of 100.0 ppm and 10.0 ppm.

Stationing the Brine Shrimps
Fresh seawater (3.0 mL) was introduced into the specimens' vials (test tubes) prepared in triplicates. Then, 0.5 mL of each prepared concentration was introduced followed by the introduction of ten brine shrimps into every vial including the control. Finally, every specimen vial was topped up with sea water until it reached 5.0 mL. The entire specimen vials were left open for 24 h.

Antimicrobial Activity of the Essential Oils
The antimicrobial assay was carried out using the agar well diffusion method. Five bacterial and five fungal species were used for the study. Three of the selected bacterial species were gram-positive (*Staphylococcus aureus, Bacillus subtilis* and *Salmonella typhi*), while the other 2 were gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*). Pathogenic fungi (*Candida albicans, Candida krusei, Candida glabrates, Aspergillus niger* and *Penicillum nostatum*) were used in the investigation. All the microbes were clinical isolates obtained from the Department of Pharmaceutical Microbiology, University of Ibadan, Nigeria. Nutrient agar was used as the medium of growth for the bacteria and sabouraud dextrose agar (SDA) [Oxiod Laboratories, UK] for the growth of fungi following the method of Washington and Sutter (1980).[13]

Statistical Analysis
Finney's probit analysis was used to determine the LC_{50} i.e., the concentration of extract that kills 50% of the shrimps within 24 h. Percentage mortality was calculated using the following equation:

$$\%\ \text{Mortality} = \frac{No.of\ dead\ nauplii}{Initial\ No.of\ life\ nauplii}\ X\ 100$$

Toxicity of the extract against the brine shrimps was determined by a statistically significant decrease in the survival of brine shrimps exposed to plant extract, relative to the survival of shrimps in the control.
The antimicrobial activity data were analysed using descriptive statistics.

Results and Discussion

Predominant bioactive constituents
The compositions of the essential oils were determined and reported earlier.[14] The major constituents of the essential oils of *M. myristica* are presented in Table 1.

Brine Shrimps Toxicity
The essential oils of *Monodora myristica* (Annonaceae) were tested for Brine Shrimps lethality and the toxicity was determined by LC_{50} (median lethal concentration) using Finney probit analysis. The stem-bark and the seeds essential oils were equally potent with the same LC_{50} of 0.1 µg/mL (Table 2). This value is low and may imply that the essential oils were toxic to the Brine Shrimps. The degrees of toxicities of the essential oils were directly proportional to the concentration. Maximum mortality (100.0%) of the brine shrimps was recorded at 1000.0 ppm and the lowest mortality recorded at 10.0 ppm of solution of the essential oils. The mechanism of action on the organisms was not clear, but essential oils are lipophilic, thus they may pass through the cell wall and cytoplasm membrane, and disrupt the structure of their different layers. Cytotoxicity appears to include such membrane damages.[14]

Antibacterial and antifungal activities
The essential oils from the seeds and stem-bark of *Monodora myristica* (Gaertn.) Dunal were evaluated for antimicrobial activity against pathogenic species of Gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis*, and *Salmolellna typhi)* and two species of Gram negative bacteria (*Escherichia coli*, and *Pseudomonas aeruginosa*). Five species of fungi were also used for antimicrobial activity of the essential oils. They were *Candida albicans, Candida krusei, Aspergillus niger, Candida glabrates* and *Penicillum nostatum.* The antibacterial activity ranges between 10.0 ± 2.0 to 18.0 ± 2.0 mm inhibition zone while the antifungal activity vary between 11.0 ± 0 to 16.0 ± 0 mm inhibition zone in a dose-dependent manner (Tables 3 and 4).
The essential oils were more effective within the concentration range of 250 - 1000 µg/mL. There was reduced level of in the antimicrobial activity of the essential oils at 62.0 µg/mL; only a small level of activity (12.0 ±2.5 mm mean diameter zones of inhibition) against gram positive bacteria was observed while no activity was recorded against gram negative bacteria and fungi. The essential oils (EOs) in this study were generally slightly more active against gram positive bacteria than gram negative bacteria which is in accord with results of most studies reported in literature on the action of whole EOs against pathogenic bacteria. According to Ratledge and Wilkinson,[15] this is to be expected because the gram-negative bacteria possess an outer membrane (lipopolysaccharide) surrounding the cell wall which restricts diffusion of lipophilic compounds through it.

Table 1: Major bioactive constituents of *M. myristica*

Plant part	Predominant bioactive constituent	Percentage occurrence (%)	Subclass
Seed	Tricyclo[5.2.1(1,5)]dec-2-ene	13.35	Monoterpene
	linalool	15.10	Monoterpene
	δ-cadinene	11.09	Sesquiterpene
	α-terpineol	3.35	Monoterpene
Stem-bark	*γ*-cadinene	31.31	Sesquiterpene
	α-cubebene	6.70	Sesquiterpene
	γ -muurolene	5.94	Sesquiterpene
	α-caryophyllene	5.42	Sesquiterpene

Table 2: Toxicity of the essential oils on Brine shrimps (*Artemia salina*)

Essential oil	% Mortality ((1000 ppm)	% Mortality (100 ppm)	% Mortality (10 ppm)	LC_{50} (µg/mL)
MMS	100	100	83.3	0.1
MMSB	100	96.7	86.7	0.1

MMS = *M. myristica* seeds, MMSB = *M. myristica* stem-bark

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 3: Antimicrobial activity of the essential oil of *Monodora myristica* seeds

Microorganism	Inhibition zone (mm)						
	Essential oil (µg x 10^2)						
	(10.0)	(5.0)	(2.5)	(1.3)	(0.6)	(0.3)	STD[a] Control
Gram negative							
E. coli	12.0 ± 2.0	10.5 ± 0.5	12.0 ± 0	0	0	0	36.0 ± 0.5
P. aeruginosa	13.5 ± 1.0	12.0 ± 0	10.0 ± 0	0	0	0	36.0 ± 0.3
Gram Positive							
S. aureus	10.0 ± 0	0	0	0	0	0	38.0 ± 0.13
B. subtilis	18.0 ± 2.0	16.0 ± 2.0	12 ± 0	10.0 ± 0	0	0	8.0 ± 0.7
S. typhi	14.5 ± 0.5	12.0 ± 0	10.0 ± 0	0	0	0	38.0 ± 0.5
Fungi							STD[b] Control
C. albicans	11.0 ± 0.	10.0 ± 0	0	0	0	0	26.0 ± 0.5
C. krusei	16.0 ± 0	10.0 ± 0	0	0	0	0	29.0 ± 0
A. niger	14.0 ± 0	10.0 ± 0	0	0	0	0	26.0 ± 0.6
C. glabrates	12.0 ± 0	10.0 ± 0	0	0	0	0	29.0 ± 2.0
P. nostatum	14 ± 1.0	12.0 ± 0	10.0 ± 0	0	0	0	26.0 ± 1.0

STD[a] = Gentamicin (10.0 µg/mL); STD[b] = Tioconazole (70.0%)

Table 4: Antimicrobial activity of the essential oil of *Monodora myristica Stem-bark*

Microorganism	Inhibition zone (mm)						
	Essential oil (µg x 10^2)						
	(10.0)	(5.0)	(2.5)	(1.3)	(0.6)	(0.3)	STD[a] Control
Gram negative							
E. coli	16.0 ± 0	12.5 ± 0.5	10.0 ± 0	0	0	0	36 ± 0.5
P.aeruginose	14.5 ± 0.5	12.0 ± 0	10.0 ± 0	0	0	0	36 ± 0.3
Gram Positive							
S. aureus	16 ± 0.5	12.0 ± 0	10.0 ± 0.5	0	0	0	38 ± 0.1
B. subtilis	18.0 ± 2.0	14.0 ± 0	12.0 ± 0	10.0 ± 0	0	0	38 ± 0.7
S. typhi	14.5 ± 0.5	12.0 ± 0.5	10.0 ± 0	0	0	0	38 ± 0.5
Fungi							STD[b] Control
C. albicans	12.0 ± 0	10.0 ± 0	0	0	0	0	26.0 ± 0.5
C. krusei	12.0 ± 0	10.0 ± 0	0	0	0	0	29.0 ± 0
A. niger	12.5 ± 1.5	12.0 ± 0	10.0 ± 0	0	0	0	26.0 ± 0.6
C. glabrates	12 ± 2.0	10.0 ± 0	0	0	0	0	29.0 ± 2.0
P. nostatum	14.0 ± 1.0	12.0 ± 0	10.0 ± 0	0	0	0	26.0 ± 1.0

STD[a] = Gentamicin (10.0 µg/mL); STD[b] = Tioconazole (70.0%)

The same pattern of dose-dependent activity was recorded in the antifungal assay, which showed higher activity at higher concentrations. Bacterial organisms were found to be more susceptible, with *Bacillus subtilis* being the most susceptible of all ten organisms.

Conclusion

Results generated from this work suggest that essential oils from the seeds and stem-bark of *Monodora myristica* were lethal to *Artemia salina* (Brine shrimps), exhibiting 100% mortality at 1000 ppm. The LC_{50} of 0.1 µg/mL for both essential oils confirm their potency and serves to predict their cytotoxicity. The antibacterial and antifungal activities of the essential oils showed that they were effective against the selected pathogenic organisms. This indicates that the essential oils from *Monodora myristica* could be a potential source of pharmaceuticals against microbial infections.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The author thanks Dr. I. A. Oladosu of the Department of Chemistry, University of Ibadan for donation of Brine Shrimps eggs.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

References

1. Ananiel K, Hudson JB Amason JT, Gbeassor, M. Investigation of medicinal plants of Togo for antiviral activity. Pharm Biol. 2000; 38:40-45.
2. Werka JS, Boehmeb AK, Setzera WN. Biological Activities of Essential Oils from Montverde, Costa Rica. Nat Prod Commun. 2007; 2:12.
3. Prakash P and Gupta N. Therapeutic uses of *Ocimum sanctum* LINN (tulsi) with a note on eugenol and its pharmacological actions: a short review. Ind J Phys Pharm. 2005; 49(2):125-131.
4. Dorman HJD, Deans SG, Noble RC, Surai P. Evaluation *in vitro* of plant essential oils as natural antioxidants. J Ess Oil Res. 1995; 7:645-651.
5. Dobre AA, Gagiu V, Petru N. Antimicrobial activity of essential oils against food-borne bacteria evaluated by two preliminary methods. Rom Biotech Lett. 2011; 16(6):1-6.
6. Kubo A and Kubo I. Antimicrobial agents from *Tanacetum balsamita*. J Nat prod. 1995; 58(10):1565-1569.
7. Fournier G, Leboeuf M, Cave A. Annonaceae essential oils: a review. J Ess Oil Res. 1999; 11:131.
8. Okafor JC. Development of forest tree crops for food supplies in Nigeria. Forest Ecol Manage. 1987; 1:235.
9. Ekeanyanwu CR, Ogu LG, Nwachukwu UP. Biochemical characteristics of the African nutmeg, *Monodora myristica.* Agric J. 2010; 5(5):303.
10. Uwakwe AA and Nwaoguikpe RNJ. Invitro and antisickling effect of *Xylopia aethiopica* and *Monodora myristica*. Med Plant Res. 2008; 2(6):110.
11. Adams RP. Identification of essential oil components by gas chromatography/mass spectroscopy. Allured Publishing Corporation, Illinois, USA. 1995; 5-115 p.
12. Krishnaraju AV, Rao-Tayi VN, Vanisree M, Tsay HS, Subbaraju GV. Assessment of bioactivity of Indian medicinal plant using brine shrimp (*Artemia salina*) Lethality Assay. Int J Appl Sci Eng. 2005; 3(2):125-134.
13. Washington JE and Sutter VL. Dilution Susceptibility Tests, Agar and microbroth Dilution procedure. Manual of Clinical Microbiology, 3rd Edition.Washington DC: Am Soc Micr. 1980; 453 p.
14. Owokotomo IA and Ekundayo O. Comparative study of the essential oils of *Monodora myristica* from Nigeria. Eur Chem Bull. 2012; 1(6):263-265.
15. Carson CF, Mee BJ, Riley TV. Mechanism of action of *Melaleca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage and salt tolerance assays and electron microscopy. Ant Agents Chemother. 2012; 46: 1914–1920.