



Preliminary Phytochemical Screening and Toxic Effect of *Melanthera scandens* Leaf Extracts Using Brine Shrimp (*Artemia salina*) Test

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Authors' contributions

This work was carried out in collaboration between both authors. Author IED designed the study, wrote the protocol, and the first draft of the manuscript. Author USE managed the literature searches and the experimental process. Author USE identified the species of plant. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of the study was to evaluate the toxicity of the crude extracts of *Melanthera scandens* leaves extracts using Brine Shrimp (*Artemia salina*) test in order to substantiate the ethnopharmacological uses of this plant in the treatment of different illnesses.

Methodology: Extractions of the dried powdered leaves of *Melanthera scandens* by maceration was carried out using ethanol and distilled water. All the extracts were subjected to preliminary phytochemical screening using standard methods while the toxicity of the extracts was evaluated using Brine Shrimp lethality assay. The percentage lethality (mortality) of the brine shrimp were evaluated in six different concentrations; 1000, 500, 250, 125, 62.5 and 31.25 µg/ml and the lethal concentration LC₅₀ for 50% mortality of brine shrimp after 24 h of exposure to the extracts was determined.

Results: The preliminary phytochemical analysis showed the presence of saponins and cardiac glycosides in both extracts. However, tannins and flavonoids were present in the aqueous extracts while phlobatanins and terpenes/steroids were detected in the ethanol extract. The Brine Shrimp

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lethality assay revealed that ethanol and aqueous extracts were effective against brine shrimp nauplii with LC₅₀ of 173.78 µg/ml and 331.13 µg/ml respectively. It was also observed that maximum mortalities took place at a concentration of 1000 µg/ml whereas least mortalities were at 31.25 µg/ml concentration.

Conclusion: Results of the phytochemical screening indicated that bioactive phytoconstituents were present in this plant and that the ethanol extract showed better toxicity against brine shrimp with LC₅₀ value 173.78 µg/ml when compared to aqueous extract with LC₅₀ value of 331.13 µg/ml. As a result of this, ethanol extract may be considered significantly active and have the potential for further investigation.

Keywords: Phytochemical analysis; brine shrimp lethality; toxicity, tannins; flavonoids.

1. INTRODUCTION

Medicinal plants are plant species or food which one or more of its constituents can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [1]. These plants constitute an effective source of both traditional and modern medicine, with about 80% of rural population relying on them as major health care [2]. It has been reported that the pharmacological characteristics of medicinal plants may be attributed to the presence of bioactive compounds such as alkaloids, tannins, flavonoids and phenolic compounds. These bioactive compounds produce definite physiological action on the human body and animal health [3]. The determination of the phytochemical profile of a plant provides evidence for the major classes of compounds in that plant [4]. When screening for biologically active plant constituents, the selection of the plant species to be studied is obviously a crucial factor for the ultimate success of the investigation. Plants used in traditional medicine are more likely to yield pharmacologically active compounds [5]. Some of the phytochemical compounds, example glycosides, saponins, tannins, flavonoids etc have antimicrobial activities [6]. Despite the numerous benefits of medicinal plants in the treatment of diseases, some of them are known to carry toxicological properties as well, and as such, the effectiveness and potential toxicity of medication used in folk medicine have to be scientifically evaluated [7,8, 9]. Numerous research studies have recently focused on both pharmacology and toxicity of medicinal plants used by humans. This is of high importance in order to achieve a safe treatment with plant products [10].

Melanthera scandens (Schmach Thonn) Roberty (Family Asteraceae) is a coarse shrub of about 1 – 4 m high, with branches quadrangular and scabrid. The leaves are alternate or opposite,

simply or variously divided, and are widely distributed across tropical Africa, Zimbabwe and Australia. The leaves are extensively used in traditional system for various ailments like stomach ulcer, ringworm, eczema, rashes, constipation, dysmenorrhoea, diabetics, malaria, piles and as a purgative and an antidote against poisoning. The leaves have been reported to be used as an emetic, cough and febrile headache medicine [11,12,13,14,15,16]. The antioxidant, *in-vitro* anti-plasmodial, anti-diabetic, anti-ulcer, anti-inflammatory, analgesic, anti-convulsant and anxiolytic activities of extracts of *Melanthera scandens* have been reported [17,18,19,20]. Since *Melanthera scandens* is commonly used as folk medicine, the aim of this research is to explore toxicity of the ethanol and aqueous extracts of the leaves on brine shrimp.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Sample

Fresh leaves of the plant *Melanthera scandens* were collected from a bush in Ididep in Ibiono Ibom Local Government Area of Akwa Ibom State and identification and authentication were carried out in the herbarium section of the Department of Botany, University of Uyo. The fresh leaves of *Melanthera scandens* were washed with water immediately after collection and then chopped into small pieces, air dried at room temperature for about 10 days and pulverised into powder form and stored in an airtight container. 2.5 kg of the leaves powder was macerated with 7 litres of 90% ethanol for 72 hrs at room temperature. After 72 hrs, the ethanol leaf extract was filtered off through a cotton plug and finally with a Whatman No. 1 filter paper. The liquid filtrate was concentrated and evaporated to dryness using rotary evaporator [19]. The weight of the ethanol crude extract obtained from the leaves *Melanthera scandens* was 6.89 g. The same procedure

described above was repeated for distilled water except that the filtrate of the aqueous extract obtained was lyophilized, and the weight obtained was 9.22 g.

2.2 Brine Shrimp Lethality Bioassay

The Brine Shrimp lethality bioassay was used to predict toxicity activity as described by [21]. Briefly, 4 mg of each extract was dissolved in 2% dimethyl sulfoxide (DMSO) and solutions of varying concentrations (1000, 500, 250, 125, 62.5, 31.25 µg/ml) were obtained by serial dilution technique using sea water. The solutions obtained were then added to pre-marked vials containing 10 live brine shrimp nauplii in 5 ml seawater. The experiments were done in triplicate. About 10 ml of DMSO in sea water and different concentrations of potassium dichromate were taken as negative and positive controls respectively. After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. LC₅₀ values were determined, based on the per cent mortality, using linear regression method from plotting % mortality against correspondent log of concentration. Biological activity was recorded as the concentration when 50% of the larvae were killed within 24 hrs. From the data obtained, the percentage lethality of Brine Shrimp nauplii for each concentrations and control was calculated.

2.3 Phytochemical Screening Methods

Preliminary phytochemical screening was performed on aqueous and ethanol extracts for tannins, phlobatannins, saponins, anthraquinones, cardiac glycosides, flavonoids, alkaloids, deoxy-sugar and terpenes using standard methods as described by [22,23,24]. Formation of colour or change in intensity of colour as well as precipitate formation was used as inference.

3. RESULTS AND DISCUSSION

3.1 Results

The results of the phytochemical screening of ethanol and aqueous extracts of *M. scandens* are presented in Table 1. The results obtained in this study revealed the presence of saponins and cardiac glycosides in both extracts. However, tannins and flavonoids were present in the aqueous extracts while phlobatannins and

terpenes/steroids were detected in the ethanol extract.

Table 1. Phytochemical screening of the water and ethanol leaf extracts of *M. scandens*

Phytochemical constituents	Ethanol extract	Aqueous extract
Tannins	-	+
Phlobatannins	+	-
Saponins	+	+
Cardiac glycosides	+	+
Flavonoids	-	+
Deoxy – sugar	-	-
Terpenes / steroids	+	-
Alkaloids	-	-

+ = Indicates presence of constituents, - = Indicates absence of constituents

The results of the Brine Shrimp lethality assay using the crude ethanol and aqueous extracts from the *Leaves of M. scandens*, the percentage mortality and the LC₅₀ values obtained for extracts and that of the positive control, Potassium dichromate are given in Table 2. Maximum mortality took place at a concentration of 1000 µg /ml for both extracts as well as for the positive control.

3.2 Discussion

The phytochemical analysis showed that various plant secondary metabolites were present in *Malenthera scandens* leaf extracts. These results are in agreement with the findings of other researchers [20,25,19,26]. Diverse uses of plants in treatment of wide variety of diseases are attributable to the presence of the phytochemicals [27]. For instance, tannins are known for their antiviral, antibacterial, antiparasitic as well as anticancer activities [28]. Glycosides, flavonoids, saponins, sterols and triterpenoids have hypoglycaemic, antioxidant, anti-inflammatory and analgesic activities [29,30,31]. These classes of compounds present from phytochemical screening are responsible for the medicinal activity of *M. scandens* against several pathogens and therefore justify the ethnopharmacological uses of this plant in the treatment of different illnesses.

Brine Shrimp lethality bioassay (BST) is an efficient, rapid and inexpensive assay for testing the bioactivity of plant extracts. It is an excellent choice for elementary toxicity investigations

Table 2. Brine shrimp lethality assay of aqueous and ethanol leaf extract of *M. scandens*

Test sample	Concentration (µg/ml)	Log concentration	% mortality	LC ₅₀
Ethanol extract	1000	3.0	90	173.78 µg/ml
	500	2.6	80	
	250	2.3	60	
	125	2.0	30	
	62.5	1.79	20	
	31.25	1.49	0	
Aqueous extract	1000	3.0	70	331.13 µg/ml
	500	2.6	60	
	250	2.3	40	
	125	2.0	20	
	62.5	1.79	10	
	31.25	1.49	0	
Potassium dichromate (Positive control)	1000	3.0	100	131.82 µg/ml
	500	2.6	90	
	250	2.3	70	
	125	2.0	40	
	62.5	1.79	20	
	31.25	1.49	10	

based on the ability to kill laboratory-cultured *Artemia naupli* [32]. Studies have demonstrated a positive correlation between the Brine Shrimp lethality and oral lethality test in mice in medicinal plant research [33].

Different pharmacological properties can be assumed on the basis of Brine Shrimp toxicity of any plant extract [34].

Brine Shrimp lethality assay after 24 hours of exposure to the crude extracts and positive control were investigated. In the present research, different measures of lethality were observed with exposure to different concentrations of the test samples. It was revealed that maximum mortality took place at a concentration of 1000µg /ml for both extracts as well as for the control, indicating that, the Brine Shrimp lethality of the these plant extracts were found to be concentration-dependent. According to Meyer's toxicity index, herbal extracts with LC₅₀ < 1000 µg/ml are considered as toxic, while extracts with LC₅₀ > 1000 µg/ml are considered as non-toxic [21]. Clarkson's toxicity criterion for the toxicity assessment of plant extracts classifies extracts in the following order: extracts with LC₅₀ above 1000 µg/ml are non-toxic, LC₅₀ of 500 - 1000 µg/ml are low toxic, extracts with

LC₅₀ of 100 - 500 µg/ml are medium toxic, while extracts with LC₅₀ of 0 - 100 µg/ml are highly toxic [35]. From the results obtained, the ethanol and aqueous leaf extracts of *M. scandens* showed good Brine Shrimp larvicidal activity based on the toxicity index by Meyer and Clarkson, with LC₅₀ values of 173.78 µg/ml and 331.13 µg/ml respectively, whereas the LC₅₀ of the positive control (potassium dichromate) was 131.82 µg/ml. [36] proposed that crude extracts resulting in LC₅₀ values of less than 250 µg/ml were considered significantly active and had the potential for further investigation. The Brine Shrimp assay result obtained in this study further corroborates the exploitation of *Melanthera scandens*. Anti-inflammatory and analgesic activities of ethanol leaf extract of *Melanthera scandens* was determined by [19]. The result obtained indicated that the leaf extract possessed anti-inflammatory and analgesic effect as a result of the presence of bioactive compounds. Anticonvulsant and anxiolytic activity of the aqueous and ethanol leaf extracts of *Melanthera scandens* in a rat model was evaluated by [20]. The results suggested that both the aqueous and ethanol extracts of *M. scandens* have anticonvulsant and anxiolytic effect in a rat model. [17] investigated the antiplasmodial and antiulcerogenic activities of

leaf extracts and fractions of *M. scandens* using standard models. It was found that both extracts and its fractions significantly reduced the parasitaemia in prophylactic, suppressive and curative models in a dose dependent manner, while it was also observed that the extracts significantly reduced mucosal damage in the indomethacin – induced ulcer. However, the variations observed in the LC₅₀ values may be attributed to the ability of ethanol to extract more of the plant active components than the aqueous solvent used. [37] equally observed that there is a significant difference in the obtained LC₅₀ results for different solvents extracts, mainly because some solvents are a poor medium for obtaining specific bioactive components (responsible for the toxicity) from the plant sample, than others.

4. CONCLUSION

The results of the preliminary phytochemical screening of ethanol and aqueous extracts of *M. scandens* indicated that the leaf extracts contained secondary metabolites such as saponins, tannins, cardiac glycosides, flavonoids, steroids/ terpenoids and Phlobatannins. In the present study, both plant extracts were found to show potent activity against brine shrimp nauplii, since their LC₅₀ values were less than 1000 µg/ml. The toxicity of the extracts further confirms the presence of these bioactive compounds, validating the ethnopharmacological uses of this plant in the treatment of different illnesses. However, more research should be carried out to elucidate the specific bioactive phytoconstituents present in this plant.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Soforowa EA. Medicinal plants and traditional medicine in Africa, 5th Ed. John Wiley and Sons. 1993;96-105.
2. WHO. National policy on traditional medicine and regulation of herbal medicines: Report of a WHO global survey. Geneva; 2005.
3. Edeoga HO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology. 2005;4(7):685–688.
4. Sreeshma LS Bindu RN. Brine shrimp lethality assay in two species of *biophytum* dc. (Oxalidaceae). International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(4):582-586.
5. Hamburger M, Hostettmann K. Phytochemistry. 1991;30:3864-74.
6. Wallace RJ. Antimicrobial properties of plant secondary metabolites. Proceed Nutritional soc, 2004;63:621–629.
7. Baker J, Borriss R, Carte B, Cordell G, Soejarto D, Cragg G, et al. Natural product discovery and development. New prospective on international collaboration. Journal of Natural Products. 1995;58: 1325-1357.
8. Cox P, Balik M. The ethnobotanical approach to drug discovery. Scientific American. 1994;270:82-87.
9. Munoz V, Sauvain M. A search for natural bioactive compounds in Bolivia through a multidisciplinary. Approach part 1. Evaluation of the anti-malarial activity of plants used by the Chacobo Indians. Journal of Ethnopharmacology. 2002;69: 127–137.
10. Parra LA, Yhebra RS, Sardiñas IG, Buela LI. Comparative study of the assay of *Artemia salina* L. and the estimate of the medium lethal dose (LD₅₀ value) in mice, to determine oral acute toxicity of plant extracts. Phytomed. 2001;8:395-400.
11. Morah FNI. Chemistry and herbal medicine. A paper presented at the plenary session of the 30th internal conference of the chemical society of Nigeria at merit house, Maitama FCT, Abuja; 2007.
12. Fernsworth NR, Bunya P. The medicinal plants recommended for private health care system. Prachachion, Bangkok. 1992; 20–33.
13. Agyara C, Asase A, Lechtenberg M, Niehues M, Deters A, Hensi A. An ethnopharmacological survey and *in vitro* confirmation of ethnopharmacological use of medicinal plant used for wound healing

- in Gosomtwi – Atwina – Kwanwoma area, Ghana. *Journal of Ethnopharmacology*. 2009;125:393–403.
14. Ajibesin KK, Ekpo BA, Bala DN, Essien EE, Adesanya SA. Ethnobotanical survey of Akwa Ibom State of Nigeria. *Journal of Ethnopharmacology*. 2008;115:387–408.
 15. Etukudo A. Ethnobotany, conventional and traditional uses of plants in Akwa Ibom State. 2003;26.
 16. Affia BF, Tonzibo ZF, Koffi AM, Chalard P, Figueredo G. Chemical composition of essential oil of *Melanthera scandens* (Schum. et Thonn.) Roberty. *World Appl. Sci. J.* 2011;15(7):992-995.
 17. Okokon J, Etebong E, Udobang J, Obot J. Antiplasmodial and antiulcer activity of *Melanthera scandens*. *Asian Pacific Journal of Tropical Biomed.* 2012;2(1):16–20.
 18. Adesegun SA, Alabi SO, Olabanji PT, Coker HA. Evaluation of antioxidant potential of *Melanthera scandens*. *Journal of Acupuncture And Meridian Studies*. 2010;3(4):267-271.
 19. Okokon J, Udoh AE, Frank SG, Amazu LU. Anti-inflammatory and analgesic activities of *Melanthera scandens*. *Asian Pacific Journal of Tropical Biomed.* 2012; 2(2):144-148.
 20. Twinomujuni SS, Oloro J, Alele PE. Anticonvulsant and anxiolytic activities of the leaf aqueous and ethanolic extract of *Melanthera scandens* in a rat model. *African Journal of Pharmacy and Pharmacology*. 2016;10(12):216-222.
 21. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp, a convenient general bioassay for active plant constituents. *Planta Medica*. 1982;45:31–34.
 22. Trease GE, Evans WC. *Pharmacognosy*, 13th ed. London: Bailliere Tindal. 1996; 683-684.
 23. Harborne JB. *Phytochemical methods*. London Chapman and Hall, Ltd. 1984;49-188.
 24. Sofowora EA. *Medicinal plant and traditional medicine in Africa*. John Wiley and Sons LTD. 2008;1-10.
 25. Fagbohun ED, Lawal OU, Ore ME. The proximate, mineral and phytochemical analysis of the Leaves of *Ocimum gratissimum* L., *Melanthera scandens* A. and *Leea guineensis* L. and their medicinal value. *International Journal of Applied Biology and Pharmaceutical Technology*. 2012;3(1):15-22.
 26. Omoyeni OA, Aterigbade E, Akinyeye RO, Olowua RA. Phytochemical screening, nutritional/anti-nutritional and amino acid compositions of Nigeria *Melanthera scandens*. *Scientific Reviews and Chemical Communication*. 2012;2(1):20-30.
 27. Ayodele SQ. The effects of herbal remedies. Paper presented at the 12th Annual Conference of Botanical Society of Nigeria. South Africa: University of Lagos; 2003
 28. Akiyama H, Kazuyasu F, Yamasaki O, Oono T, Iwatsuki K. Antibacterial action of several tannins against *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy*. 2001;48:487-491.
 29. Augusti KT, Cherian S. Insulin- sparing action of leucopelargonidin derivative isolated from *Ficus bengalosis* Linn. *Indian J. Exp. Biol.* 2008;33:608-611.
 30. Argal A, Pathak A. A KCNS activity of *Calotropis gigantea* roots. *J. Ethnopharmacology*. 2006;106:142-145.
 31. Belch JF, Belch PA. *Prescription for nutritional healing*. New York: A Very Penguin Puntam Inc. 2000;267-270.
 32. Carballo JL, Hernández-Inda ZL, Pérez P, García- Grávalos MDA. Comparison between two brine shrimp assays to detect *in vitro* cytotoxicity in marine natural products. *BMC Biotechnol.* 2002;2:17.
 33. Logarto PA, Silva YR, Guerra SI, Iglesias BL. Comparative study of the assay of *Artemia salina* L. and the estimate of the medium lethal dose (LD₅₀ value) in mice, to determine oral acute toxicity of plant extracts. *Phytomedicine*. 2001;8:395–400.
 34. Hatano T, Edamatsu R, Hiramatsu M, Mori A, Fujita Y, Yasuhara. Effects of interaction of tannins with coexisting substances. VI. Effects of tannins and related polyphenols on superoxide anion radical and on DPPH radical. *Chem Pharma Bull.* 1989;37: 2016–2021.
 35. Clarkson C, Maharaj VJ, Crouch NR, Grace OM, Pillay P, Matsabisa MG, et al. *In vitro* antiplasmodial activity of medicinal plants native to or naturalized in South Africa. *J Ethnopharm.* 2004;92:177-191.

36. Rieser MJ, Gu ZM, Fang XP, Zeng L, Wood KV, McLaughlin JL. Five novel mono-tetrahydrofuran ring acetogenins from the seeds of *Annona muricata*. *Journal of Natural Products*. 1996;59: 100-108.
37. Lalisian JA, Nuñez OM, Uy MM. Brine shrimp (*Artemia salina*) bioassay of the medicinal plant *Pseudelephantopus spicatus* from Iligan City, Philippines. *Int Res J Biol Sci*. 2014;3:47-50.

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