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Preliminary qualitative phytochemical screening, acute toxicity study and cytotoxicity tests on methanolic leaf extract of *Vernonia migeodii*

Solomon C. David [1] *, Samuel C. Attama [2], Timothy U. Obetta [1], Gideon E. Onunwa [3]

[1] Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

[2] Department of Veterinary Pharmacology and Toxicology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

[3] Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.

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Abstract

Many people worldwide still rely on herbal medications for their health care needs. *Vernonia migeodii* leaves have shown medicinal activity against some common diseases. There is however a dearth of information on its phytochemical contents, acute toxicity as well as its cytotoxicity. The present study qualitatively screened methanol leaf extract of *V. migeodii* for its phytochemical constituents, and also evaluated the acute toxicity and cytotoxicity of the extract. The leaves of *V. migeodii* were collected, air dried, then pulverized and extracted by cold maceration in 70% methanol for 48 hours. The extract was concentrated in a hot air oven at 40°C. Qualitative phytochemical screening of the extract was done following standard procedures. Eighteen albino mice were used for the acute toxicity study following Lorke's method, and brine shrimps were used for the cytoxicity test. During the acute toxicity study, at day 14, the mice groups given higher doses were humanely sacrificed and their liver, stomach and kidney were processed for histopathology. Results of the phytochemical analysis revealed the presence of saponins, phenolic acid, terpenes, flavonoids and alkaloids in the extract. No obvious sign of toxicity was recorded in the mice groups during the acute toxicity study. The LD_{50} of the extract was thus taken to be above 5000 mg/kg. In the cytotoxicity test using brine shrimps, death was recorded in the group having the highest dose, with 80% survival rate. The methanol leaf extracts of *V. migeodii* was thus considered to be rich in medicinally useful phytochemicals, and safe/not acutely toxic at the highest given dose of 5000 mg/kg in mice.

Keywords: Phytochemical screening; *Vernonia migeodii*; Methanol leaf extract; Cytotoxicity; Acute toxicity.

***Correspondence:** Solomon C. David; E-mail: solomon.david@unn.edu.ng; Phone: +2347034398164

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Introduction

Many people worldwide, especially in developing countries, still rely on the use of natural products and herbs for health care purposes, as a result of their availability, lower cost as well as accessibility, in comparison to modern medications (Iwu *et al*., 1999; Togola *et al*., 2005; Idu *et al*., 2007; Nwauzoma and Dappa, 2013). From earlier reports in available literature, about 1000 plants had been documented based on their usage in healthcare (Borchardt, 2002), mostly, in their crude forms (Ipek, 2020).

Vernonia migeodii (VM) is one of the plants known to have medicinal value. It is commonly known by the Yorubas of Nigeria as ęru ewum (easily translated as "slave of the bitter leaf) (Burkill, 1985). The Igbos of Nigeria refers to it as Onugbu agu (bush bitter leaf) (Ifeoma *et al*., 2017). *Vernonia migeodii* belongs to the family Asteraceae, the same as *Vernonia amygdalina* (bitter leaf). It is a perennial herb with erect striations and sparsely pubescent stems from woody stock. It grows to about 2 – 4 feet in height with sessile leaves and rounded base. It is widely distributed around the South East part of Nigeria and many West African countries, and its spread is usually affected by weather in various areas. The known folkloric uses of the roots, stem and leaves of *Vernonia migeodii* include treatment of many ailments such as malaria, diarrhoea and skin infections (Orabueze *et al*., 2017).

There is no information in available literature on the phytochemical constituents, acute toxicity and cytotoxicity of leaf extracts of *V. migeodii*. The present study is a preliminary qualitative phytochemical screening of the methanol leaf extract of *V. migeodii* and evaluation of its acute toxicity and cytotoxicity.

Materials and Methods

Plant Collection and Extraction: *Vernonia migeodii* leaves were collected between July to September 2023 from its natural habitat in Orba, Nsukka, Enugu State, Nigeria. It was identified by a taxonomist at the Bioresources Development and Conservation Programme, Aku Road Nsukka, Enugu State, Nigeria. The leaves were dried under mild sunlight and pulverized into coarse powder of about 1 mm in diameter. Extraction was by soaking 2 kg of the dried leaves in 70% methanol for 48 hours with intermittent shaking at two hour intervals after which it was filtered with Whatman No. 1 filter paper. The filtrate was then concentrated in a hot air oven at 40°C. The percentage yield was calculated using the standard formula. The extract was stored in a refrigerator.

Phytochemical Screening: Phytochemical screening of the extract was carried out according to the method of Trease and Evans (1996), and Harborne (1998). One gramme of *Vernonia migeodii* extract was dissolved in 100 ml of distilled water in a beaker. The solution was filtered with Whatman No. 1 filter paper to obtain a clear filtrate which was used in testing for the presence of saponins, tannins, flavonoids, alkaloids, terpenes, steroids, phlobatannins and glycosides.

Acute Toxicity Test: The Lorke's two step method (Lorke, 1983) was used for the acute toxicity test. A total of 18 albino mice were used for the two phases of the acute toxicity test. In phase one, nine mice were randomly assigned to three groups, and mice in each group received 10, 100 and 1000 mg/kg of the extract per os. The mice were observed every two hours for 24 hours to monitor their behavior and if mortality will occur. Mice used for the phase two of the acute toxicity study were also randomly assigned to three groups of three mice each, and the doses administered to them were 1600, 2900 and 5000 mg/kg of the extract, respectively. They

were also observed every two hours for the first 24 hours, and later daily for 14 days. After the 14th day, the mice were humanely sacrificed, and the liver, kidney and stomach were collected and processed for histopathology following standard procedures. All through the study, the mice were cared for and handled humanely following standard institutional animal care and use guidelines.

Cytotoxicity Test: The brine shrimp assay as earlier described by Michael *et al*. (1956) and later developed by several groups (Vanhaecke *et al*., 1981; Sleet and Brendel, 1983) was adopted for the cytotoxicity test. Sea water was used for the incubation of the brine shrimp and also throughout the test. Sea water was put in a container that was unequally divided and shrimp eggs were added to the large compartment of the container which was darkened by covering it with a black masking tape. The illuminated side attracts shrimp larvae (nauplii). The shrimp was allowed to hatch and mature for 24 hours at room temperature. Five dilutions of the extract were made; 10 mg/ml (10,000 ppm), 1 mg/ml (1000 ppm), 1 mg/10 ml (100 ppm), 1 mg/100 ml (10 ppm), and 1 mg/1000 ml (1 ppm). For each concentration, three replicates were prepared, i.e. for each dilution, three Petri dishes were used making a total of 15 petri dishes for the extracts; another three Petri dishes were used for the control (0 mg/ml extract), making up a total of 18 Petri dishes. Two millilitre of each diluted extract was transferred to the labeled Petri dishes corresponding to 10,000 ppm, 1000 pm, 100 ppm, 10 ppm and 1 ppm. After 24 hours, the shrimp eggs were hatched. With the help of a Pasteur pipette, 10 shrimps were added to each Petri-dish, making it 30 shrimps per dilution. The Petri dishes were maintained under illumination. At the expiration of 24 and 36 hours, the number of surviving shrimps were counted with the aid of a magnifying glass and recorded.

Statistical Analysis: The resultant dose-response data from the cytotoxicity test was analyzed by probit analysis (Finney, 1971) to provide an estimate of the median concentration (LC_{50})

Results

The methanol leaf extract of *Vernonia migeodii* was dark brown in colour, odourless and of low viscosity. The extraction yield was 7.4% w/w.

The results of the qualitative phytochemical screening of the methanolic leaf extract of *Vernonia migeodii* is presented in Table 1. The phytochemical constituents of the extract were saponins, phenolic acid, terpenes, flavonoids and alkaloids. No tannins and phlobatannins were detected in the extract (Table 1).

Table 1. Qualitative phytochemical constituents of *Vernonia migeodii* methanolic leaf extract.

Phytochemical constituents	Presence (+) or Absence (-)
Saponins	+
Tannins	-
Flavonoids	+
Alkaloids	+
Terpens/Steroids	+
Phlobatannins	-
Glycosides	+

In the cytotoxicity test, death was recorded at the highest concentration of 10 mg/ml (10,000 ppm) of the extract after 24 and 36 hours. There were also signs of dullness prior to death. There was no death and no abnormal behaviors recorded at other concentrations (Table 2).

Table 2: Results of the cytotoxicity test of *Vernonia migeodii* methanol leaf extract using brine shrimp.

Extract concentration level (PPM)	Initial No. of Artemia nauplii	No. of nauplii dead after 24 hours	No. of dead nauplii after 36 hours	Observed abnormal behavior	% Survival
10000 ppm	30	6	6	Dullness	80%
1000 ppm	30	0	0	-	100%
100 ppm	30	0	0	-	100%
10 ppm	30	0	0	-	100%
1 ppm	30	0	0	-	100%
0 ppm (Control)	30	0	0	-	100%

Table 3: Result of the acute toxicity test on methanolic leaf extracts of *Vernonia migeodii* in mice.

Dose (mg/kg)	No. of mice used.	No. of mice dead after 24 hours.	No. of mice dead after 14 days.	% of mice that survived.	Clinical signs recorded.
5000	3	0	0	100	Ventral recumbency
2900	3	0	0	100	Ventral recumbency
1600	3	0	0	100	-
1000	3	0	0	100	-
100	3	0	0	100	-
10	3	0	0	100	-

In the acute toxicity study, there was no death recorded even at the highest dose of 5000 mg/kg. However, there were signs of depression characterized by ventral recumbency and dullness at doses of 2900 mg/kg and 5000 mg/kg. This was noticed in the first two hours post-administration of these doses, and stopped thereafter. The detailed result of the acute toxicity test is presented in Table 3.

During the histopathological examination, there were no obvious lesions on the stained sections of the stomach, liver and kidney of the mice given the highest doses (Figures 1, 2 and 3).

Discussion

The 7.4% w/w yield of the methanolic leaf extract of *V. migeodii* as recorded in this study is comparable and relatively higher than the 5.1% w/w yield reported by Abdulmalik *et al*. (2016) for a related species, *Vernonia amygdala*. Certain factors as genetic,

environmental conditions, duration of the growth period, flowering time and ripening time has been reported to significantly influence the yield and bioactive compounds in medicinal plants (Valyaie *et al*., 2021).

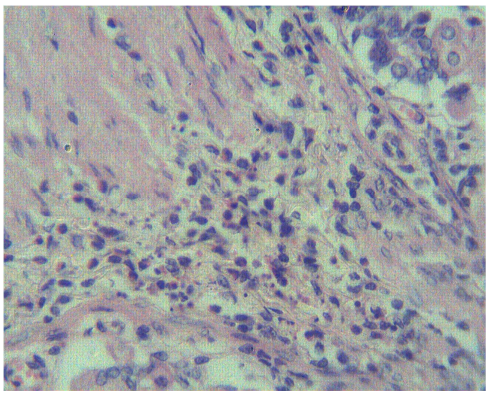

Figure 1. Stained section of the stomach of mice given 5000 mg.kg of *Vernonia migeodii* methanol leaf extract, [H & E, × 400].

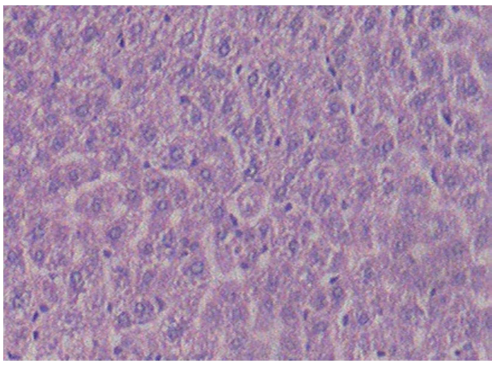

Figure 2. Stained section of the liver of mice given 5000 mg/kg of *Vernonia migeodii* methanol leaf extract [H & E, × 400].

The presence of the phytochemicals such as saponins, phenolic acid, terpenes, flavonoids and alkaloids in *V. migeodii* extract as recorded in this study suggests that the plant extract may have medicinal value. Flavonoids and phenolic acids for instance have been reported to exhibit activities against free radicals and protective effects against cancer, allergies and inflammation (Ballard and Marostica, 2019). Saponins, which was also detected in the extract has been reported to have hypoglycemic, anti-viral and anti-fungal activities (Brull *et al*., 2012; Akihisa *et al*., 2016). Terpenes on the other hand have been reported to have anti-cancer effects and neuroprotective activity (Zhao *et al*., 2016).

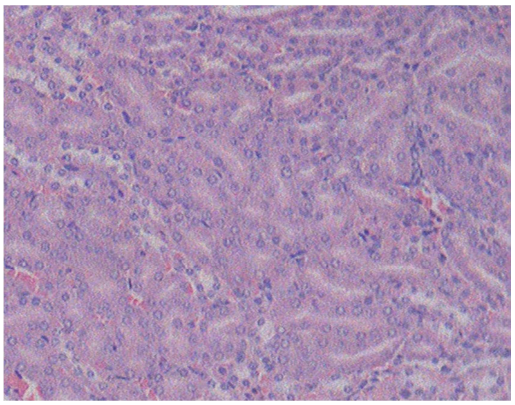

Figure 3. Stained section of the kidney of mice given 5000 mg.kg of *Vernonia migeodii* methanol leaf extract. [H & E, × 400]

The results of the cytotoxicity test, which showed that the methanolic leaf extract of *V. migeodii* had an LC_{50} in brine shrimp that is higher than 10 mg/ml, implies that the extract is safe, and suggests that it is non-toxic (Meyer *et al*., 1982). This finding concurs with the recommended cut off points for assessing cytotoxicity (Geran *et al*., 1972).

The finding in the acute toxicity study that the LD_{50} of the extract in mice is above 5000 mg/kg implies that the extract is acutely safe in vivo in mice, and this LD_{50} places the extract of *Vernonia migeodii* on the category of products that are considered acutely non-toxic. The depression and ventral recumbency recorded in the group given 2900 and 5000 mg/kg is worthy of note, and should guide the future use of such high doses. The absence of obvious histopathological lesions in the stomach, liver and kidney of the mice given these high doses further underscores the safety and non-toxic nature of the extract.

Based on the results of the study, it was concluded that methanol leaf extract of *Vernonia migeodii* contains phytochemicals of medicinal value, and is non-cytotoxic in vitro and not acutely toxic in vivo in mice.

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Conflict of interest

The authors declare no conflict of interest.

References

Abdulmalik O, Oladapo OO and Bolaji MO (2016). Effect of aqueous extract of *Vernonia amygdalina* on atherosclerosis in rabbits. *ARYA Atherosclerosis*, 12(1): 35 – 40.

Akihisa T, Zhang J and Tokuda H (2016). Potentially chemopreventive triterpenoids and other secondary metabolites from plants and fungi. *Studies in Natural Products*, 51: 1 – 50.

Ballard CR and Marostica MR (2019). Bioactive compounds. Health benefits of flavonoids. Woodhead Publishing, Cambridge, UK, pp. 185 – 201.

Borchardt (2002) Natural product chemistry for drug. *Discover Drug News Perspective,* 15: 187.

Brull FB, Mensink RP, Sleinbusch MF and Husche C (2012). Beneficial effects of sitostanol on the attenuated immune function in asthma patients. PLOS One, 7(10): e46895

Burkill HM (1985). The Useful Plants of West Tropical Africa. 2nd edition, Volume 1, Families A – D. Royal Botanical Gardens, Kew. University Press of Virginia, pp. 960.

Finney DJ (1971). Probit analysis. 3rd edition, Cambridge University Press, Cambridge.

Geran RI, Greenberg NH, McDonald MM and Abbot BJ (1972). Protocols for screening chemical agents and natural products against animal tumors and other biological systems. *Cancer Chemotherapy Reports*, Part 33: 1 – 17.

Harborne JB (1998). Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. Chapmann and Hall, London, UK, pp. 129 – 134.

Idu M, Omogbai EKI, Aghimien GE, Amaechina F, Timothy O and Omonigho SE (2007). Preliminary phytochemistry and antimicrobial properties of *Stachytarpheta jamaicensis* (Linn.) Vahl stem. *Research Journal of Medicinal Plants* 1(4): 149 – 153.

Ifeoma O, Sunday AA, Herbert AB, Felix N (2017). Ethno botanical survey of medicinal plants and herbal formulations used in management of malaria in Nsukka South East Nigeria. Nigeria *Journal of Natural Products and Medicine*, 21: 66 – 81.

Ipek S (2020). Importance of ethnopharmalogical studies in drug discovery: Role of medicinal plants. *Photochemistry Reviews*, 19(12): 1199 – 1209.

Iwu MM, Duncan AR, Okunji CO (1999). New antimicrobials of plant origin. In: Janick J (Ed.), Perspectives on New Crops and New Uses, ASHS Press, Alexandria, VA., USA, pp. 457 – 462.

Lorke D (1983) A new approach to practical acute toxicity testing. *Archives of Toxicology*, 54: 275 – 287.

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE and McLaughlin JL (1982).

Brine shrimp: A convenient general bioassay for active plant constituents: *Planta Medica*, 45: 31 – 34.

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Michael AS, Thompson CG and Abramovitz M (1956). *Artemia salina* as a test organism for a bioassay. *Science* 123: 464.

Nwauzoma AB and Dappa MS (2013). Ethnobotanical studies of Port Harcourt metropolis, Nigeria. *International Scholarly Research Notices*, Article ID 829424: 1 – 11.

Orabueze C, Adesegun SA, Nwafor F and Coker HA (2016). Ethnobotanical survey of medicinal plants and herbal formulations used in management of malaria in Nsukka, South East, Nigeria. *Nigeria Journal of Natural Products and Medicine*: 21(1): 66 – 81.

Sleet RB and Brendel K (1983). Brine shrimp, *Artemia salina*: a potential screening organism for initial teratology screening. *Proceedings of the Western Pharmacology Society*, 26: 169 – 170.

Togola, AT, Diallo DS, Barsett H and Paulsen BS (2005). Ethnopharmacological survey of different uses of seven medicinal plants from Mali, (West Africa) in the regions Doila, Kolokani and Siby. *Journal of Ethnobiology and Ethnomedicine*, 1: 7

Trease GE and Evans M (1996). A Textbook of Pharmacognosy, 14th edition, Baillie, Tindal and Caussel, London, pp. 144 – 148.

Valyaie A, Aziz M, Kashi A, Sathasivam R, Park SU, Sugiyama A, Motobayashi T and Fujil Y (2021). Evaluation of growth, yield, and biochemical attributes of bitter gaurd (*Mormordica charantia* L.) cultivars under Karaj conditions in Iran. *Plants*, 10(7): 1370.

Vanhaecke P, Persoone G, Claus C and Sorgoloos P (1981). Proposal for short term toxicity test with Arthemia nauplii. *Ecotoxicology and Environmental Safety*, 5: 387 – 392.

Zhao D, Jiang L, Li H, Yan P and Zhang Y (2016). Chemical components and pharmacological activities of terpene natural products from the genes paeonia. *Molecules*, 21(10): 1362.