



Evaluation of Preliminary Cytotoxic and Growth Inhibitory Effects of *Melastomastrum Capitatum* (Vahl.) Fern. (Melastomataceae) Leaf Methanol Extract by Bench-Top Bio-Assay

C.A. Ukwubile^{1*}, A. Ahmed², U.A. Katsayal³, J. Yau⁴

^{1,2,3}Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria.

⁴Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria.

***Corresponding Author:** C.A. Ukwubile, Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria.

Abstract:

Context: *Melastomastrum capitatum* is indicated in traditional herbal medicine as one of the plant used in treatment of cancer and tumor as well as other diseases in Mambila plateau Taraba State Nigeria. The plant is unique for its sour-to-sweet taste of the leaves.

Aim: To evaluate the preliminary cytotoxic and growth inhibitory effects of *M. capitatum* leaf methanol extract.

Methods: Cytotoxic and growth inhibitory effects of the methanol extract of leaf was evaluated using tadpoles (*Raniceps ranninus*) and the radicle length of guinea corn (*Sorghum bicolor*) seeds respectively.

Results: *M. capitatum* leaf methanol extract (MCE) produced 20 % (2.3±0.2) mortality at a concentration of 20 µg/mL and was eventually increased to 100 % mortality at the concentration of 320 µg/mL over a period of 2 h in the tadpoles. The extract displayed a concentration-dependent cytotoxic effect on the tadpoles. In the growth inhibitory study, an average growth length of 64.97±8.1 mm was produced by the radicle in the control seeds after 96 h with percentage growth inhibitory effect of 0 % . At MCE concentration of 16µg/mL, the extract showed growth inhibitory percentage of 87.87 % on the length of the radicle, and lowest at 1µg/mL concentration of the extract. In all the studies, cytotoxic and growth inhibitory effects were showed by the plant extract in a concentration-dependent fashion.

Conclusion: The study showed that *M. capitatum* leaf possessed cytotoxic and growth inhibitory effects, thus can act as a cytotoxic and growth inhibitory agent to cancer and tumor cells respectively in traditional medicine.

Keywords: *Melastomastrum capitatum*, Cytotoxic, Growth inhibitory, *Raniceps ranninus*, *Sorghum bicolor*.

1. INTRODUCTION

The plant *Melastomastrum capitatum* belongs to the family *Melastomataceae* which is a taxon of dicotyledonous flowering plants commonly found in the tropics. *Melastomataceae* are annual or perennial herbs, shrubs or small trees with simple opposite leaves with a characteristic variation pattern. The main veins which are usually 5-9 are palmate at the base and secondary veins between them are scalariform, parallel and regularly spaced (Burkill, 1997).

Melastomastrum capitatum is a shrubby herb that grows up to 1.25 m high, and it is found in dry situations and stream-banks in Nigeria especially in Mambila Plateau Taraba State (Hutchison and Dalziel, 1958), Guinea, Mali, Uganda and Angola. In Nigeria, it is locally called "Belkon" by the Fulani tribe (In Mambila Plateau Nigeria) who use the leaf to treat cancers traditionally. A large part of the plant has sweet to sour taste. The leaf-sap diluted into a little water is used in Ivory Coast as a

sedative. Its leaf can reduce cholesterol, acts as an analgesic, and cleans the blood vessels in traditional medicine. In South-south Nigeria, the leaves have been used to heal many wounds.

The leaves are somewhat distinctive, being opposite decussate and usually contain sweet and sour tastes, with 3-7 longitudinal veins arising either from the base of the blade (inner veins diverging above base of blade) veins diverging or pinnately nerved with three or pairs of primary veins diverging from the mid-vein at succession point above the base. Flowers are perfect and borne either singly or in terminal or axillary; paniculate cymes (Hutchings, 1996). A number of *Melastomastrum capitatum* are regarded as invasive species once naturalized in tropical and subtropical environments outside of their normal range. The leaf extract contains mainly glycosides, alkaloids and carbohydrates. The leaf extract possessed analgesic and anti-inflammatory properties as well as antihypercholesterolemic activity (Ukwubile and Elsie, 2015). The leaf methanol extract has been shown to possess analgesic and anti-inflammatory activity in Swiss albino mice. The leaves are used as anti-rheumatic agent, cure stomach aches, purification of blood vessels and blood as well as for alleviating diuresis, as sedatives, correcting pulmonary problems. The plant contains alkaloids, glycosides, carbohydrates, sterols, and tannin (Ukwubile *et al.*, 2015).

Botanically, it is also a woody herb or shrub up to 2 m or 5 m high with the following features:

Stems: It is robust, erect or weak and flexuous, sparsely scabrid to densely strigose.

Leaf : The lamina is ovate with green and purple colours, up to 13 cm long and 5 cm wide, apex acute, base cuneate to shortly attenuate, strigose on both surfaces; nerves 5, impressed above, sub-prominent beneath; petiole up to 1.6 cm long.

Inflorescence: There are two or more flowers enclosed in involucre of bracts, sessile; outer bracts leafy; inner bracts persistent, oblong, acute, membranous with tufts of long hairs towards the base, and producing fruits with semi-naked seeds.

Calyx: It is 11 mm long and 5 mm in diameter, crimson, glabrous except for a few bristles at the base; lobes lanceolate, 7 mm long, 2.5 mm wide, acuminate, margins sparsely ciliate.

Petals: Broadly obovate, 20 mm long, and 15 mm wide.

Stamens: It is long in nature up to 22 mm long,

Anthers: They are 9 mm long, mauve, free part of connective 4 mm long with two lobes 1–2 mm long.

Ovary: It is usually 5 mm long, glabrous except for the apical crown of bristles.

Style: It is 12 mm long (Ukwubile and Manasseh, 2016; WHO, 2002; Yang *et al.*, 1998).



Fig1. Pictorial view of *M. capitatum* (Vahl.) A & R Fern (Melastomataceae) in its natural habitat at Mambila plateau, Taraba State (March, 2018).

Geographical distribution: In Nigeria, *M. capitatum* grows throughout the year in the edges of water canals and valleys in Mambila plateau Sardauna Local Government Area of Taraba. It is one of the most dominant shrubs in the area with occasionally coloured leaves. The presence of water in this area and cold climate essentially favors their flourish. Apart from the Mambila plateau, the plant also grows well in Ogurugu Uzo-Uwani Local Government Area of Enugu State; where it is majorly found in marsh land and wet areas especially in shallow streams. They also found in Ibaji Local Government Area of Kogi State, Borgu Local Government Area of Niger State, Southern part of Kaduna State, Edo State, South-west Nigerian States of Ondo, Ekiti, Lagos, Oyo and Osun .

Ethnomedicinal Uses: In Mambila plateau, the leaves are the main part of the plant use in traditional medicine where it is used for blood purification, as remedy for stomach ache, and as antitumor and anticancer agent (Note these claims are have not been scientifically proven or ascertained yet. They are traditional claims by the Fulani tribe in Mambila Plateau, Taraba State, Nigeria). The leaf has been shown to display analgesic and anti-inflammatory properties, and antihypercholesterolemic in mice (Hajiahaalipour *et al.*, 2015). Other uses of the plant are not documented or known yet.

This present study was therefore, carried out in order to evaluate the preliminary cytotoxic and growth inhibitory effects of *M. capitatum* leaf extract on tadpoles and guinea corn radicle.

2. MATERIALS AND METHODS

2.1. Collection and Identification of Plant

Fresh leaves of were collected in the morning hour from Mambila plateau, Sardauna Local Government Area of Taraba State in February, 2018, and was identified by Mr. Namadi Sunusi of the Herbarium Unit, ABU Zaria, with a voucher specimen number of *ABU2761* deposited at the herbarium. *M. capitatum*

2.2. Preparation of Plant Materials

The collected leaves of *M. capitatum* were air-dried at room temperature inside the laboratory to avoid decomposition of some chemical substances by sunlight for two weeks. Dried sample was pounded and ground into fine powder with the aid an electronic blender. The powdered sample was weighed and kept in an air tight container until for further usage.

2.3. Extraction of the Plant Materials

The powdered leaves weighing 800 g was extracted for 48h in 1000 mL of methanol using Soxhlet apparatus. The extract was concentrated in vacuo in rotary evaporator to obtain a gel-like extract. Final yield of extract was 7.5 % (final weight was 60 g). The extract was stored in desiccators for further use.

2.4. Cytotoxic Evaluation of *M. Capitatum* Leaf Methanol Extract

Using a method obtained from Ayinde *et al.* (2011) with slight modifications. Ten tadpoles were selected into a 250 mL capacity beakers, containing 15mL of water from the source of the tadpoles which was made up to 49mL with distilled water. The volume was made up to 50 mL with 0.5 μ L, 1 μ L, 2 μ L, 4 μ L and 8 μ L of the extract dissolved in 5% Dimethyl sulfoxide (DMSO) in water thereby making the concentration of 20, 40, 80, 160 and 320 μ g/mL respectively. The controls for each of the experiment were not treated with the extract and the mortality rate of the tadpoles was observed for a minimum of 24 h.

2.5. Determination of Growth Inhibitory Effect of *M. Capitatum* Leaf Methanol Extract

10 mL of 1 μ g/mL, 2 μ g/mL, 4 μ g/mL, 8 μ g/mL and 16 μ g/mL of methanol extract was dissolved in 5 % DMSO in water, and was poured into 9cm wide Petri dishes laid with cotton wool and Whatman No 1 filter paper. Twenty seed were tested for viability by pre-soaking in 50 mL distilled water and spread on each plate and incubated in a dark cupboard. The lengths (mm) of the radical emerging from the seeds were taken at 24, 72, and 96 h. The control seeds were treated with 10mL of 5% Dimethyl sulfoxide in distilled water containing no extracts (Ayinde and Agbakwuru, 2010; Ayinde *et al.*, 2011).

3. STATISTICAL ANALYSIS

The data obtained were expressed as mean \pm SE. One way analyses of variances (one-way ANOVA) was used to test for significance difference. $P > 0.05$ was considered statistically significance.

4. RESULTS AND DISCUSSION

Production of tumor cells is characterized by an uncontrolled multiplication of the cells. This can be linked to the rapid growth and multiplication exhibited by the meristematic cells of a germinating seed or a growing radicle. The present experiment was conducted to evaluate the cytotoxic and growth inhibitory effect of leaf methanol extract of *M. capitatum* on tadpoles (*Raniceps ranninus*) and guinea corn (*Sorghum bicolor*) radicals respectively.

Results of the current experiment revealed that *M. capitatum* leaf methanol extract has significant growth inhibitory effect on guinea corn radicle. It was observed that the guinea radicals in the control containing 10mL distilled water had the highest length compared to those containing different concentrations after 24, 48, 72 and 96 h respectively. The result also revealed that the length of the guinea corn radicals decreased with increase in concentration of the plant extract (Table 1). Tumor cells are generally known to grow and divide rapidly at an abnormal rate (Kligerman, 1973). This is in line with the rapid growth observed in the guinea corn radicle which is due to the presence of the meristematic cells at the root or shoot apex of plants. The guinea corn radicle were observed to grow geometrically within few hours interval as seen in Table 1.

The ability of the leaf extract of *M. capitatum* to inhibit the growth of guinea corn radicle as seen in the result indicated that the plants extract can be used to inhibit the growth of tumor cells which has similar growth trend with the guinea corn radicle. Cytotoxicity study carried out also revealed that the extract has significant cytotoxic effect on tadpoles. In the control experiment containing ten tadpoles (50mL distilled water), there was no mortality recorded. However, in the beaker containing various concentrations of the extract (20, 40, 80, 160 and 320 $\mu\text{g/mL}$), high mortality rates were recorded after 30 minutes while the beaker containing the highest concentration of the extract (320 mg/mL) recorded 100 % mortality. The result also revealed that the mortality rate increased with increase in concentration. (Table 2).

Research shows than cancer cells divide relentlessly forming solid tumors and one able to spread from one part of the body to another thereby invading other tissues. This can be linked to the fast growing nature of the tadpoles. The ability of the plant extract to kill the tadpoles within 30 minutes indicates that this extract can have significant cytotoxic effect on cancer cells. These observations are similar to the works by (Madaughinji *et al.*, 1991), where it was concluded that the plant under observation could be used in treating tumor related ailments.

Although this investigation requires further tests using appropriate human cell lines, the result obtained so far have indicated the claimed ethnomedicinal use of this plant in treating tumor-related ailments. The methanol leaf extract was observed to reduce the rate of germination of the seeds with increasing concentration. It has been reported that at high concentration *M. capitatum* has ability to inhibit Hela cell survival compared with vehicle-treated control (Obuotor and Onajobi, 2000; Xiaorong *et al.*, 2015; Ju *et al.*, 2007; Vogel, 1991). The length of the seed radicles increasing after the incubation period of 24hours to 96hours, while those of control seeds radicles increased progressively. It can be inferred that the methanol extract of *M. capitatum* was observed to elicit concentration dependent reduction in the length of the radicles that emerged from the guinea corn seeds treated with the extract. The cytotoxicity effect was indicated by an initial slowdown in the movement of the tadpoles and subsequent of movements, indicated by complete submergence of an organism and turning upside down.

Table1. Growth inhibitory effect of methanol extract of *M. capitatum* on *Sorghum bicolor* (guinea corn) radicle

MCE	Time (hour)				%inhibition
	24	48	72	96	
D/H ₂ O	14.6 \pm 1.30	20.30 \pm 3.40	23.37 \pm 2.50	26.80 \pm 3.60	-
1 $\mu\text{g/mL}$	9.00 \pm 1.0	12.80 \pm 1.40	17.67 \pm 3.40	21.40 \pm 1.17	20.15

Evaluation of Preliminary Cytotoxic and Growth Inhibitory Effects of *Melastomastrum Capitatum* (Vahl.) Fern. (Melastomataceae) Leaf Methanol Extract by Bench-Top Bio-Assay

2 µg/mL	7.50 ± 3.12	9.43 ± 3.60	9.37 ± 2.00	12.20 ± 5.31	54.48
4 µg/mL	6.0 ± 1.00	8.83 ± 2.00	10.33 ± 4.04	11.53 ± 3.50	56.98
8 µg/mL	2.20 ± 0.43	2.20 ± 1.00	8.0 ± 3.70	8.83 ± 4.16	67.05
16 µg/mL	2.25 ± 0.20	2.05 ± 0.32	2.95 ± 0.32	3.25 ± 0.32	87.87

Results are means ± SE of three replicate experiments, MCE (*Melastomastrum capitatum* extract), D/H₂O (distilled water), p < 0.05 (one-way ANOVA).

Table2. Cytotoxicity of leaf methanol extract of *M. capitatum* on *Raniceps ranninus* (Tadpoles)

Time (minutes)		
MCE	Mortality rate in 30 minutes	% mortality
50 mL D/H ₂ O	-	-
20 µg/mL	2.33 ± 0.27	20.00
40 µg/mL	3.33 ± 0.27	30.00
80 µg/mL	6.67 ± 0.27	70.00
160 µg/mL	8.33 ± 0.54	80.00
320 µg/mL	9.67 ± 0.54	100.00

Results are mean ± SE of ten tadpoles in each group to the nearest percentage (i.e. n= 10), D/H₂O (distilled water), % mortality = (No of dead tadpoles/ total nos of tadpoles in a beaker) x 100.

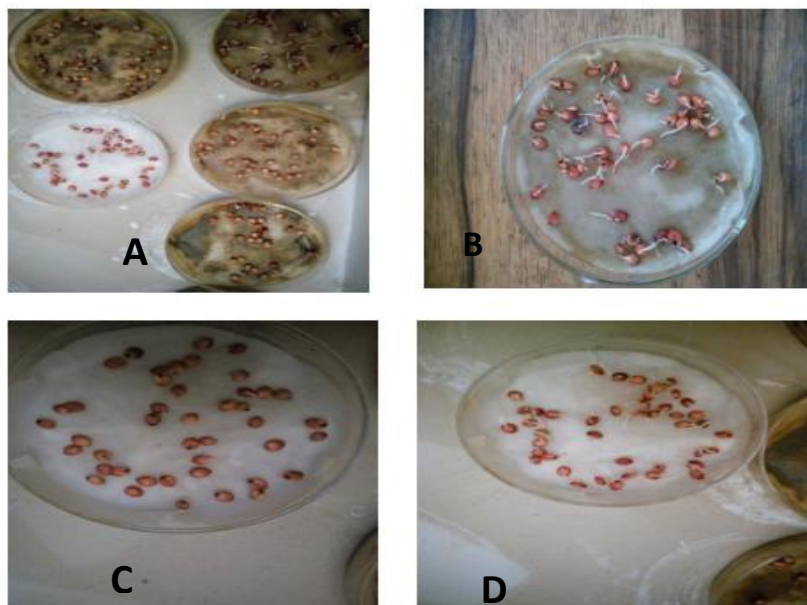


Plate1. Growth inhibitory study: A, B & C are treated plates, D is control plate



Plate2. Cytotoxicity study: E is treated group while F is control group.

5. CONCLUSION

From the result obtained in this work, it is concluded that *M. capitatum* leaf methanol extract was a potential for causing both cytotoxic and anti-proliferative (growth inhibitory) effect on fast

proliferating cells, and hence cancerous cells. This result thus justifies the acclaimed use of the leaf an anti-tumor and anti-cancer agents in traditional medicine.

ACKNOWLEDGEMENT

The authors are to grateful to Mr. Geoffrey Victor who provided us with tadpoles and also to Dr. Ikpefan E.O for his assistance during this work.

REFERENCES

- [1] Ayinde, B.A, Omogbai, E.K, Ikpefan, E.O. (2011). Comparative cytotoxic and anti-proliferative effects of *Persea americana* Mill (Lauraceae) leaf stem and root barks. *Nigeria Journal. Pharm Sci.*:10:16:26
- [2] Ayinde, B.A., Agbakwuru, U. (2010). Cytotoxic and growth inhibitory effect of the methanol extract of *Struchium sparganophora* Ktze (Asteraceae) leaves *Pharmacognosy Magazine*; 6:29-37.
- a. Botanic Garden Kew: 166-179.
- [3] Burkill, H.M(1997). The useful plants of tropical West Africa. 43rd Edition. Royal
- [4] Hajiaghaalipour, F., Kauimath, M.S., Sanus, J., Rajarajeswaran, J.(2015). White tea (*Camellia sinensis*) inhibits proliferation of the colon cancer cell line HT.29, activates caspases and Protect DNA of normal cells against oxidative damage. *Food Chem*; 169:401-410.
- [5] Hutchings, A. (1996). Zulu Medicinal Plants. An Inventory. University of Natal Press, Pietermaritzburg, pp 300 – 325.
- [6] Hutchison, J.D, Dalziel, J.M (1958). Flora of west tropical Africa, London, Vol. 1 Part
- [7] Ju, J., Lu, G., Lambert, J.D., Yang, S.(2007). Inhibition of carcinogenesis by tea constituents semen. *Cancer Biol.*,01.:013
- [8] Kligerman, M.M. (1973). Cancer Medicine, Ed by Holland J.F., Frei EIII, Lea &Febiger, Philadelphia.541-565
- [9] Madaughinji, J., Chnag, C., Smith, D.I.(1991). “Bench-top” Bio assay for the discovery of bioactive natural products chemistry. Elsevier Science Pub.
- [10] Obuotor E.M, Onajobi, F.D.(2000). Preliminary evaluation of cytotoxic properties of *Raphia hookeri* fruit mesocarp. *Fitoterapia*;71:190-2.
- [11] Ukwubile, C.A and Elsie, J.A (2015). Analgesic and Anti-inflammatory Activities of *Melastomastrum capitatum* (Vahl) A. Fern. & R. Fern.(Melastomataceae) Leaf Methanol Extract. *American Journal of Biology and Life Sciences*;3(5):151-154.
- [12] Ukwubile, C.A., Agu, M.O., Agabila, J.E. (2015). Phytochemical Screening and Acute Toxicity Studies of *Melastomastrum capitatum* (Vahl) A. & R. Fern. (Melastomataceae) Leaf Methanol Extract. *American Journal of Biological Chemistry*; 3(4):57-62. <http://www.openscienceonline.com/journal/ajbc>
- [13] Ukwubile, C.A., and Manasseh, F.M. (2016). Application of *Melastomastrum capitatum* A. & R. Fern. (Melastomataceae) loaded-exosome as analgesic drug carrier in acetic acid-induced Swiss albino mice. *Molecular Biology Research and Innovations*; 1: 19-23. doi:10.5281/zenodo.218558
- [14] Vogel, H.G (1991). Similarities Between Various Systems of Traditional Medicine. Considerations for the future Ethno pharmacology. *J. Ethno pharmacology*;35:179-90.
- [15] WHO(2002). WHO Traditional Medicine Strategy 2002-2005; Pp.594-99.
- [16] Xiaorong, L., Zhongheng, C., Yuanyua, Z., Wei, L., Hao, T., Baibiao, D., Jian, D., Bin, L. (2015). Comparative characterization of green and black tea cream: *Physicochemical and phytochemical nature*. *Food Chem*, 173:432-440. doi: 10:1016/j. food chem.2014.10.048.
- [17] Yang, G., Liao, J., Kim, K., Yurkow, E.J, Yang, C.S.(1998). Inhibition of growth and induction of apoptosis in human cancer cell lines by tea polyphenols. *Carcinogen*. 19(4):611-616. doi:10:1093/Carcin/19.4.611.

Citation: Ukwubile, C.A. et al.,(2018). Evaluation of Preliminary Cytotoxic and Growth Inhibitory Effects of *Melastomastrum Capitatum* (Vahl.) Fern. (Melastomataceae) Leaf Methanol Extract by Bench-Top Bio-Assay. *International Journal Of Medicinal Plants And Natural Products (IJMPNP)*, 4(4), 42-47. doi: DOI: <http://dx.doi.org/10.20431/2454-7999.0404004>

Copyright: © 2018 Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.