

## ***In Vitro* Antioxidant Activity of *Melastomastrum capitatum* (Vahl) A. & R. Fern. (Melastomataceae) Leaf Methanol Extract by DPPH Radical Scavenging Activity**

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**Abstract:** Antioxidant activity of methanol extract of *Melastomastrum capitatum* leaf was evaluated using DPPH antioxidant assay. The methanol extract of the plant showed potent radical cation scavenging activity. The maximum inhibitory concentration ( $IC_{50}$ ) and radical cation scavenging activity of the plant was found to increase in concentration dependent fashion from 100, 200, 300, 400 and 500  $\mu\text{g/mL}$ . This study indicates significant free radical scavenging potential of the plant of *M. capitatum* which can be exploited for the treatment of various free radical mediated ailments like cancers and tumours.

**Keywords:** *In vitro*, Antioxidant activity, *Melastomastrum capitatum*, DPPH scavenging.

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### **1. INTRODUCTION**

Antioxidants are substances which are used as foods or as additives to foods whether in smaller amount or bigger amount, which are capable of preventing the oxidation of oxidizing agents which are capable of releasing free oxygen radicals in the body [1]. These substances have the potentials to inhibit specific oxidizing enzymes that can react with oxygen radicals, thereby causing damage to molecule in human cells [2].

Antioxidants act in the body in various ways by; reducing localized oxygen concentration, preventing the commencement of chain by scavenging radicals, breaking down lipid peroxides to peroxy and alkoxy radicals, reducing peroxides by converting them to non-radicals product, and breaking chain to prevent hydrogen reduction [3]. Currently, due to high toxicity of synthetic antioxidants, natural antioxidants of plant origin have gained popularity among natural product researchers.

*Melastomastrum capitatum* is a dicot plant belonging to the family *Melastomataceae*. It is locally called "Belkon" in Fulani language in Nigeria's North-East, Taraba State. Anti-inflammatory and analgesic activity as well as anti-hyper-cholesterolemic activities of the leaf methanol extract in albino mice has been reported by Ukwubile *et al.* [4]. The crude leaf methanol extract contains mainly glycosides, alkaloids, tannins and carbohydrates. In traditional medicine, the plant is used as anti-rheumatic agents, to treat stomach ache, for blood purification, for treating diuresis, for correcting intestinal and pulmonary problems [4].

This present study was carried out to evaluate the antioxidant activity of *M. capitatum* leaf methanol extract *in vitro* by DPPH scavenging radical assay.

### **2. MATERIALS AND METHODS**

#### **2.1. Materials**

DPPH, air dried leaves of *Melastomastrum capitatum*, ascorbic acid, beaker, micro pipette, distilled water, micro cuvette, test tube rack, test tube, spatula, UV/VIS spectrophotometer, ATI CO<sup>TM</sup> Model, methanol, filter paper, Kim white, mortar and pestle, measuring cylinder, separating funnel, etc.

#### **2.2. Methods**

##### *2.2.1. Collection and Identification of Plant*

Fresh leaves of *Melastomastrum capitatum* were collected in the evening hour from Mambila Plateau Sardauna Local Government Area Taraba State, and was authenticated by Mr. Cletus A. Ukwubile

(Biology Unit) of the Department of Science Laboratory Technology. A plant press was prepared and was deposited with voucher number "MELA001" in the herbarium of Biology Unit of Science Laboratory Technology Department, Federal Polytechnic, Bali, Nigeria.

### 2.2.2. Preparation and Extraction of Plant Material

The leaves of *Melastomastrum capitatum*, were air-dried at room temperature (40°C) for two weeks and was reduced into fine powder using electronic blender. 600g of the powder was defatted in 700 mL petroleum ether and then extracted with separating funnel by cold maceration techniques. The extract was then filtered using Whatman No 1 filter paper. The filtrate was concentrated, *in vacuo* at room temperature. After this, the methanol extract was further partitioned successively using solvents in increasing order of polarity from the eluotropic series in this order: carbon tetrachloride, chloroform, acetone, ethyl acetate and methanol. The final weight of the methanol leaf extract was calculated from the formula below:

$$\% \text{ yield} = (\text{Final weight of powder}/\text{initial weight of powder}) \times 100.$$

### 2.2.3. Antioxidant and Free Radicals Scavenging Assay

The method of Brain-Williams *et al.* [7] was used in a methanol solution of 2, 2-diphenyl-picrylhydrazyl (DPPH) radicals (concentration  $1.0 \times 10^{-4} \text{ M}$ ). The test extract was added in concentrations of 100, 200, 300, 400 and 500 µg/mL. The reaction mixture was shaken vigorously and kept in the dark for 30 min. The absorbance of the solution were measured using ATICO™ Model UV-Vis spectrophotometer at 546.0 nm wavelength against a blank without DPPH. Decreasing of DPPH solution absorbance indicated potential scavenging activity while the increase was given as DPPH radical scavenging activity. This activity is given as % DPPH radical scavenging activity that is calculated in the equation below:

$$\% \text{ DPPH radical scavenging} = \frac{\text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

DPPH solution without sample solution was used as control. All tests were run in triplicate and average. Ascorbic acid was used as reference drug [5-8].

## 3. RESULTS AND DISCUSSION

The DPPH analysis (% scavenge) showed a higher percentage of radical sequestration with statistical difference. In the concentration of 100 µg/mL, the DPPH scavenge, showed a higher concentration of  $91.70 \pm 14.63$  and at the concentration of 500 µg/mL, the DPPH scavenge was  $25.90 \pm 1.97$ , which means that the lower the concentration 100 µg/mL the higher the DPPH scavenging ( $91.70 \pm 14.63$ ), and the higher the concentration (500 µg/mL) the lower the DPPH scavenging ( $25.90 \pm 1.97$ ) of the plant extract. The inhibitory concentration showed that the lower the concentration (54.2%). At the concentration of 500 µg/mL, the inhibitory concentration was 87.1%. The antioxidant activity of *M. capitatum* revealed that the plant showed higher antioxidant activity at a higher concentration (Table 1).

The presence of non-antioxidant food component (amino acid and uronic acid) may interfere in the quantification of antioxidant activity in food [9]. Studying the antioxidant capacity of Gallic acid, using DPPH with different solvents (water, methanol/water, methanol acetone/water) found different among the method. However the DPPH assays did not demonstrate any interference due to solvent polarity. Begetti *et al.* [9] quantified the antioxidant activity of *M. capitatum*, which showed high antioxidant activity for the DPPH method as demonstrated by the leaf extracts. Total phenol has antioxidant activity, acting in the neutralization of free radicals, and contributing to the control of oxidative stress in pancreatic islets of cancer mice. The result regarding antioxidant activity showed that methanol extract of *M. capitatum* has strong antioxidant activity suggesting antioxidant property, as demonstrated by the *in vitro* study [10].

**Table 1.** Antioxidant activity of *Melastomastrum capitatum* methanol leaf extract

| Conc. (µg/mL) | DPPH Scavenging (nm) | I C <sub>50</sub> (%) |
|---------------|----------------------|-----------------------|
| Control       | $200 \pm 7.49$       | -                     |
| 100           | $91.70 \pm 1.61$     | 54.2                  |
| 200           | $70.40 \pm 1.14$     | 64.8                  |
| 300           | $64.40 \pm 1.72$     | 67.8                  |
| 400           | $63.80 \pm 1.75$     | 68.1                  |
| 500           | $25.90 \pm 1.97$     | 87.1                  |

Abs (546 nm), ascorbic acid is the reference drug

#### **4. CONCLUSION**

The study showed that methanol leaf extract of *M. capitatum* has a higher antioxidant activity. It is possible that the presence of major compounds found in these extract reported (ellagic acid, gallic acid and rutin) by previous researchers, might have contributed to the antioxidant effect of the extract. Overall, the result are very promising and may demonstrate the action of compound present in *M. capitatum* leaf with antioxidant property.

However, despite the antioxidant property found during this study , further study are necessary to determine the respond of methanol leaf extract, and the antioxidant signaling process of organs responsible for this, which will consolidate the results for future drug development.

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