Antioxidant Activity, Total Phenolic and Flavonoid Contents of the Methanol Whole Plant Extract of *Elytraria marginata* (Vahl)

'Ogbiko C', Dabai M.U', Amanabo M2, Okoh E.V.C' and Bature B. H'

1Department of Pure and Applied Chemistry, Faculty of Science, Usmanu Danfodiyo University Sokoto, Nigeria
2Department of Biochemistry, Faculty of Natural Science, Ibrahim Badamasi Babangida University Lapai, Nigeria

**ABSTRACT**

*Elytraria marginata* (Vahl) is used in Nigeria folk medicine as febrifuges, pulmonary problems and cancer related ailments. This study evaluated scientifically the phytochemical screening, antioxidant, total phenolic and flavonoid contents of the whole plant extract with a view to validate its folkloric usage. The phytochemical constituents were ascertained using standard procedures. In vitro antioxidant properties of methanol whole plant extract (MWPE) were evaluated using the free radical scavenging activities by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), nitroblue tetrazolium (NBT) and ferric reducing assays with ascorbic acid as control. Total phenol and flavonoid contents of the various extracts were determined using gallic acid and quercetin as standards. 

**Keywords:** *Elytraria marginata*, antioxidant activity, polyphenol compounds, flavonoids

*Author for correspondence: E-mail: cyril.ogbiko@gmail.com; cyril.ogbiko@udusok.edu.ng. Tel: 234-8177757199

**Received:** April, 2017; **Revised version accepted:** August, 2017

**Abstracted by:** Bioline International, African Journals online (AJOL), Index Copernicus, African Index Medicus (WHO), Excerpta medica (EMBASE), CAB Abstracts, SCOPUS, Global Health Abstracts, Asian Science Index, Index Veterinarius

**INTRODUCTION**

Free radicals are chemical compounds which contain an unpaired electron spinning on the peripheral layer around the nucleus (Auudy et al., 2003). They contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and repercussion injury of many tissues, a central nervous system injury, gastritis and cancer (Kumpulainen et al., 1999). Those generated from oxygen are called reactive oxygen species (ROS) like superoxide anion radicals (O₂⁻), hydroxyl radicals (OH⁻), peroxyl radicals (ROO⁻) as well as non-free radicals like (H₂O₂) and singlet oxygen (¹O₂). Those generated from nitrogen are called reactive nitrogen species (RNS) like nitric oxide (NO⁻) and peroxynitrite anion (ONOO⁻) (Auudy et al., 2003). Antioxidants on the other hand are group of substances that when present in low concentrations compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substance while preferentially being oxidized themselves (Auudy et al., 2003). Aside their role as health benefactors, antioxidants are added in foods to prevent or delay oxidation of food, initiated by free radicals formed during their exposure to environmental factors such as air, light and temperature (Cook and Samman, 1996).

The antioxidant activity of phenolics is mainly due to their redox potentials hence allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Farrukh et al., 2006). A number of synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) have been extensively added to foodstuffs, although their use has raised serious concerns because of their toxicity (Myojin et al., 2008; Saha et al., 2004), so there is considerable interest in preventive medicine and in the food industry in the development of natural antioxidants obtained from botanical sources, especially herbal plants.

Medicinal plants are an important source of antioxidants (Horax et al., 2005). Secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers (Ito et al., 1983). There is no data on the
antioxidant activity, total phenolic content and total flavonoid content of the plant hence, the reason for the study.

MATERIALS AND METHODS

Chemicals: Methanol, (analytical grade) was obtained from J.T. Baker (USA). Riboflavin, Folin and Ciocalteau reagent, gallic acid, potassium acetate, quercetin, potassium ferricyanide, phosphate buffer, trichloroacetic acid, ferric chloride, and nitro blue tetrazolium, d,l-methionine and Triton X-100 were obtained from Sigma Chemicals (St. Louis, USA). 2, 2-Diphenyl picrylhydrazyl (DPPH), Na$_2$CO$_3$, AlCl$_3$ were obtained from Fluka Chemicals, Germany.

Plant materials: Fresh whole plants of *E. marginata* for this experiment were collected in June 2014, at a forest in Benin City, Edo State Nigeria. The plant was identified and authenticated by the Forest Research Institute, Ibadan, Nigeria and a herbarium copy deposited in the same institute.

Processing of the plant material and preparation of methanol whole plant extracts: The air dried and powdered leaves (1.5 kg) were extracted exhaustively with 5.5 L methanol using cold maceration. The whole plant extract (77.55 g) was obtained. The extract was preserved at 4°C till use.

Determination of Plant Extract Yield: The yield of evaporated dried extracts based on dry weight basis was calculated from the following equation: Yield (g/100 g of dry plant material) = (W$_1$ × 100) / W$_2$. Where W$_1$ was the weight of the extract after the solvent evaporation and W$_2$ was the weight of the dry plant material.

Phytochemical analysis: Simple chemical tests to detect the presence of carbohydrates, proteins and secondary metabolites were done in accordance with standard methods (Osawa and Namiki, 1981; Iwu, 1993; Burkill, 1985).

Analysis of Total Phenol Content (TPC): TPC in the extract was determined according to the Folin-Ciocalteu procedure (Engel et al., 2011) with slight modifications. The extract solution (0.5 ml) with a concentration of 1000 μg/ml was added to 4.5 ml of deionized distilled water and 0.5 ml of Folin Ciocalteu’s reagent. The solution was maintained at room temperature for 5 minutes followed by the addition of 5 ml of 7 % sodium carbonate and 2 ml of deionized distilled water. The thoroughly mixed samples were incubated for 90 minutes at 23°C. The absorbance was measured by spectrophotometer at 750 nm. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of extract. Gallic acid was used as positive control. The standard curve was prepared by gallic acid in five different concentrations (50, 100, 150, 250 and 500 mg/L).

Determination of Total Flavonoid Content (TFC): The total flavonoid content in the plant extracts was determined by the Aluminum Chloride method as described (Dorman et al., 2003). 0.5 ml of the extract (5 g/L) was mixed with 1.5 ml of methanol and then, 0.1 ml of 10 % aluminum chloride was added, followed by 0.1 ml of potassium acetate and 2.8 ml of distilled water. The mixture was incubated at room temperature for 30 min. The absorbance was measured by a spectrophotometer at 415 nm. The results were expressed as milligrams Quercetin equivalents (QE) per gram of extract (mg QE/g dry extract). Quercetin was used as positive control and the standard curve was prepared by quercetin in different concentrations (12.5, 25, 50, 80 and 100 mg/L).

Antioxidant Activities: Various methods are often recommended in the estimation of antioxidant activity of compounds in plant extracts since one assay method is insufficient to elucidate possible mechanisms of antioxidant effects (Erkan et al., 2008).

DPPH Free Radical Scavenging Activity: The ability of methanol extract of *E. marginata* to scavenge 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radicals was estimated as previously described (McDonald et al., 2001) with slight modifications. The extracts (3 ml) with five different concentrations (100.0, 200.0, 300.0, 400.0, and 500 μg/ml) were mixed with 1 ml of a 0.1 mM methanolic solution of DPPH. The absorbance was measured by a spectrophotometer at 517 nm at 30 minutes intervals against a blank (pure ethanol). The percentage of radical scavenging activity was calculated using the formula:

$$DPPH \text{ radical inhibition (\%)} = \frac{1 - (A_{\text{test}} / A_{\text{control}})}{100}$$

Where $A_{\text{control}}$ is the absorbance of the control and $A_{\text{test}}$ is the absorbance of the sample extracts. Ascorbic acid was used as a reference standard with the same concentrations.

Ferric reducing power assay: Ferric reducing or antioxidant power was determined as described earlier (Ebrahimzadeh et al., 2008). Briefly, 100 μL of the extract (100–500 μg/mL) were mixed with 2.5 mL of 200 mmol/L phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide were incubated at 50°C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid were added, and the tubes were centrifuged at 10,000 rpm for 10 min. 5 mL of the upper layer were mixed with 5.0 mL distilled water and 1 mL of 0.1% ferric chloride. The absorbance of the reaction mixtures was measured at 700 nm. Ascorbic acid was used as a positive control.

Nitroblue tetrazolium (NBT) assay: Superoxide anion scavenging activity was determined as described earlier (Jain et al., 2008). The reaction was performed in 50 mmol/L phosphate buffer (pH 7.8) containing concentrations of 100–500 μg/mL of the extract, 1.5 mmol/L riboflavin, 50 mmol/L nitroblue tetrazolium (NBT), 10 mmol/L d,l-methionine, and 0.025% (v/v) Triton X-100. The reaction was initiated by illuminating the reaction mixture; the absorbance of formazan was recorded at 560 nm, and the percentage scavenging activity was described as the inverse of the produced formazan. Ascorbic acid was used as a positive control.

Statistical Analysis: Results were expressed as means ± standard deviations (SD). Statistical comparisons were made...
Antioxidant, Phenolic and Flavonoid contents of E. marginata

using the student t-test, one-way analysis of variance (ANOVA) using SPSS statistics 17.0 software package.

RESULTS

Percentage yield of crude extracts
Approximately, 77.55 g (5.17%) viscous mass was obtained from 1.50 kg powdered whole plant of E. marginata 72 h of cold maceration in methanol.

Preliminary phytochemical screening
Preliminary phytochemical analysis showed the presence of major classes of secondary metabolites such as tannins, steroids, alkaloids, flavonoids, carbohydrate, glycosides, cardiac glycosides, saponins, terpenoids and phenols.

Phenolic and Flavonoids contents
The total phenolic content of the methanol whole plant extract, calculated from the calibration curve \( R^2 = 0.998 \), was 44.14 ± 1.62 gallic acid equivalents/g, and the total flavonoid content \( R^2 = 0.999 \) was 36.24 ± 2.22 rutin equivalents/g (Table 1).

In vitro antioxidant assay
The graphical representation of the DPPH, NBT and Ferric reducing power is presented in figure 1a – c respectively.

Table 1
<table>
<thead>
<tr>
<th>Total phenolics content*</th>
<th>244.14 ± 1.62</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total flavonoids content**</td>
<td>66.24 ± 2.22</td>
</tr>
</tbody>
</table>

*mg gallic acid equivalent (mgGAE)/g extract  
**mg Quercetin equivalent (mgQE/g extract)  
Values are means of three biological replicates.

DISCUSSION

Phenolic compounds are potent antioxidant mainly due to their reduced properties which allow them to act as metal chelators, absorb and neutralize free radicals (Pourmorad et al., 2006). The capability of flavonoids to interact with protein phosphorylation and the antioxidant, iron-chelating, and free radical scavenging activity may account for the wide pharmacological profile of flavonoids (Kumaran and Karunakaran, 2007). Reported researches have established that phenolic compounds exhibit biological activities such as antioxidant, anti-diabetic, hepatoprotective, anti-inflammatory, antimicrobial, anticancer among others (Falodun and James, 2011), hence substantiate the findings of this research.

Based on the results of the total phenol and flavonoid content in the whole plant of E. marginata, it can be proposed that the biological activity exhibited could be attributed to the presence of flavonoids and other phenolics in it. The results of the antioxidant activity of the three methods employed demonstrated a considerable high antioxidant activity in a concentration dependent manner but this was lower than that of the standard ascorbic acid because ascorbic acid is a known and potent antioxidant agent used in medicines.
Conflict of interest
All authors declare that no conflict of interest exists

REFERENCES


