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Research Article

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

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ABSTRACT

Elytraria marginata (Vahl) isused in Nigeria folk medicine as febrifuges, pulmonary problems and cancer related ailments. This study evaluated scientifically the phytochemical screening, antioxidant, total phenolic and flavonoids contents of the whole plant extract with a view to validate its folkloric usage. The phytochemical constituents were ascertained using standard procedures. In vitroantioxidant 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 positive controls. Phytochemical analysis of MWPE showed presence of major classes of phytochemicals. *In vitro* antioxidant activities of the crude extracts were significant and comparable with the standard ascorbic acid.The high contents of total phenolic compounds (244.14 ± 1.62 mg GAE/g of dry extract) and total flavonoids (66.24 ± 2.22 mg QE/g of dry extract) indicated that these compounds contribute to the antioxidative activity. The present study, hence validates that the MWPE of *Elytraria margi*nata might have good potential as a source for natural health products due to its antioxidant activities

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

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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 (O_2^-), hydroxyl radicals (OH^-), peroxyl radicals ($ROO^\bullet$) as well as non-free radicals like (H_2O_2) and singlet oxygen ($^1O_2$). Those generated from nitrogen are called reactive nitrogen species (RNS) like nitric oxide ($NO^\bullet$) 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_2CO_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^{o}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) = $(W_1 \times 100) / W_2$. 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-picryl-hydrazyl (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:

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

Where A_{control} is the absorbance of the control and A_{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 of1% potassium ferricyanide were incubated at 50◦C for20 min. Then, 2.5 mL of 10% trichloroacetic acid were added, and the tubes were centrifuged at 10,000 rpm for10 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 700nm. 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

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

Total phenolics and flavonoids content of methanol whole plant extract of *E. marginata*

Total phenolics content*	244.14 ± 1.62
Total flavonoids content**	66.24 ± 2.22

*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.

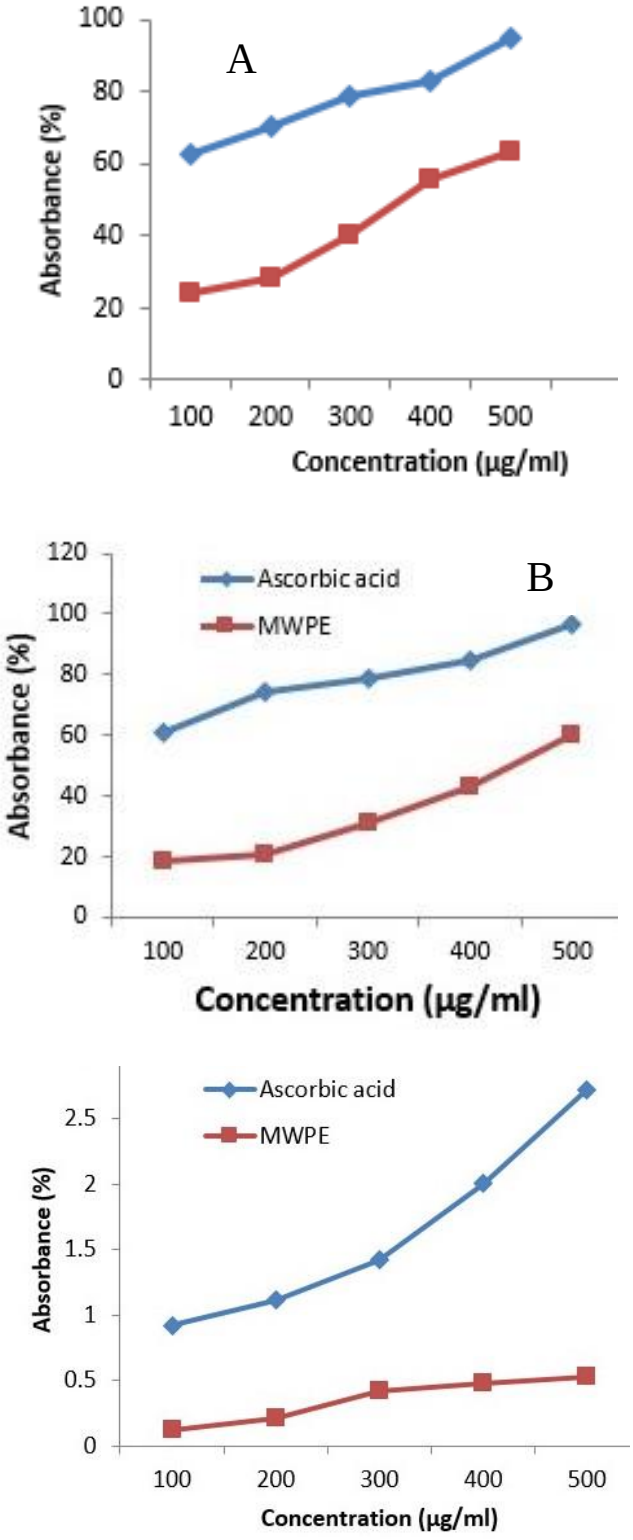

Figure 1

(A) DPPH Free radical scavenging activity of the methanol whole plant extract (MWPE) of *E. marginata*. Ascorbic acid was included as a positive control. Each value is the mean ± standard deviation. (B) Superoxide scavenging activity of the methanol whole plant extract (MWPE) of *E. marginata*. Ascorbic acid was included as a positive control. Each value is the mean ± standard deviation. (C)Ferrous scavenging activity of the methanol whole plant extract (MWPE) of *E. marginata*. Ascorbic acid was included as a positive control. Each value is the mean ± standard deviation.

Conflict of interest

All authors declare that no conflict of interest exists

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