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Phenolics Content and Antioxidant Capacity of Extracts and Fractions of *Vernonia blumeoides* (Asteraceae)

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ABSTRACT

Leaf extract and fractions of *Vernonia blumeoides* were evaluated for total phenolics content (Folin-Ciocalteu method), free radical scavenging activity (1, 1-diphenyl-2-picrylhydrazyl radical assay), total antioxidant capacity (Phosphomolybdate assay) and ferric reducing power. The results of the phenolics content expressed in mg/100 g of Gallic Acid Equivalent (GAE) showed that the n-butanol fraction has significantly ($p<0.05$) higher phenolics content (410±0.8) than the chloroform fraction and ethanol extract. The radical scavenging activity of the extract and solvent fractions displayed strong concentration dependent activity. But it was also observed that the ethyl acetate fraction showed highest activity in all concentration tested ranging from 70.56 to 99.04%. However, the total antioxidant capacity (mg g^{-1} ascorbic acid) showed that n-butanol fraction has the highest capacity (60.0 mg g^{-1}). The results also showed that the n-butanol extract has strongest reducing ability (2.105±0.109 nm) at higher concentration which is comparable to that of Gallic acid at all the concentrations tested. Phytochemical screening on the extracts revealed the presence of flavonoids, saponins and tannins. The results suggest that the plant especially the n-butanol and ethyl acetate fractions are very rich in antioxidant compounds worthy of further investigations.

Key words: Free radical, *Vernonia blumeoides*, reducing power, phytochemicals

INTRODUCTION

Reactive Oxygen Species (ROS) are oxygen centered free radicals that exist in different forms such as superoxide (O_2), peroxyl (ROO), alkoxyl (RO), hydroxyl (HO) and Nitric Oxides (NO). They are generated during normal metabolism and energy production in the body (Pietta, 2000) and have the potentials of reacting with almost all types of molecules in living cells (McCord, 1985). Although the organisms possesses intracellular defense mechanisms such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) which arrest the damaging properties of the ROS (Halliwell *et al*., 1995; Sies, 1993), continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body beyond its capacity to control them and cause irreversible oxidative damage (Tseng *et al*., 1997). This situation has been linked to the progression and/or deterioration of a number of metabolic diseases including diabetes, cancer and cardiovascular diseases (Moussa, 2008). Hence the need for agents that could combat the oxidative stress mediated events in such diseases cannot be overemphasized. Medicinal plants and

vegetables consumed as food are widely accepted as sources of antioxidants substances. This is because they contain secondary metabolites that can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction in human systems which therefore, appear to be very important in the prevention of many diseases (Halliwell *et al.*, 1995). Plants containing phenolics, flavonoids and polypropanoids have the capacity to scavenge free radicals, due to their proton donating ability (Benabadji *et al.*, 2004).

Vernonia species are important herbal recipes in African ethno medicine because they are widely used for the treatment of various diseases across the continent. It is referred to as the bitter genus, distributed across northern Nigeria and widely used in traditional medicine for the treatment of malaria, stomach pain and other infectious diseases. *Vernonia* species are characteristically known to contain large amount of sesquiterpene lactones, with several reported biological activities (Kumari *et al.*, 2003; Kuo *et al.*, 2003; Koul *et al.*, 2003). Other compounds have also been isolated from *Vernonia* genus, including flavonoids (Huang *et al.*, 2003), steroids (Tchinda *et al.*, 2003) and polysaccharides (Nergard *et al.*, 2004).

In view of the widely use of *Vernonia* species as herbal recipe and the increasing awareness of herbal remedies as potential sources of antioxidants, it is important to understand its potentials antioxidant capacity which will provide additional knowledge for maximum utilization. We have previously reported the potentials of Nigerian medicinal plants as sources of phenolics antioxidants (Aliyu *et al.*, 2009a-c, 2010). This study was designed to evaluate the *in vitro* total phenolics contents and antioxidant activity of solvent fractions of *V. blumeoides* leaf.

MATERIALS AND METHODS

Chemicals and reagents: Deionized water, Folin-Ciocalteu phenol reagent (Fluka, UK) Gallic acid (Fluka, UK), 1, 1-diphenyl-2-picrylhydrazylradical (DPPH) (Sigma-Aldrich Co.), Trichloroacetic acid (Sigma-Aldrich Co.), anhydrous ferric chloride, potassium ferricyanide, anhydrous sodium carbonate, Ascorbic acid and all other chemicals were of analytical grade BDH Chemical Laboratory (England, UK).

Plant material: Whole plant of *V. blumeoides* was collected in the month of September, 2009 at Dakace village along Jos road, Zaria. It was authenticated at the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria. A Voucher specimen (No.1784) was deposited there.

Extraction and preliminary fractionation: Powdered leaves sample (200 g) of *V. blumeoides* was extracted exhaustively with ethanol on a soxhlet extractor for four hours. The extract was filtered using Whatman filter paper No. 2 and concentrated on a Büchi rotary evaporator at 45°C which afforded 40.50 g of the crude ethanol extract. Fractionation of antioxidant compounds was carried out as reported by Cho *et al.* (2003). Crude extract (20 g) was suspended in 10% aqueous methanol and filtered. The aqueous soluble portion was partitioned with chloroform (3×200 mL), ethyl acetate (3×200 mL) and n-butanol (3×200 mL) which afforded 2.5, 3.8 and 4.0 g of the fractions respectively as shown in Fig. 1.

Free radical scavenging activity: The determination of the radical scavenging activity of each of the crude extracts and solvent fractions was carried using the DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay as described by Mensor *et al.* (2001) with a slight modification. To 1.0 mL of

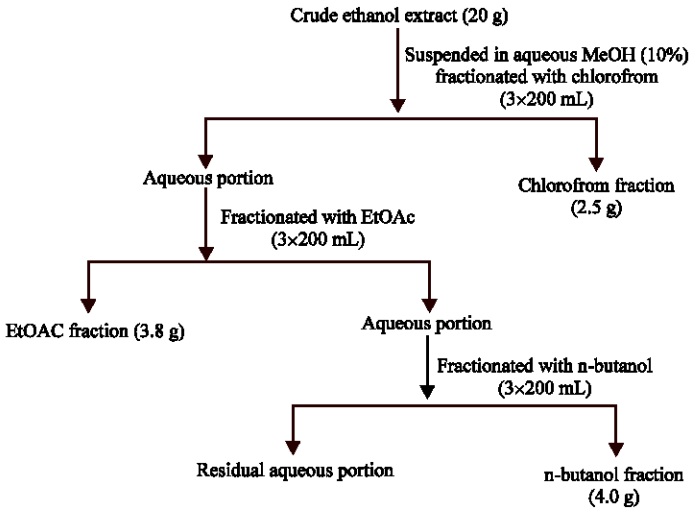

Fig. 1: Fractionation of antioxidants compounds from *Vernonia blumeoides*

DPPH (0.25 mM) in methanol was added to 2.0 mL solution of various concentrations of extract, fractions or standard (1000, 500, 250, 125, 62.5 and 31.25 μg mL^{-1}). The reaction mixture was then allowed to stand at room temperature in a dark chamber for 30 min. The change in colour from deep violet to light yellow was then measured at 518 nm on a spectrophotometer. The decrease in absorbance was then converted to percentage radical scavenging activity (% RSA) using the formula:

$$\%RSA = 100-\{[(Abs_{sample}-Abs_{blank})\times 100]/Abs_{control}\}$$

Blank is a Methanol (1.0 mL) plus sample solution (2.0 mL), Negative control is a DPPH solution (1.0 mL, 0.25 mm) plus methanol (2.0 mL), ascorbic acid and Gallic acid were used as standards.

Total antioxidant capacity: The total antioxidant capacity of the extracts and fractions were evaluated by the phosphomolybdenum method according to the procedure described by Prieto *et al.* (1999). The assay is based on the reduction of Mo (VI) to Mo (V) by the extracts and fractions and subsequent formation of green phosphate/Mo (V) complex at acid PH. A 0.3 mL of various concentration of extract or fractions (1000, 500, 250, 125, 62.5 and 31.25 μg mL^{-1}) was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer (Jenway 6025) against blank after cooling to room temperature. Methanol (0.3 mL) in the place of extract is used as the blank. The total antioxidant activity is expressed as the number of gram equivalents of ascorbic acid.

Total phenolics content: Total phenolics content of the extracts were determined using the method of McDonald *et al.* (2001) with slight modifications. Calibration curve was prepared by mixing ethanol solution of Gallic acid (1 mL; 0.025-0.400 mg mL^{-1}) with 5 mL Folin-Ciocalteu reagent (diluted tenfold) and sodium carbonate (4 mL, 0.7 M). Absorbance values were measured at 765 nm using a UV-VIS spectrophotometer (UVmini_1240, Shimadzu Corporation, Kyoto, Japan) and the standard curve was plotted. One milliliter of each of the extract solution in methanol (5 g L^{-1}) was also mixed with the reagents above and after 30 min the absorbance was

measured to determine the total phenolic contents. All determinations were carried out in triplicate. The total phenolics components in the extracts in Gallic Acid Equivalents (GAE) were calculated by the following formula: T = C.V/M; where T is a total phenolic contents, milligram per gram of sample extract, in GAE; C is the concentration of Gallic acid established from the calibration curve, mg mL^{-1}; V is the volume of extract, milliliter; M is the weight of sample extract (g).

Reducing power assay: This was determined according the method of Oyaizu (1986). The extract, fractions or standard (1 mL) of various concentrations (1000, 500, 250, 125, 62.5 and 31.25 μg mL^{-1}) were mixed with phosphate buffer (pH 6.6, 0.2 M, 2.5 mL) and potassium ferricyanide (1%, 2.5 mL). The mixture was incubated at 50°C for 20 min. Trichloroacetic acid (10%, 2.5 mL) was added to the mixture. A portion of the resulting mixture was mixed with $FeCl_3$ (0.1%, 0.5 mL) and the absorbance was measured at 700 nm in a spectrophotometer (Jenway 6025). Higher absorbance of the reaction mixture indicated reductive potential of the extract.

Statistical analysis: The experiments were carried out in triplicate and results are given as Mean±standard deviation. The data in all the experiments were analyzed for statistical significance using student's t-test and differences were considered significant at $p<0.05$.

RESULTS

Phytochemical screening of Ethanol Extract (EE) and solvent fractions of *V. blumeoides* revealed the presence of secondary metabolites such as steroids/terpenes, saponins, flavonoids, tannins and alkaloids. Anthraquinones were found absent in all the fractions and crude ethanol extract (Table 1). The results of the free radical scavenging activity of the DPPH assay expressed as percentage radical scavenging (%RSA) indicates that Ethyl Acetate Fraction (EAF) has the highest activity at all concentrations tested ranging from 70.56 to 99.04%. However, the total antioxidant capacity expressed as mg g^{-1} of ascorbic acid showed that n-butanol fraction has the highest capacity (60.0 mg g^{-1}), whereas chloroform and ethyl acetate fractions having 58 mg g^{-1} and 57 mg g^{-1} respectively (Table 2). Phenolic contents of the n-butanol fraction were higher

Table 1: Percentage recovery and phytochemical analysis of extract and fractions of *V. blumeoides*

Samples	EE	CHF	EAF	NBF
% Recovery	20.25	12.5	19.0	20.0
Alkaloids	+	+	-	-
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Tannins	+	-	+	+
Anthraquinones	-	-	-	-
Steroids/terpenes	+	+	+	+

EE: Ethanol extract, CHF: Chloroform fraction, EAF: Ethyl acetate fraction, NBF: n-butanol fraction

Table 2: Total phenolics content and total antioxidant capacity of *V. blumeoides*

Samples	Total phenolics content mg/100 g Gallic acid equivalent	Total antioxidant capacity mg g^{-1} Ascorbic acid equivalent
Ethanol crude extract	110.0±0.3	40.0±0.05
Chloroform fraction	14.0±0.3	58.0±0.01
Ethyl acetate fraction	340.0±0.2	57.0±0.03
n-butanol fraction	410.0±0.8	60.0±0.02

(410 mg/100 g GAE) than those of ethyl acetate fraction (340 mg/100 g GAE) and ethanol extract (110 mg/100 g GAE). The reducing power assay on the other hand indicates that the ethanol extract has the highest activity in all the concentrations tested. Although differences in activity between the extract and the solvent fractions in most cases are not statistically significant ($p<0.05$).

DISCUSSION

Vernonia bluimodes is commonly used to traditionally treat many diseases whose pathogeneses are, among other factors, linked to oxidative stress. However, information on antioxidant potentials of this plant that could be relevant in the treatment of such diseases has not been investigated. In this study, we report the antioxidant potentials of some solvent fractions of *V. blumoides* leaves. The free radical chain reaction is widely accepted as a common mechanism of lipid peroxidation. The use of DPPH, a relatively stable radical, is based on the measurement of the scavenging ability of antioxidants towards the stable radical which reacts with suitable reducing agents. The electrons become paired off and solution loses colour stochiometrically depending on the number of electrons taken up (Baskar *et al.*, 2007). On the other hand, the electron donating capacity, reflecting the total reducing power of bioactive compounds or fractions, is associated with antioxidant activity (Ak and Gulcin, 2008). In this study, DPPH radical scavenging and total reducing power methods were respectively used to determine the radical scavenging and electron donating abilities of all the solvents fractions. It is evident from our results that EAF and NBF contain more promising radical scavenging and reducing power agents which might further indicate, at least in this experimental condition, that the more polar components of the leaves part of this plant are more relevant in the antioxidant action and perhaps other therapeutic actions of this plant (Fig. 2-4).

Phytochemicals are currently receiving increased attention because of interesting new findings regarding their biological activities (Cho *et al.*, 2003). The flavonoids, saponins and tannins consistently detected in the EAF and NBF could implicate these classes of phytochemicals as important bioactive agents of the plant and could be responsible for the observed antioxidant activity in particular or the therapeutic action of this plant as a whole. This is because these

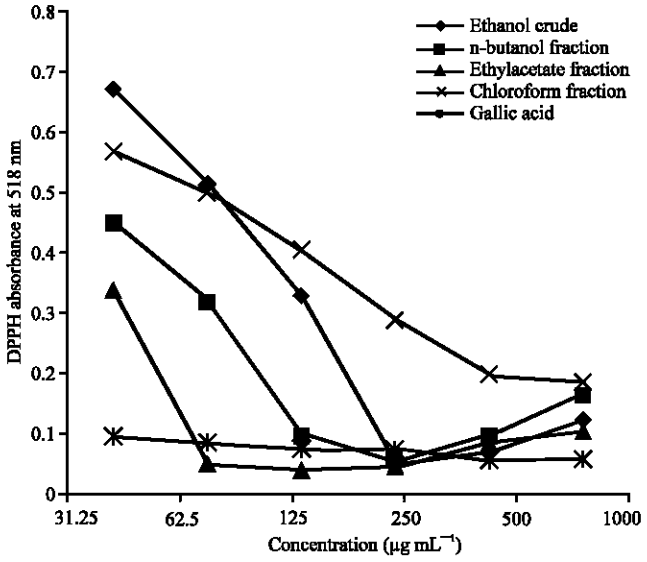

Fig. 2: DPPH radical scavenging with increasing concentration

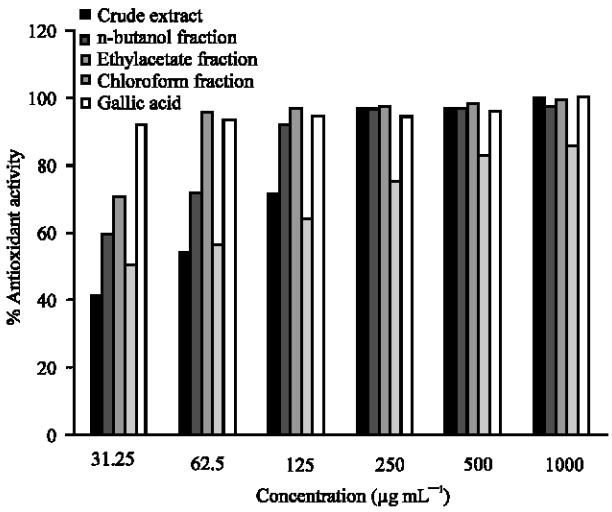

Fig. 3: Percent scavenging activity of solvent fractions of *V. blumeoides* leaf

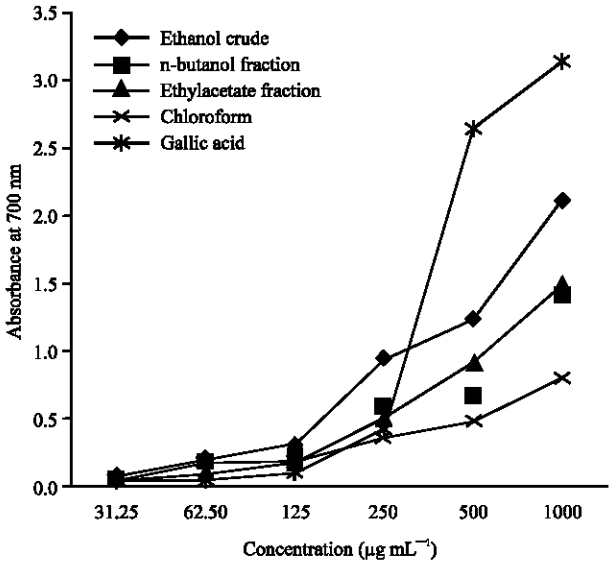

Fig. 4: Reducing power assay of Crude extract and solvent fractions of *V. blumeoides*

fractions displayed higher antioxidant activity and possess similar phytochemicals profile. The high phenolics content of EE, EAF and NBF indicates high antioxidant potentials because the phenolics constituents react with active oxygen radicals such as hydroxyl radical (Husain *et al*., 1987), superoxide anion radical (Afanas'ev *et al*., 1989) and lipid peroxy radical (Terol *et al*., 1986). Literature reports showed that there is high correlation between antioxidant activity and phenolics content (Odabasoglu *et al*., 2004). The EAF and NBF were found to possess higher phenolics content than CHF and although correlation analysis was not performed, our data also suggests high correlation between total phenol content and antioxidant potential considering the higher antioxidant activity observed with the EAF and NBF than CHF in most of the assays.

We concluded that *V. bluimodes* leaves; especially the more polar fractions contain potent antioxidant substances. It will be expedient to further characterize the polar components of this plant.

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