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Review Article

Antioxidant Potential of *Vernonia occephala*

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Abstract

In this study, antioxidant activities of ethanolic extracts, ethyl acetate, n-butanol and chloroform fraction of *Vernonia oocephala* were evaluated using DPPH free radical scavenging assay, total phenolic contents, reducing power assay and total antioxidant capacity. The free radical scavenging activity expressed as percentage antioxidant activity was highest in ethyl acetate fraction of (EC_{50} = 20.0 ± 0.005 µg/ml). The total phenolic content was highest in ethyl acetate fraction (106.0 ± 0.003 mg/g GAE). Also, the ethyl acetate fraction exhibited the highest total antioxidant capacity (100 mg g^{-1} ascorbic acid equivalent). The chloroform fraction exhibited the highest reducing power at all concentrations. There is no significant difference between the standard and the sample ($P \leq 0.05$). The results of this study have shown that *Vernonia oocephala* contains antioxidant chemical substances and therefore can be considered as a good source of natural antioxidants for medicinal uses.

Keywords: *Vernonia occephala; Antioxidant Activity; DPPH; Folin-Ciocalteu*

Introduction

Plants as sources of medicinal compounds have been playing vital role in maintenance of human health since the existence of man. Over 50% of all modern clinical drugs are of natural product origins [1] and natural products play an important role in drug development programs of the Pharmaceutical Industries [2]. In developing countries, especially in rural area, people usually turn to traditional healers when in diseased conditions and local preparation of plant origins are often presented for use [3].

Investigations into the chemical and biological activities of plants during the two centuries have yielded compounds for the development of modern synthetic organic chemistry and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents [4,5].

Free radicals are formed during oxidative and nitrodative stress. These free radicals which include reactive oxygen species (ROS) such as ($OH^{\cdot}$), the superoxide anion radical ($O_2^{\cdot}$), hydrogen peroxides (H_2O_2) and peroxyl ($ROO^{\cdot}$) and reactive nitrogen species (RNS) such as nitroxide ($NO^{\cdot}$), peroxy nitrite ($OONO^{\cdot}$), nitrous acid (HNO_2) and dinitrogen trioxide (N_2O_3) among others are primarily generated during normal metabolism and energy production in the body system [6]. Oxidative and nitrodative stress plays a role in the etiology of human diseases including heart disease neurodegenerative diseases, cancer, aging process, among others [7-9]. In recent years, there has been increasing in the presence and availability of compounds in plant materials that may possess bioactive properties, in particular, antioxidant activity [10-12]. Therefore, antioxidants are substances which when present in minute quantity can inhibit or prevent oxidation.

The plant *Vernonia oocephala* is distributed across Northern part of Nigeria. It is an erect perennial shrub used in the folk medicine to treat malaria and a number of unspecified infectious diseases [13]. A study of phytochemical screening with native plants from Nigeria revealed the presence of secondary metabolites: alkaloids, flavonoids, saponins, tannins, glycosides, cardiac glycosides, steroids and triterpenes [14]. The antibacterial activity of the ethanolic extracts of leaves of *V. oocephala* was reported against the clinical bacterial strains, *K. pneumoniae, S. pyogenes, S. aureus, C. ulcerans*, methicillin resistant *S. aureus* and *P. mirabilis* by the disk diffusion and broth microdilution methods [14]. Though, many antioxidant research works have done on Vernonia amygdalina but no work has so far been reported on *Vernonia occephala*.

Aim of the Study

This research aimed to determine the antioxidant potential of *Vernonia oocephala* through radical scavenging activity, total phenolic content, total antioxidant capacity and reducing power assay.

Materials and Methods

Chemicals and reagents

Deionized water, Folin-Ciocalteu phenol reagent (Fluka, UK) gallic acid (Fluka, UK), 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) (Sigma-Aldrich Co.), anhydrous sodium carbonate, Ascorbic acid and all other chemicals were of analytical grade BDH Chemical Laboratory (England, UK).

Plant materials

The plant, *V. oocephala* (Asteraceae), was collected alone Zaria-Jos road, Kaduna state. It was identified at the Herbarium of the Biological Science Department, Ahmadu Bello University Zaria, Nigeria. The plant was air dried and pulverized into coarse powder.

Extraction/fractionation

Powdered samples (200g) of *V. oocephala* was soaked in ethanol and left on the laboratory bench for four weeks, with continuous swirling on daily basis. On the fourth week, the solution was filtered using Whatman number 1 filter paper and the filtrate was dried on a rotary evaporator until all the ethanol was removed. The percentage recoveries of the extract/fractions were calculated using:

% recovery = (Weight of extract)/(Weight of dried plant material) x 100

The fractionation of the extract was carried out according to the procedure described by Cho., *et al* [15]. A dried crude extract (20g) of the *V. oocephala* was dissolved in aqueous methanol (10%) and extracted with chloroform (3 × 200 ml). The organic layer (chloroform) was separated from the aqueous layer (using separating funnel) and concentrated to dryness using rotary evaporator. This is the chloroform fraction. The resultant aqueous portion was further extracted with n-butanol (3 × 200 ml) and ethyl acetate (3 × 200 ml) to obtain the n-butanol and ethyl acetate fraction respectively.

DPPH free radical scavenging activity

The determination of the free radical scavenging activity of crude extract and solvent fractions were carried out using the DPPH (1, 1-diphenyl-2-picrylhydrazyl radical) assay as described by Mensor., *et al*. [16] with a slight modification. Various concentrations of 1000, 500, 250, 125, 62.5 and 31.25 μgml^{-1} of sample extract in methanol were prepared. 1.0 ml (0.25 Mm) DPPH in methanol was added to 2.0 ml solution of the extracts and standard and allowed to stand at room temperature in a dark chamber for 30 minutes. The change in colour from deep violet to light yellow was then measured at 518 nm on the spectrophotometer. The decrease in absorbance was then converted to percentage antioxidant activity (% AA) using the formula:

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

Blank = Methanol plus sample solution (2.0 ml). Negative control = DPPH solution (1.0 ml, 0.25 mM) plus methanol (2.0 ml), Gallic acid was used as standard.

Determination of total phenolics

The total phenolic contents of extract and fractions were determined using the method of Macdonald., *et al*. [17] with slight modification. Calibration standard was prepared by dissolving ethanol solution of Gallic acid (l ml; 0.025 - 0.4 mg/ml) with 5 ml folin-ciocalteu reagent (diluted tenfold) and sodium carbonate (4 ml, 0.7 M). Absorbance values were measured at 765 nm and the standard curve was drawn. 1 ml of extract/fraction (1 g/200 ml) was also mixed with the reagents above and after 30 minutes the absorbance was measured to determine the total phenolic contents. The total phenolic content in the extract was expressed in Gallic acid equivalents (GAE) and was calculated by the following formula: T = (C.V)/M, Where T = Total phenolic contents, milligram

per gram of plant extract, GAE, C = The concentration of Gallic acid obtained from the calibration curve, milligram per milliliter; v = The volume of extract, milliliter; M = The weight of methanol plant extract, gram.

Reducing power assay

This was determined according to the method of Oyaizu [18]. The extracts and standard of various concentrations 1 ml (1000, 500, 250, 125, 62.5 and 31.25 µg ml^{-1}) were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%); the mixture was incubated at 50°C for 20 min. Trichloroacetic acid (2.5 ml, 10%) was added to the mixture with $FeCl_3$ (0.1%, 0.5 ml) and the absorbance values were measured at 700 nm with a spectrophotometer (Jenway 560). Higher absorbance of the reacting mixture indicates higher reducing power of the extracts/fractions.

Determination of total antioxidant capacity

The antioxidant activity of the extract/fractions was evaluated by the phosphomolybdenum method according to the procedure described by Prieto., *et al* [19]. The assay is based on the reduction of Mo (VI)-Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. A 0.3 ml extract/fraction was mixed with 3 ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 minutes. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer after cooling to room temperature. The antioxidant activity is expressed as the number of gram equivalent of ascorbic acid.

Statistical analysis

All determinations were replicated three times and the results expressed as mean ± SD.

Results and Discussion

Extraction

The result of the extractive yields is presented in table 1. From the table, the highest recovery was obtained in butanol fraction while the lowest recovery was observed in chloroform fraction. The yields may be due to the choice of solvents used during the extractive process.

Sample	EE	BUF	CHF	EAF
Weight (g)	39.0	4.0	2.5	4.4
% Recovery	19.75	20.0	12.5	22.0

Table 1: Weight of crude extract/fractions and percentage recovery from V. oocephala.
Key: EE: Ethanol Extract; BUF: Butanol Fraction; EAF: Ethyl Acetate Fraction; CHF: Chloroform fraction.

Radical scavenging activity

It is well understood that the potential of plants as antioxidant sources is related to the types of phytochemicals contain in them. Plant extracts containing polyphenols or any other antioxidant components have the ability to be donors of hydrogen atoms or electrons and to capture the free radicals [20]. DPPH assay is one of the most common and effective tests used to demonstrate the ability of the components of the extracts to act as donors of hydrogen atoms or radical scavengers [21,22].

Figure 1 and table 3 show the result of DPPH scavenging activity of extract/fraction of *V. oocephala*.

Figure 1: % Scavenging activity of *Vernonia oocephala.*

From figure 1, the ethyl acetate fraction of *V. oocephala* has the highest percentage antioxidant activity at all concentrations except at concentration 1000 µg/ml where ethanol crude extract showed higher activity. The lowest activity was observed in chloroform fraction at all concentration except at concentration 125 and 250 µg/ml where crude ethanol extract showed lowest activity. This may be due to weak polarity of chloroform solvent. The results of this study revealed that there is no significant difference between

the activity of the extract/fractions and Gallic acid standard. However, it is worthy of mentioning that the ethyl acetate fraction was more effective than the standard at 125 and 250 µg/ml. The result demonstrated that *V. oocephala* has great potential to donate hydrogen to terminate free radical reaction. The activity of extract and fractions is in the order: EAF > BUF > EE > CHF. This is supported by the result of EC_{50} as shown in table 2. The higher the EC_{50} the lower is the scavenging activity of the extract/fractions. From the table, the EC_{50} is in the order of: Gallic acid < EAF < BUF < EE < CHF.

Extract /fractions/standard*	EC_{50} (μgml^{-1}) ± SD
Crude ethanol	34.0 ± 0.022
Chloroform	45.0 ± 0.024
Ethyl acetate	20.0 ± 0.005
n- butanol	42.0 ± 0.014
* Gallic acid	18.0 ± 0.001

Table 2: Effective concentration (EC_{50}) of V. oocephala.
The values are mean of determined ± standard deviation.
$EC_{50:}$ Effective concentration sufficient to obtain 50% maximum effect.

Total phenolic content of *V. oocephala*

Table 3 shows the result for total phenolic content of the extract/fractions.

Samples	Total phenolic content (mg/g GAE)
Ethanol crude extract	27.6 ± 0.002
Ethyl acetate fraction	106.0 ± 0.003
Chloroform fraction	36.0 ± 0.005
n- butanol fraction	61.0 ± 0.002

Table 3: Total phenolic content of V. oocephala.
The values are mean of determined ± standard deviation.
GAE: Gallic Acid Equivalent.

From the table, it can be observed that the total phenolic content varied in the crude and fractions of the sample. The ethyl acetate fraction showed highest content (106.0 ± 0.003 mg/g GAE) and the crude extract was lower (27.6 ± 0.002 mg/g GAE). This is in agreement with highest percentage antioxidant activity observed in ethyl acetate fraction. The result of the analysis shows that there is a relationship between the phenol content of medicinal plants and antioxidant activity.

Reducing power assay

Table 4 shows the result of reducing power assay of extract and fractions.

Conc (µg/ml)	EE	BUF	EAF	CHF	Gallic acid
31.25	0.008 ± 0.002	0.063 ± 0.003	0.022 ± 0.003	0.062 ± 0.002	0.040 ± 0.002
62.5	0.011 ± 0.002	0.080 ± 0.002	0.040 ± 0.003	0.072 ± 0.002	0.047 ± 0.003
125	0.017 ± 0.002	0.088 ± 0.004	0.064 ± 0.001	0.077 ± 0.007	0.104 ± 0.009
250	0.035 ± 0.005	0.120 ± 0.008	0.069 ± 0.004	0.134 ± 0.006	0.418 ± 0.026
500	0.068 ± 0.009	0.172 ± 0.002	0.073 ± 0.003	0.224 ± 0.004	2.636 ± 0.160
1000	0.131 ± 0.006	0.269 ± 0.013	0.156 ± 0.007	0.458 ± 0.009	3.133 ± 0.015

Table 4: Reducing power of V. oocephala.
The values are mean of determined ± standard deviation.

From table 4, the reducing power assay treats the antioxidants contained in the samples as reductants in a redox-linked reaction and the value reflects the reducing power of the antioxidant. The reducing power of *V. oocephala* was higher at 1000 µg/ml (0.458 ± 0.009 nm) and lower at 31.25 µg/ml (0.008 ± 0.002 nm) for chloroform and ethanol fraction respectively (Table 4). This result shows that chloroform fraction possesses most reductive potential compared to other fractions. Though, the trend observed here is quite different from radical scavenging activity in which ethyl acetate fraction possessed the highest activity. The reductive potential of the extract/fractions is as follow: CHF > BUF > EAF > EE.

Total antioxidant capacity of *V. oocephala*

Table 5 shows the result of antioxidant capacity of the extract/fractions.

The total antioxidant capacity was due to the effective reduction of Mo (VI) to Mo (V) complex by the antioxidant compound and was expressed in ascorbic acid equivalent. The ethyl acetate fraction of *V. oocephala* was highest (100 mg/g) and the ethanol crude

Sample	Equivalent to ascorbic (mg/g)
Ethanol crude	62.0
n-butanol fraction	96.0
Chloroform fraction	80.0
Ethyl acetate fraction	100.0

Table 5: Shows the result of antioxidant capacity of the extract/fractions.

was lower (62.0 mg/g). The activity is in the order: EAF > BUF > CHF > EE. The highest total antioxidant capacity exhibited by ethyl acetate fraction is in agreement with high percentage antioxidant and total phenolic content observed in the fraction. This inferred that apart from phenolic components, the fraction also contains some non-phenolic antioxidant compounds with the potential of scavenging free radicals [23]. This is in conformity with the phytochemical analysis carried out by Aliyu., *et al* [14].

Conclusion

The research studied exhaustively the antioxidant potential of plant *Vernonia oocephala*. The results suggest that the crude ethanol extracts and solvent fractions from the species possessed considerable antioxidant activities as demonstrated by DPPH free radical, total phenolic content, reducing power assay and total antioxidant capacity. It was observed that free radical scavenging activity of the species is a function of concentration, that is, the higher the concentration used, the higher is the activity. The EC_{50} of the test samples compared favorably with that of a standard Gallic acid, thus implying that both plant crude extracts and solvent fractions contained compounds with strong radical scavenging and antiradical generating effects. Therefore, they may have great relevance in the prevention and therapies of diseases related to free radical reactions.

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