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Antioxidant activity of *Eucalyptus globulus* Labill root extracts

Basil Nse Ita**Abstract**

E. globulus is used in folk medicine to treat various diseases. This study investigated the antioxidant activity of ethanol (EtE), ethylacetate (EaE) and aqueous (AqE) root extracts of *E. globulus* by measuring its DPPH, ABTS radical scavenging, H₂O₂ scavenging, reducing power and metal chelating activities. EtE showed the highest DPPH (IC₅₀ = 15.80 μg/mL), ABTS (IC₅₀ = 41.87 μg/mL) and reducing power (IC₅₀ = 8.41 μg/mL). AqE demonstrated the best H₂O₂ activity (IC₅₀ = 18.10 μg/mL), while EaE exhibited the best metal chelating ability (IC₅₀ = 13.52 μg/mL). Total phenolics and flavonoids were highest in EtE. These results indicate that *E. globulus* roots can be used as an alternative source of natural antioxidants with the potential to reduce oxidative stress related diseases.

Keywords: *E. globulus* root extracts, antioxidant assay, total phenolics, flavonoids

Introduction

Reactive oxygen species (ROS) such as hydroxyl, peroxy and superoxide radicals are produced continuously during normal physiological processes in biological systems. Oxidative stress arises when there is an imbalance between the production of these ROS and the ability of the body to scavenge them. These species cause damage to biological molecules such as lipids, protein and nucleic acids and have been implicated in the pathogenesis of various diseases including cancer, diabetes, cardiovascular diseases, neurodegenerative diseases and aging [1-3]. One way of tackling this challenge is by the use of exogenous antioxidants that can effectively scavenge these ROS. Synthetic analogues such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) have been implicated to be toxic or mutagenic [4], therefore the search for plant - derived antioxidants becomes an effective therapeutic strategy to combat diseases associated with oxidative stress [5].

Eucalyptus globulus belongs to the family Myrtaceae. This plant is native to Australia and Tasmania and have been successfully cultivated in many regions, including Nigeria. The plant is a good source of timber, fuel, paper pulp, essential oils and is rich in phytochemicals such as flavonoids, alkaloids, tannins and propanoids [6]. In folk medicine, the plant is used to treat asthma, respiratory infections, fungal infections, diabetes and as herbal tea [5-7]. Different biological activities have been reported for this plant. The essential oil from leaves of *E. globulus* is used to treat diabetes, pulmonary tuberculosis and asthma. Also, antibacterial, antioxidant, anti-inflammatory, anthelmintic and anticancer activities of oils from its leaves, fruits, buds and barks have been documented [5, 8-9]. However, there are limited outcomes on the plant roots. Therefore, the aim of this research was to evaluate the antioxidant activity of *E. globulus* root extracts using different systems and their correlation with contents of total phenolics and flavonoids in the extracts.

Materials and Methods**Plant Sample**

Roots of *E. globulus* were obtained from a forest reserve in Akwa Ibom State, and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Nigeria. The roots were washed, shade dried and pulverized. 200g of this powder was macerated in 1.5L ethylacetate, ethanol and water separately for 24 hrs at room temperature. The filtrates were concentrated in a rotary evaporator, while the aqueous extract was freeze dried to obtain the crude ethylacetate (EaE), ethanol (EtE) and aqueous (AqE) extracts respectively.

Chemicals

1,1- diphenyl-2-picryl hydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), disodium salt of 3-(2-pyridil)-5,6-bis (4- phenylsulfonic acid)-1,2,4-triazine

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(ferrozine), Folin Ciocalteu reagent, gallic acid, quercetin, butylated hydroxyanisole (BHA), trichloroacetic acid (TCA) were purchased from Sigma – Aldrich. All other reagents were of analytical grade.

Evaluation of Antioxidant activity

The antioxidant activity of *E. globulus* root extracts was evaluated by several models. The DPPH, ABTS radical scavenging activity, H₂O₂ scavenging activity, reducing power and metal chelating activity were tested.

Evaluation of DPPH activity

Precisely, 1mL of each extract at varying concentrations was mixed with 1mL of 0.004% methanol solution of DPPH. The mixture was shaken vigorously and allowed to stand for 30 min at room temperature in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 517nm. The procedure was repeated for the blank and control. The radical scavenging activity was calculated using the equation:

DPPH scavenging effect (%) = $[(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100$. Sample concentration providing fifty percent inhibition (IC₅₀) was calculated from the graph plotting inhibition percentage against extract concentration. BHA was used as standard [10].

Evaluation of ABTS activity

ABTS⁺ was produced by reacting 7mM ABTS solution (absorbance = 0.7 + 0.02 at 734nm) with 2.45 mM potassium persulfate and the mixture allowed to stand at room temperature for 12h in the dark. 2.94 mL of ABTS solution was mixed with 60μL of each extract and incubated at 37°C for 20 min in the dark. After incubation, the absorption was read at 734nm. The percentage inhibition was calculated using the equation:

$$\% \text{ inhibition} = [A_{\text{blank}} - A_{\text{sample}}]/A_{\text{blank}} \times 100$$

Sample concentration providing fifty percent inhibition (IC₅₀) was calculated from the graph plotting inhibition percentage against extract concentration. BHA was used as standard [11].

Evaluation of hydrogen peroxide scavenging activity

Extracts of varying concentration (1mL) was mixed with 400μL of H₂O₂ (5mM) in phosphate buffer (pH 7.4; 100mM) and incubated for 20 min, after which the absorbance was read at 230nm against a blank. The H₂O₂ inhibition (%) was determined using the equation:

$$\text{H}_2\text{O}_2 \text{ scavenging activity (\%)} = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100.$$

Vitamin C was used as positive control [12].

Evaluation of reducing power

The reducing power of the root extracts was determined according to the method of Oyiazu (1986) [13]. Each extract (10 -100μg/mL) in ethanol (2.5mL) was mixed with 2.5mL of 200mM sodium phosphate buffer (pH 6.6) and 2.5mL of 1% potassium ferricyanide and the mixture incubated at 50°C for 20 minutes. Thereafter, 2.5 mL of 10% trichloroacetic acid (w/v) was added and the mixture centrifuged at 200g for 19 minutes. The upper layer (5mL) was mixed with 5mL of deionised water and 1mL of 0.1% ferric chloride and the absorbance measured at 700nm against a blank. A higher absorbance indicated a higher reducing power. IC₅₀ value

(μg/mL) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation. Ascorbic acid was used as positive control.

Evaluation of metal chelating activity

Metal chelating activity was determined according to the modified method of Decker and Welch (1990) [14]. Briefly, 0.5mL of each extract at varying concentrations was mixed with 0.05mL of 2mMFeCl₂ and 0.1mL of 5mM ferrozine and the total volume made to 2mL with methanol. This mixture was shaken vigorously and left standing at room temperature for 10min. After the mixture had reached equilibrium, the absorbance of the solution was measured spectrophotometrically at 562nm. The percentage inhibition rate of ferrozine – Fe²⁺ complex formation was calculated using the formula:

Scavenging activity (%) = $[(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$ where A_{control} = absorbance of ferrozine – Fe²⁺ complex, and A_{sample} = absorbance of sample. EDTA was used as positive control.

Determination of total phenolic content

Total phenolic content was determined using the Folin-Ciocalteu reagent. Briefly, 10μL of each extract was taken and the volume made to 2mL with distilled water. 0.5mL of Folin-Ciocalteu reagent was added and the sample incubated for 3 min. This was followed by the addition of 2mL Na₂CO₃ (20% w/v), placed in boiling water for 1 min and allowed to cool to room temperature. The absorbance of this mixture was then read at 765nm, subtracting the absorbance of the control. Total phenolic content was expressed in mgGAE/g extract based on a standard calibration curve of gallic acid [15].

Determination of flavonoid content

Flavonoid content of the extracts was determined according to the method of Kumar *et al.* (2013) [16]. Briefly, each plant extract (10μL) was diluted with distilled water and the total volume made up to 2mL and kept at room temperature for 3 min. At the end of this period, 3mL of 5%NaNO₂ and 0.3mL of 10% AlCl₃ was added and incubated for a further 6 min. Then, 2mL of 1M NaOH was added and the final volume adjusted to 10mL with distilled water. The absorbance of this mixture was read at 510nm. Flavonoid content was expressed in mgQE/g extract based on a standard calibration curve prepared from quercetin.

Statistical analysis

Experiments were repeated in triplicate. The data were subjected to analysis of variance (ANOVA) using Statistical Package for the Social Science (SPSS version 20.0, IBM Corp, USA). with levels of significance maintained at 95% for each test.

Results and Discussion

Apart from being sources of food, plants are also rich sources of phytoconstituents such as phenolics, flavonoids, alkaloids and carotenoids which play vital role in the maintenance of good health, disease control and neutralizing toxic substances. These phytoconstituents have demonstrated biological activities in numerous *in vitro* and *in vivo* experiments [1-3, 5]. In this study, the antioxidant activity of *E. globulus* root extract was evaluated by measuring its DPPH, ABTS radical scavenging activities, H₂O₂ scavenging activity, metal chelating and reducing power. In the DPPH assay, the DPPH radical scavenging ability of the extracts increased in a dose -

dependent manner (Fig. 1a). At 10 $\mu\text{g/mL}$, EaE, EtE and AqE of *E. globulus* root scavenged 40.6, 43.8 and 42.9% of the DPPH radical respectively and this increased to 70.3, 79.2 and 75.3% respectively at 200 $\mu\text{g/mL}$. However, the scavenging abilities of EaE and AqE did not differ significantly. IC₅₀ values (Table 1) indicated the following order: EtE (IC₅₀ =

15.80 $\mu\text{g/mL}$) > AqE (IC₅₀ = 18.24 $\mu\text{g/mL}$) > EaE (IC₅₀ = 22.46 $\mu\text{g/mL}$). Abifarín *et al.* (2019) [17] found significantly higher IC₅₀ values of 0.927 and >1mg/mL respectively for acetone and aqueous root extracts of *C. africanus*. Also, Khan *et al.* (2013) [1] reported an IC₅₀ value of 41 $\mu\text{g/mL}$ for methanolic extract of *M. alba* root bark.

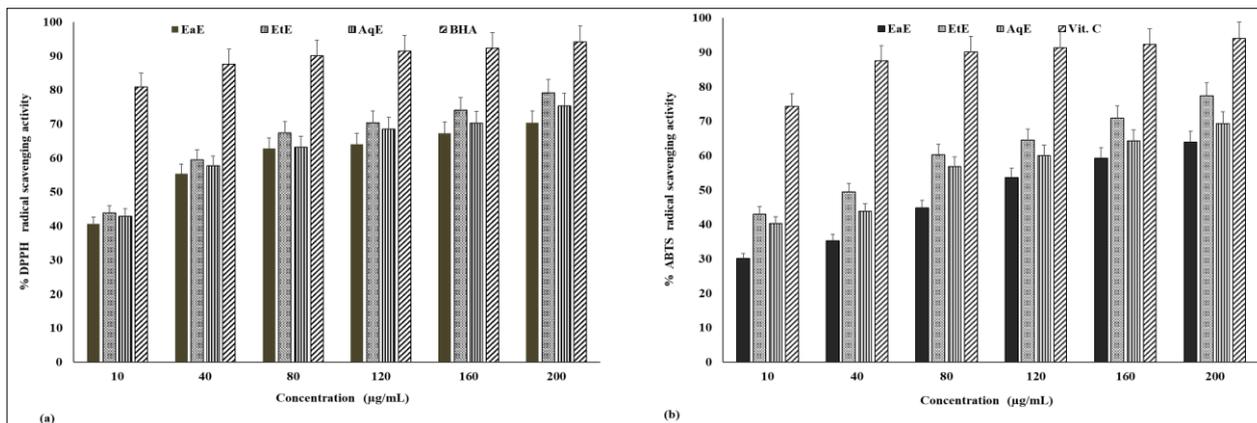


Fig 1: (a) DPPH and (b) ABTS radical scavenging activities of *E. globulus* root extracts

Similarly, Do *et al.* (2014) [18] reported higher IC₅₀ values of 679.67, 70.06 and 88.06 $\mu\text{g/mL}$ for water, ethanol and acetone root extracts respectively of *L. aromatica*, while Sembiring *et al.* (2018) [19] reported IC₅₀ value of 135.7 $\mu\text{g/mL}$ for 70% ethanolic extract of *C. bonduc* root extract. The result of our study demonstrates that root extracts of *E. globulus* are potent scavengers of DPPH radical by proton donation, with EtE being the most active. Differences in phytogeographic region,

plant matrix, solvent, pH and extraction conditions may be responsible for the observed variation in DPPH activity [19]. All tested extracts demonstrated ABTS radical scavenging ability in a concentration - dependent manner (Fig. 1b). EaE, EtE and AqE exhibited low scavenging activity (30.11, 43.02 and 40.26% respectively) at 10 $\mu\text{g/mL}$ and moderate to high scavenging activity (63.87 to 77.34%) at 200 $\mu\text{g/mL}$. The highest ABTS activity was demonstrated by EtE (IC₅₀ = 41.87 $\mu\text{g/mL}$). Our results

Table 1: Antioxidant activity, total phenolics and flavonoids of *E. globulus* root extracts

Parameter	<i>E. globulus</i>			Control		
	EaE	EtE	AqE	BHA	EDTA	Vit C
DPPH activity*	22.46	15.80	18.24	4.72	-	-
ABTS activity*	102.52	41.87	59.01	-	-	4.50
H ₂ O ₂ activity*	105.04	64.32	18.10	-	-	6.02
Reducing power*	105.66	8.41	15.16	-	-	5.02
Metal chelating activity*	13.52	26.44	60.05	-	4.02	-
Total Phenolics ^a	43.16	102.33	66.78	-	-	-
Flavonoids ^b	14.28	31.25	20.64	-	-	-

*IC₅₀ ($\mu\text{g/mL}$) is the effective concentration where ABTS, DPPH and H₂O₂ radical is scavenged by 50%, ferrous ion is chelated by 50% and the absorbance is 0.5 for reducing power. IC₅₀ was obtained using the regression equation. a in mgGAE/g, b in mgQE/g.

suggest that the extracts possessed good hydrogen donating abilities and correlates with ABTS radical scavenging ability reported for root extracts of *C. africanus* [17], while Ullah *et*

al. (2019) [20] reported lower values for various root extracts of *A. hypogaea*.

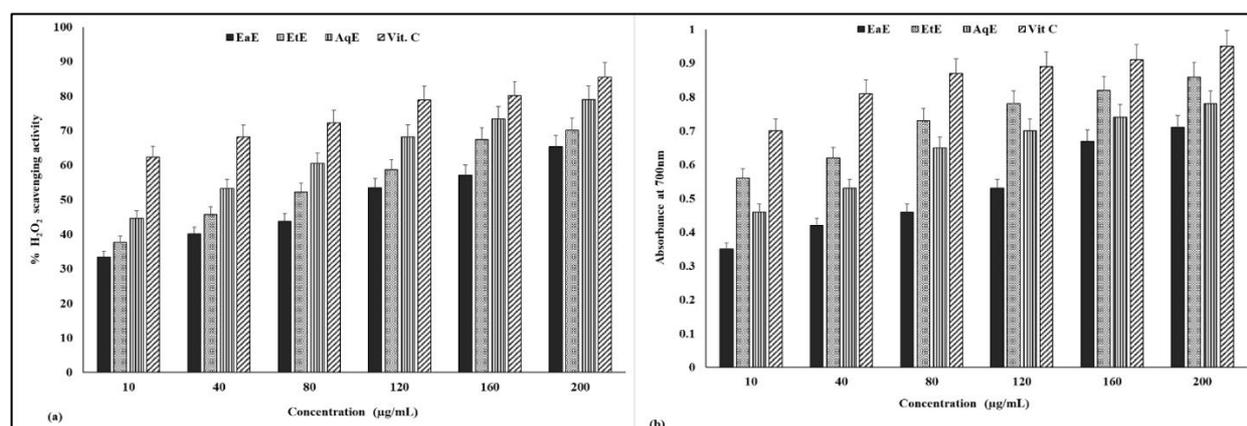


Fig 2: (a) H₂O₂ scavenging activity and (b) reducing power of *E. globulus* root extracts

Fig. 2a depicts the H₂O₂ scavenging activity of the extracts. This test is important because H₂O₂ can cross membranes and oxidize compounds. It may also generate hydroxyl radicals, therefore the removal of H₂O₂ in cells or food systems becomes imperative [21]. Like the DPPH and ABTS radical scavenging activities, H₂O₂ scavenging activity also increased in a dose-dependent manner. At 10 µg/mL, EaE, EtE and AqE scavenged 33.45, 37.66 and 44.59% of H₂O₂ respectively, and this increased to 65.37, 70.12 and 79.01% respectively at 200 µg/mL. AqE showed the strongest ability to scavenge H₂O₂ (IC₅₀ = 18.10µg/mL) and this differed significantly ($p < 0.05$) with EaE and EtE (Table 1). However, the observed activity of the extracts was lower than Vitamin C (IC₅₀ = 6.02µg/mL). The reducing power of *E. globulus* root extracts which also serves as a measure of antioxidant activity is presented in Fig.2b. The extracts demonstrated electron - donating ability in a dose - dependent manner (Fig. 2b). The absorbance ranged from 0.35 to 0.71 for EaE, 0.56 to 0.85 for EtE and 0.46 to 0.78 for AqE, with EtE having the best reducing ability at all concentrations. A higher absorbance indicates a higher reducing power. The lowest reducing ability was observed in EaE with an IC₅₀ value of 105.66µg/mL. Generally, our result suggests that root extracts of *E. globulus* exhibited promising reducing power, with EtE being the most potent (IC₅₀ = 8.41µg/mL), and this was close to the reducing power of Vitamin C (IC₅₀ = 5.02µg/mL). In comparison with other works, similar reducing power have been reported for *L. aromatica* extracts [19].

The metal chelating activity of the extracts (Fig. 3) was also evaluated. This test is important because antioxidant activity is also measured by the ability of extracts to chelate transition metals such as iron, which catalyze hydroperoxide decomposition and Fenton type reactions producing hydroxyl radicals that could contribute to oxidative stress [22-24].

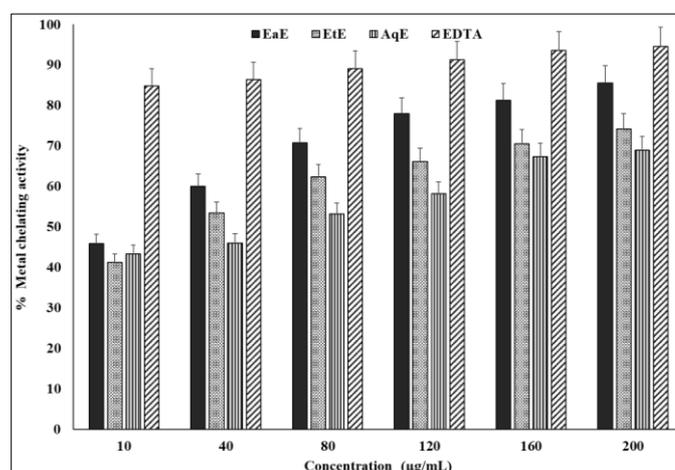


Fig 3: Metal chelating activity of *E. globulus* root extracts

In this study, the extracts demonstrated potent metal chelating ability, and this increased with increasing extract concentration. EaE had the highest metal chelating ability at all concentrations, with an IC₅₀ value of 13.52µg/mL (Table 1), while AqE demonstrated the lowest metal chelating activity with an IC₅₀ value of 60.05µg/mL. The result obtained from this study indicate that the extracts are able to chelate transition metals that may cause oxidative damage by either catalyzing Fenton reactions or hydroperoxide decomposition reactions. The number of hydroxyl group and the phenolic structure is reported to enhance the metal chelating and scavenging activity of extracts [25].

Total phenolic and flavonoid contents of the extracts (Table 1) varied with solvent. EtE had the highest total phenolic content (102.33mgGAE/g) which was significantly higher than AqE (66.78mgGAE/g) and EaE (43.16mgGAE/g). Similarly, flavonoid was highest in EtE (31.25mgQE/g) and lowest in EaE (14.28mgQE/g). Lower phenolic and flavonoid contents were reported for root extracts of *A. hypogaea* [20].

Result obtained from the antioxidant assays, total phenolics and flavonoid contents of *E. globulus* extracts were compared and correlated with each other (Table 2). Total phenolics showed good relationship with DPPH ($R^2 = 0.9294$), ABTS ($R^2 = 0.8747$) and reducing power ($R^2 = 0.7021$). Flavonoids showed good relationship with DPPH ($R^2 = 0.9155$), ABTS ($R^2 = 0.8569$) and total

Table 2: Correlation between antioxidant assays, total phenolic and flavonoids*.

	DPPH	ABTS	H ₂ O ₂	MC	RP	TP	FL
DPPH	1						
ABTS	0.9914	1					
H ₂ O ₂	0.3568	0.4477	1				
MC	0.1703	0.2454	0.9546	1			
RP	0.9079	0.9543	0.6602	0.4484	1		
TP	0.9294	0.8747	0.1316	0.0243	0.7021	1	
FL	0.9155	0.8569	0.1045	0.0169	0.6781	0.9993	1

phenolics ($R^2 = 0.9993$), suggesting that content of total phenolics and flavonoids in the extracts were responsible for the observed DPPH and ABTS activities. This is in agreement with reports in literature [23, 25]. However, total phenolics showed poor relationship with H₂O₂ ($R^2 = 0.1316$) and metal chelating activity ($R^2 = 0.0243$). Similarly, poor relationship existed between flavonoids with H₂O₂ ($R^2 = 0.1045$) and metal chelating activity ($R^2 = 0.0169$), suggesting that other compounds such as carotenoids and tocopherols may also contribute to the observed antioxidant activity [23].

Conclusion

E. globulus root may be regarded as a rich source of phenolics and flavonoids. Antioxidant assays indicated that the extracts exhibited a broad spectrum of activities and could effectively scavenge free radicals, chelate metals and had good reducing power, particularly the ethanol extract. These results suggest that *E. globulus* root may serve as a new source of natural antioxidants with the potential to safeguard against oxidative stress.

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