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Antioxidant Activity of *Senna alata* Root Extracts

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ABSTRACT

Senna alata is used traditionally to treat various ailments. The antioxidant potential of acetone, ethanol and aqueous root extracts of *Senna alata* was studied by measuring its DPPH and ABTS radical scavenging abilities, its ferric reducing power and metal chelating activity as well as its polyphenolics content. The ethanol extract had high content of total phenolics and flavonoids with values of 78.21 mgGAE/g and 39.29 mgQE/g and exhibited the best antioxidant activity in the DPPH and ABTS assays (IC_{50} = 45.18 and 39.14 µg/mL respectively). In addition, the aqueous extract had more potent metal chelating and reducing power than the other extracts. These results suggest that *Senna alata* root could serve as new sources of antioxidants that can help prevent oxidative stress.

1. Introduction

Medicinal plants are used in many cultures around the world for the treatment of various ailments and diseases and are still important sources of health care, particularly amongst the low income populace. This is because they are natural sources of phyto-therapeutic constituents and also act as leads for synthesis of more potent drugs. Numerous diseases are associated with oxidative stress, arising from the elevated levels of reactive oxygen species (ROS) within the organism. These free radicals can disturb normal body processes by damaging lipids, proteins and DNA, and have been implicated in an array of diseases including AIDS, cancer, malaria, diabetes, neurodegenerative diseases, etc. Therefore, the search for new phyto constituents that can reduce oxidative stress becomes imperative.

In Nigeria, *Senna alata* (synonym *Cassia alata*) is used as a medicinal plant. Traditionally, the plant organs are used to remedy fungal infections, sickle cell anaemia, skin infections, diabetes mellitus, malaria and as a purgative amongst others[1-3]. Biological activities such as antimicrobial, anti-inflammatory, analgesic, antitumour, antioxidant and immune stimulating activities have been reported for its leaf, root-bark, flower and seed extracts. Also, secondary metabolites, including ellagitannin, n-hexadecanoic acid, naphthalene, phenolic acids, purine, xanthone have been identified in this plant [4-5]. However, in spite of its folkloric importance, few reports exist on the antioxidant potential of this plant root. This study therefore evaluates the antioxidant activity of various root extracts of *Senna alata* in different *in-vitro* models.

2. Experimental Methods

2.1 Plant Material

Roots of *S. alata* were obtained from a forest reserve area in Akwa Ibom State, Nigeria, washed, and cut into pieces. The roots were shade dried, and pulverized with a blender. 1000 g of this powder was macerated in 2.5 L acetone, ethanol and water separately for 24 hrs at room temperature. The filtrate was concentrated *in vacuo*, while the aqueous extract was freeze-dried to obtain the crude acetone (AcE), ethanol (EtE) and aqueous (AqE) extracts respectively.

2.2 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 (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.

2.3 Determination of Total Phenolic Content

Total phenolic content was determined using the Folin-Ciocalteu reagent. Briefly, 10 µL of each extract was taken and the volume made to 2 mL with distilled water. 0.5 mL of Folin-Ciocalteu reagent was added and the sample incubated for 3 min. This was followed by the addition of 2 mL Na_2CO_3 (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 765 nm. Total phenolic content was expressed in mgGAE/g extract based on a standard calibration curve of gallic acid [6].

2.4 Determination of Flavonoid Content

Flavonoid content of the extracts was determined according to the method of Kumar et al [7]. Briefly, each extract (10 µL) was diluted with distilled water to a total volume of 2 mL and kept at room temperature for 3 min. At the end of this period, 3 mL of 5% $NaNO_2$ and 0.3 mL of 10% $AlCl_3$ was added and incubated for a further 6 min. Then, 2 mL of 1 M NaOH was added and the final volume adjusted to 10 mL with distilled water. The absorbance of this mixture was read 510 nm. Flavonoid content was calculated using a standard calibration curve prepared from quercetin.

2.5 Evaluation of DPPH Activity

Precisely, 1 mL of each extract at varying concentration was mixed with1mL 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 517 nm. The procedure was repeated for the blank and control. The radical scavenging activity was calculated using the equation:

$$\text{DPPH scavenging effect (\%)} = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

Sample concentration providing fifty percent inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. BHA was used as a standard [8].

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2.6 Evaluation of ABTS Activity

$ABTS^{+}$ was produced by reacting 7 mM ABTS solution (absorbance = 0.7± 0.02 at 734 nm) 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 60 µL 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:

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

Sample concentration providing fifty percent inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. BHA was used as a standard [9].

2.7 Evaluation of Reducing Power

The reducing power of each extract was determined according to the method of Oyiazu [10]. Extract concentration (10-150 µg/mL) in ethanol (2.5 mL) was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL 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 200 g for 19 minutes. The upper layer (5 mL) was mixed with 5 mL of deionised water and 1 mL of 0.1% ferric chloride and the absorbance measured at 700 nm against a blank. A higher absorbance indicated a higher reducing power. IC_{50} 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.

2.8 Evaluation of Metal Chelating Activity

Metal chelating activity was determined according to the modified method of Decker and Welch [11]. Briefly, 0.5 mL of each extract at varying concentration was mixed with 0.05 mL of 2 m $MFeCl_2$ and 0.1 mL of 5 mM ferrozine and the total volume made to 2 mL 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 562 nm. The percentage inhibition rate of ferrozine – Fe^{2+} complex formation was calculated using the formula:

$$\text{Scavenging activity (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where $A_{control}$ = absorbance of ferrozine – Fe^{2+} complex, and A_{sample} = absorbance of sample. EDTA was used as positive control.

2.9 Statistical Analysis

All experiments were performed in triplicate. Microsoft Excel was used for all statistical analysis.

3. Results and Discussion

Evaluation of contents of phytoconstituents, which are known to possess antioxidant activity in roots of *Senna alata* showed that the plant is rich in phenolics and flavonoids; however these varied with the extracting solvent. Generally, total phenolics and flavonoids was higher in the ethanol extract (78.21 mgGAE/g and 39.29 mgQE/g respectively) than the aqueous and acetone extracts. Observed trend was ethanol extract > aqueous extract > acetone extract (Table 1). Our values are higher than reports for roots of *Rheum ribes* and *Hypochaeris radicata* [12, 13]. The high levels of polyphenolics in *Senna alata* roots confers on it useful biological properties. Phenolic compounds are potent antioxidants that can scavenge free radicals, chelate transition metals, act as hydrogen donors as well as prevent peroxide formation [14], and may therefore provide protection against diseases associated with elevated levels of free radicals in the body.

The DPPH assay is widely used to evaluate the antioxidant activity of plant extracts which can act as free radical scavengers or hydrogen donors. Extracts of *Senna alata* roots showed potent DPPH radical scavenging ability in a concentration - dependent manner. At 10 µg/mL, the acetone, ethanol and aqueous extracts scavenged 23.1%, 45.2% and 35.1% of the DPPH radical respectively, while at 150 µg/mL, 69.1%, 89.6% and 78.5% of the stable radical was scavenged (Fig. 1). Overall, the ethanol extract exhibited higher scavenging ability (IC_{50} = 45.18 µg/mL) than the other extracts. In comparison with other works, Benhammou et al [15] reported a lower EC_{50} value of 0.57 mg/mL for methanolic root extract of *Anabasis articulata*, while Chidambaram et al [16] reported a higher IC_{50} value of 78 µg/mL for *Codariocalyx motorius* root extract.

Table 1 Total phenolics, flavonoid, antioxidant, reducing power and metal chelating activity of *Senna alata* root extracts

	Extracts			Controls		
	AcE	EtE	AqE	BHA	EDTA	Vit. C
Total phenolics (mgGAE/g)	21.42	78.21	46.3	-	-	-
Flavonoids (mgQE/g)	9.65	39.29	26.17	-	-	-
DPPH activity *	82.42	45.18	61.15	1.21	-	-
ABTS activity *	64.93	39.14	48.37	2.86	-	-
Reducing power*	73.08	43.45	41.68	-	-	4.20
Metal chelating activity*	86.08	86.28	74.23	-	0.60	-

*IC_{50} value in µg/mL is the effective concentration where DPPH and ABTS radical is scavenged by 50%, ferrous ion is chelated by 50% and the absorbance is 0.5 for reducing power. IC_{50} was obtained using the regression equation

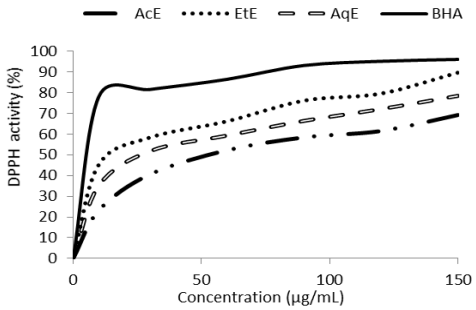

Fig. 1 DPPH radical scavenging activity of *Senna alata* roots

Similar results were obtained for the ABTS radical cation scavenging activity, with a range from 28.5% to 93.2%. The ethanol extract exhibited the highest ABTS radical cation scavenging ability amongst all the extract and its activity at 150 µg/mL, (93.2%) was close to BHA (96.1%) (Fig. 2). Observed order was ethanol extract > aqueous extract > acetone extract. Kumar et al [7] reported higher ABTS activity for extracts of *Eulophia nuda*. Our result indicate the ability of *Senna alata* root extracts to quench proton radicals, which is also an important indicator of antioxidant activity.

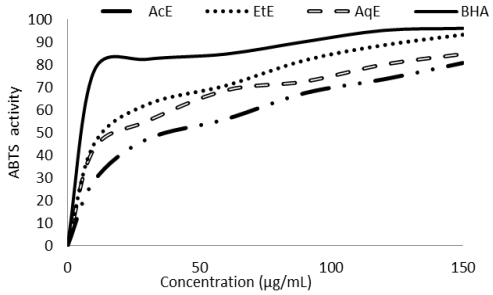

Fig. 2 ABTS scavenging activity of *Senna alata* extracts

Fig. 3 shows the reducing power of *Senna alata* root extracts. This test is important as it depicts the ability of extracts to break the free radical chain by hydrogen donation [7]. From this study, *Senna alata* root extracts exhibited promising reducing power. Like the antioxidant assay, this increased with increasing concentration of the extracts, with the aqueous extract being the most potent. Observed tend was aqueous extract > ethanol extract > acetone extract. Result obtained from this study show that *Senna alata root* is rich in reductones such as polyphenolic compounds that are capable of electron donation.

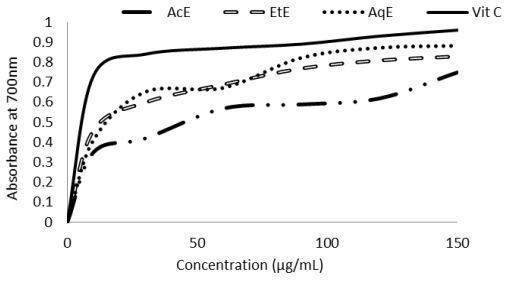

Fig. 3 Reducing power of Senna alata root extracts

Furthermore, the ability of the extracts to chelate transition metals such as iron was evaluated. This is important because iron is considered a pro-oxidant in lipid oxidation as it accelerates the oxidation of lipids by

breaking down hydrogen and lipid peroxides to free radicals through the Fenton process [12]. As shown in Fig. 4, *Senna alata* root extracts exhibited potent metal chelating potentials in a dose dependent manner, with the aqueous extract being the most active (IC_{50} = 74.23 µg/mL). However, its activity was inferior to EDTA (IC_{50} = 0.60 µg/mL).

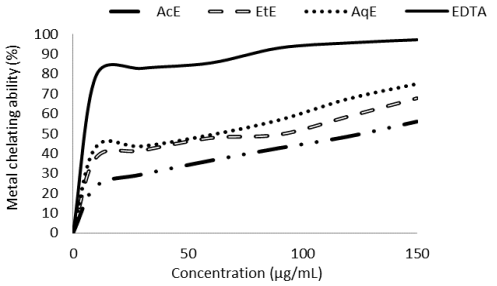

Fig. 4 Metal chelating activity of *Senna alata* root extracts

Vijayatajan and Rajasekara [17] reported lower chelating ability of 3.67 and 7.86 mg/g for methanol and acetone root extracts of *Moringa concanensis*. Also Jamuna et al [13] reported lower chelating activity for methanol root extracts of *Hypochaeris radicata*, while higher chelating ability was reported for *Rheum ribes* root extracts [12]. Our result indicate that root extracts of *Senna alata* can play pertinent role in preventing oxidative damage by chelating Fe^{2+} that may be involved in lipid peroxidation processes such as catalysing the Fenton type or similar decomposition reactions.

4. Conclusion

In the present investigation, *Senna alata* root extracts exhibited potent DPPH and ABTS scavenging abilities, could effectively chelate Fe^{2+} ions and had good reducing powers. In addition, the extracts were rich in phenols and flavonoids. Overall, the ethanol extract exhibited the highest antioxidant activity while the aqueous extract showed better reducing and metal chelating activities. Therefore, crude extract from roots of *Senna alata* could serve as promising sources of new antioxidants.

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