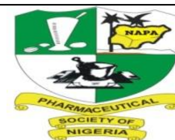
ORIGINAL PAPER



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A Comparison of the Antioxidant Activity with the Total Phenolic and Total Flavonoid contents of the Leaves and Stem-bark of *Anogeissus leiocarpa* (DC.) Guill& Pirr. (Combretaceae)

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: *Anogeissus leiocarpa* is used in traditional medical practice for the treatment of many diseases such as asthma, malaria, diabetic ulcers, dysentery, blood clots, cough, tuberculosis, parasitic infections and general body pain. Free radicals are known to play a major role in the aetiology of several these diseases. Thus, radical scavenging as well as antioxidant potentials of the plant are good indications of its effectiveness in disease management.

Objective: This study evaluates the antioxidant activity of the extracts from the leaves and stem bark of *Anogeissus leiocarpa..*

Materials and Methods: Dried powdered leaves and bark were extracted with methanol. The extracts were subjected to DPPH Free Radical Scavenging Activity, Ferric Reducing Antioxidant Power (FRAP), Total Antioxidant Capacity (TAC), ABTS Cation Decolourization, Cupric Reducing Antioxidant Capacity (CUPRAC), and Metal Chelating (MC) assays. The Total Phenolic Content (TPC), Total Flavonoid Content (TFC), total anthocyanidin Content (tAC), and proanthocyanidin Content (pAC) were also determined.

Results: The extract and ethyl acetate fraction of the stem bark had more antioxidant potential. The stem bark extract had an IC_{50} of 12.44$\pm$2.32 in the DPPH radical scavenging assay, the ferric reducing antioxidant power of 98.43$\pm$1.63 and ABTS radical decolourisation activity of 96.55$\pm$0.54 while the DPPH radical scavenging activity and the ABTS radical decolourisation effect for ascorbic acid and trolox were 39.25$\pm$ 1.11 and 379.24 $\pm$ 0.08 respectively. The Total Antioxidant Capacity as well as the Total Phenolic Content and Total Flavonoid Content was highest in the ethyl acetate fraction of the bark.

Conclusion: The extract of *A. leiocarpa* showed good antioxidant activity which compares well with the high phenolic content and thus have potential for use in the management of several diseases.

Keywords: *Anogeissus leiocarpa.* Antioxidant, Radical Scavenging, Total Phenolic, Total Flavonoid

INTRODUCTION

Reactive oxygen species (ROS) are made up of free radicals such as superoxide anions and hydroxyl radicals as well as non-free radicals like hydrogen peroxide and singled oxygen (Gulcin *et al.,* 2002b). They are produced by living organisms and react with several biological molecules (Senevirathne et al., 2006). Thus, free radicals have been implicated in the aetiology of several diseases including aging (Hyun *et al*., 2006), atherosclerosis (Upston *et al*., 2003), Alzheimer's disease (Smith *et al.,* 2000), alcohol induced liver disease (Arteel, 2003), cancer (Kinnula and Crapo, 2004), cardiovascular disease (Singh and Jialal, 2006), neural disorders (Sas *et al.,* 2007), mild cognitive impairment (Guidi *et al*., 2006), Parkinson's disease (Bolton *et al.,* 2000), and ulcerative colitis (Ramakrishna *et al*., 1997).

Antioxidants are known to regulate various naturally occurring oxidative reactions in the tissues. They are inhibitors of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body (Valko et al., 2007). According to literature, these are substances that when present in low concentration compared to those of the oxidizable substrates significantly delay or inhibit the oxidation of that substance (Murthy, 2001). The human body has a complex system of antioxidant defences both enzymatic and non-enzymatic which counteract the harmful effects of free radicals and other oxidants (Uttara et al., 2009). Protection against free radicals is achieved by high intake of dietary antioxidants (Young and Woodside 2001). Substantial evidence also indicates that foods containing antioxidants and possibly in particular the antioxidant nutrients may be of major importance in disease prevention (). It is however believed that a combination of antioxidants, rather than single entities, may be more effective in disease prevention (Alam et al., 2013). Benefits of antioxidants include improving the quality of life by preventing or postponing the onset of degenerative diseases (Coley et al., 2008) and savings substantial cost of health care delivery (Rosenfeld et al., 2012).

Plant phenolics, such as flavonoids, phenolic acids, tannins, lignans and lignin, which are found in leaves, flowering tissues, and woody parts, are known to have antioxidant activity mainly due to their redox properties, which allow them to act as reducing agents or hydrogen-atom donors(Amarowicz et al., 2004). Thus, they function as free-radical scavengers and chain breakers, complexers of pro-oxidant metal ions and quenchers of singlet-oxygen formation (Kessler *et al.,* 2003,) and helps in converting the radicals to less reactive species (Mandal et al., 2009). Antioxidants are very relevant because they prevent food rancidity as well as protect human body against damage by reactive oxygen species (ROS) (Loliger, 1991). Non enzymatic antioxidants are frequently added to the food to prevent lipid oxidation (Moure et al., 2001). However, many lipid antioxidants can have pro-oxidant effect on other molecule under certain circumstances (Atmaca 2004) thus, antioxidants for food and therapeutic purposes must be characterized carefully.

MATERIALS AND METHODS

Collection of plant material

Fresh plants materials were collected from the Botanical garden of the University of Ibadan, Nigeria. The plant was identified and authenticated by Mr Oba at Forest Herbarium Ibadan with FHI NO 109890. They were dried, powdered, extracted with 100% methanol and concentrated *in vacuo*. The extracts were stored appropriately until required.

Antioxidant Assays

DPPH Free Radical Scavenging Activity

The antioxidant activity for the extracts was assessed on the basis of their radical-scavenging effect of the stable 1,1- diphenyl-2-picrylhydrazyl (DPPH at 0.03mM in MeOH) free radical. (Brand-Williams *et al.,* 1995). The extracts were dissolved in MeOH and 150µL of different concentrations of extracts tested was incubated with 150 µL DPPH at room temperature in the dark for 30 min in a 96-well microliter plate. After incubation, decrease in absorption for each solution was measured at 517 nm using a micro plate reader. Ascorbic acid was used as reference. The percentage of the DPPH radical scavenging is calculated using the equation:

$$\% \text{ inhibition} = [1 - (A_1 / A_0) \times 100]$$

Where;

A_0 is the absorbance of the control and

A_1 is the absorbance of the extracts

A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50 % inhibition was determined and expressed as IC_{50} value.

Ferric Reducing Antioxidant Power (FRAP):

The assay was conducted according to the method developed by Benzie and Strain, (1999). 1mL of prepared FRAP reagent was mixed with 50µL of extracts. The absorbance at 593 nm was recorded after a 30 min incubation at 37 °C. FRAP values were obtained by comparing the absorption change in the test extracts with those obtained from a standard drug (Ascorbic acid) and expressed as mg of Ascorbic acid equivalents per g of extract.

ABTS Radical Cation Decolourization Assay

Antioxidant activity was measured as described by Ree *et al.* (1999). $ABTS^+$ radical cations were prepared by adding solid manganese dioxide (80

mg) to a 5 mM aqueous stock solution of $ABTS^+$ (20 mL using a 75 mM Na/K buffer of pH 7). Samples were diluted appropriately according to antioxidant activity in Na/K buffer pH 7. Diluted extracts were mixed with 2000µL of $ABTS^+$ radical cation solution in different test tubes, and absorbance was read at 734 nm after 6 min using spectrophotometer. Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid), a water-soluble analogue of vitamin E was used as an antioxidant standard. A standard calibration curve was constructed for Trolox at 1.56, 3.125, 6.25, 25, 50 and 100 µL concentrations. Trolox Equivalent Antioxidant Capacity (TEAC) values were calculated from the Trolox standard curve and percentage inhibition determined using the formula below:

$$I(\%) = [100 \times (A_0 - A_1)/A_0]$$

Where A_0 is the absorbance of the control, A_1 is the absorbance of the extract/standard, respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample required for % 50 inhibition was determined and expressed as IC_{50} value.

Cupric ion Reducing Antioxidant Capacity (CUPRAC) Assay

This was done according to Apak *et al*. (2008), 100µmL of 10mM of $CuSo_4$, 100µL of 7.5mM neocuproine and 100µL of buffer ($NaAc+(NH_4)_2SO_4$ solution were added into the wells of the micro plate. This procedure was repeated for freshly prepared Trolox of varying concentration (100µL, 80µL, 40µL, 20µL and10µL). The prepared solutions were mixed and incubated at room temperature for 30 min. The absorbance at 450 nm was determined using micro plate reader against the negative control (buffer solution +working reagent). The antioxidant capacity was calculated as mg of Trolox equivalents/g of extract (TEAC values)

Metal Chelating Activity Assay

The chelation of ferrous ions was estimated using the method of Viuda-Martos *et al.,* 2010. 100µL of the extract was added to a solution of 100µL ferrous sulphate (2 mM). The reaction is started by the addition of 100µL of Ferrozine (5 mM) and incubated in the dark at room temperature for 15 min. Absorbance was then measured at 560 nm. EDTA was used as a positive control.

Total Antioxidant Capacity Assay (TAC)

Total antioxidant capacity was measured according to the method reported by Prieto *et al.,* 1999 with slight modifications. 100µL of extract and 100 µL of ascorbic acid (as standard) were incubated in a test tube with 1mL (0.6 M sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate) in a thermal block at 95°C for 90 min. After incubation, samples were cooled to room temperature and absorbance was measured at 695 nm against a blank. A typical blank reagent solution used for the sample and it was incubated respectively at 1 mg/ml concentration under the same conditions as the rest of the samples. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid. All experiments were conducted in triplicate and values are expressed ascorbic acid equivalents in mg per g of extract.

Estimation of Total Phenolic Content (TPC)

Total Phenolic Content was determined using method of Gulcin *et al.,* (2004). 100µL of the extract was placed in a test tube (in triplicates), 900µL of distilled water and 200µL of Folin reagent respectively was added to the extract. The mixture was vortex and allowed to stand for 5min on the desk. Thereafter, 300µL of distilled water was added to the mixture and incubated at room temperature for 1hour 30min. Absorbance of the navy-blue colour formed was read at 750nm using visible spectrophotometer. Garlic acid was used as reference.

Estimation Total Flavonoid Contents (TFC)

Into the micro plate wells was introduced 150µL of the extract (in triplicates), 50µL of aluminium chloride and 100µL of sodium acetate buffer respectively were added to the each of the wells. Absorbance was taken at 412nm. (Milauskas *et al.,* 2004). The reference drug was Quercetin.

Estimation of Total anthocyanidin content

This was done according to method of Lee et al., (2005). 0.5mL of each of the tested samples, 2mL of buffers was added into a test tube (in triplicates), and incubated at room temperature for 30 minutes. Absorbance of test portion diluted with potassium chloride buffer pH 1.0 buffer and sodium acetate buffer pH 4.5 buffer was determined using visible spectrophotometer at both 520 and 700 nm.

Anthocyanin pigment concentration, expressed as cyanidin-3-glucoside equivalents was calculated as follows:

Anthocyanin pigment (cyanidin-3-glucoside equivalents, mg/L) =

$$\frac{A \times MW \times DF \times 10^3}{\varepsilon X 1}$$

Where A = (*A*520nm – *A* 700nm) pH 1.0 – (*A*520nm – *A*700nm) pH 4.5;

MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor established in DF; l = path length in cm; ε = 26 900 molar extinction coefficient, in L x mol^{-1} x cm^{-1}, for cyd-3-glu; and 10^3 = factor for conversion from g to mg

Estimation of pro anthocyanidin content

Proanthocyanin was determined by the method of Porter *et al.,* (1986). 0.25mL of extract and 2.25mL of buffer (1M HCl in Butanol) was added into a test tube. The mixture was incubated at 85°C for 90 minutes, at the end of incubation period the mixture was left on the desk to cool. Change in absorbance was determined against the control (which contain the extract plus buffer incubated at room temperature for 90 minutes also) at 550nm. The proanthocyanin content was reported as mg proanthocyanidin /mL using the formula:

$$[PA] = \frac{\Delta A \times Mw \times DF \times 10^3}{\varepsilon X 1}$$

Where ΔA = absorbance of the sample – absorbance of the control; Mw = molecular weight of Proanthocyanidin (287g/mol); DF = dilution factor established in DF; l = path length in cm ε = 17360 molar extinction coefficient, in mL x mol^{-1} x cm^{-1}, for proanthocyanidin.

RESULTS AND DISCUSSION

DPPH radical scavenging assay results

The dose dependent 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of the crude methanol, ethyl acetate and N-hexane fractions of both the leaves and the bark extracts of *A. leiocarpa* in this study is presented in Table 1. The scavenging activity is expressed as a percentage of the ratio of the decrease in absorbance of the test solution to that of DPPH solution and methanol (instead of the plant extract). The IC_{50} values (concentration of the extract that is able to scavenge half of the DPPH radical) are presented in Figure 1. All of the methanol and ethyl acetate fractions of the plant extracts gave scavenging activity above 50% at the concentration of 50µg/mL while N-hexane fractions gave less than 50% scavenging activity with the IC_{50} ranging from 12.44±2.32 to 173.61±2.47 µg/mL. The lowest IC_{50} value was obtained from the ethyl acetate fraction of the bark extract showing that this particular fraction has the highest scavenging activity. Ethyl acetate fraction of the leaves, crude methanol extract of both the leaves and bark extracts also gave lower IC_{50} values when compared with the N-hexane fractions of both extracts.

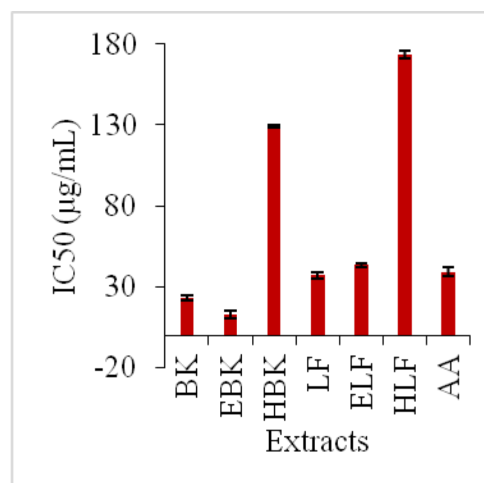

Figure 1: IC_{50} Values of the DPPH Free Radical Scavenging Activity

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Table 1: Percentage DPPH Free Radical Scavenging Activity (FRSA) of the methanol extracts and fractions of the leaf and stem bark of *A. leiocarpa* at different concentrations

Extracts	Percentage FRSA at different concentration (μg/mL)				
	200	100	50	25	12.5
BK	87.9 ± 0.54	87.63 ± 0.8	87.9 ± 0.65	56.42 ± 2.19	19.04 ± 5.37
EBK	81.6 ± 0.98	81.65 ± 0.97	82.95 ± 0.66	79.00 ± 0.22	50.16 ± 2.42
NBK	76.67 ± 0.24	41.27 ± 0.98	19.96 ± 0.31	5.11 ± 0.87	6.86 ± 0.81
LF	88.20 ± 0.26	88.52 ± 0.16	72.04 ± 8.25	27.51 ± 0.46	8.20 ± 0.35
ELF	76.71 ± 0.41	80.04 ± 0.31	57.07 ± 2.09	30.33 ± 1.30	12.92 ± 0.74
NLF	56.58 ± 3.61	28.98 ± 5.34	15.59 ± 2.63	4.10 ± 0.75	0.057 ± 1.62
AA	90.13 ± 0.12	89.86 ± 0.19	86.49 ± 1.62	46.33 ± 4.44	17.46 ± 4.77

AA: Ascorbic acid, BK: methanol extracts of the bark, EBK: Ethyl acetate fraction of the bark, NBK: N-hexane fraction of the bark, LF: methanol extract of the leaf, ELF: Ethyl acetate fraction of the leaf, NLF: N-hexane fraction of the leaf. Values are expressed as mean percentage activity ± SEM (Standard error of mean) n =3

Ferric reducing antioxidant power (FRAP) results

This method measures the ability of antioxidants to reduce ferric iron. It is based on the reduction of the complex of ferric iron and 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4- diene chloride (TPTZ) to the ferrous form at low pH. This reduction is monitored by measuring the change in absorption at 593 nm, using a spectrophotometer.

In this study, ferric reducing antioxidant power was determined by the reduction of the complex of ferric iron and 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4- diene chloride (TPTZ) to the ferrous formed at low pH. It is reported as mg Ascorbic acid equivalent/g by reference to standard curve (8.0575x + 0.2092, r^2 = 0.957). The FRAP values of the leaves ranged from 52.02± 1.40 to 98.31±0.17mgAAE/g while that of the bark ranged from 79.90±4.17 to 98.43±0.15mg AAE/g. Crude methanolic extract of the stem bark had the highest value of 98.43 followed by the ethylacetate (bark and leaves) fractions having the same values of 98.31. This indicates that both the leaves and the stem bark has high ferric reducing power.

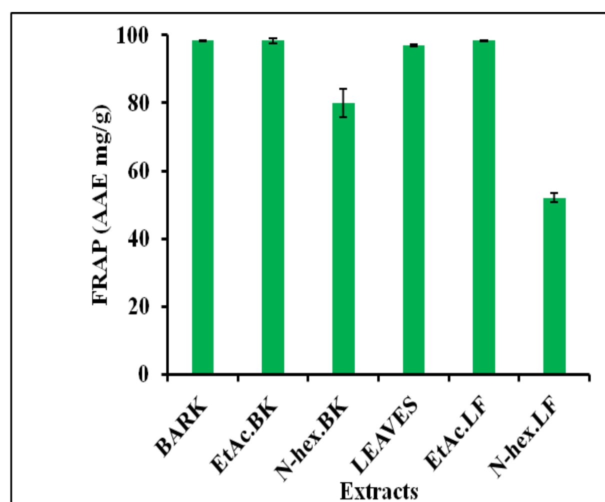


Figure 2: Ferric reducing Antioxidant power of the methanol extracts and fractions of the leaf and stem bark of *Anogeissus leiocarpa*

FRAP and DPPH antioxidant activity of the stem-bark has been previously reported by (Arbab, 2014).

ABTS radical decolourisation activity assay results

This method is used to measure the loss of colour when an antioxidant is added to the blue–green chromophore $ABTS^{\cdot +}$ (2, 2-azino-bis (3-ethylbenzthiazoline- 6-sulfonic acid)). The antioxidant reduces $ABTS^{+}$ to ABTS and decolorize it. A dose dependent $ABTS^{+}$ radical scavenging activity of the crude methanol and ethyl acetate fraction of the leaves and bark extract of *Anogeissus leiocarpa* was observed in this study. The decolourisation activity was expressed as a percentage of the ratio of the decrease in absorbance of the plant extracts to that of ABTS solution without the plant extracts. The IC_{50} ranged from 108.70±1.60 to 412.02±2.80 µg/mL. The lowest IC_{50} value was obtained for the crude methanol extract of the bark showing that it had highest percentage radical decolourization activity (RDA) of all the samples tested with a value of 96.55±0.54% at 250 µg/mL which is greater than the standard, Trolox which gave 36.00±3.25%. The ethyl acetate fraction of the leaves gave 33.74 ± 0.77% at 250 µg/mL.

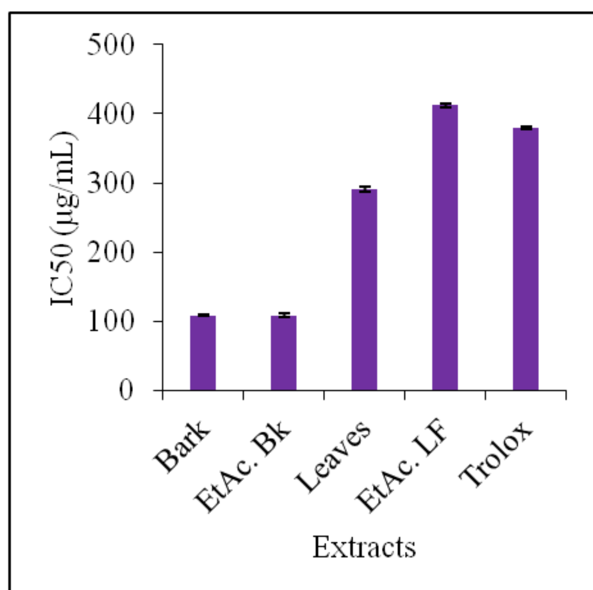
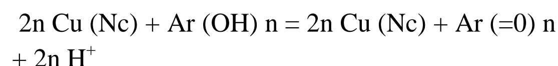


Figure 3: IC_{50} Values for the ABTS Radical Decolourization Activity

Cupric reducing antioxidant capacity (CUPRAC)

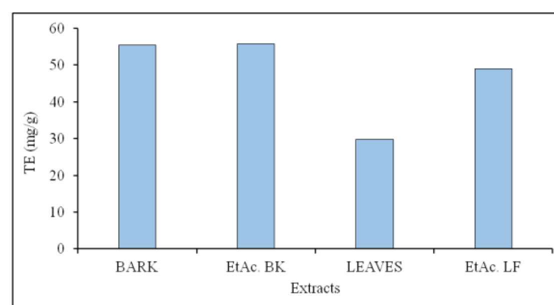
Bis (neocuproine) copper (II) chloride [Cu (II)-Nc] which is the chromogenic oxidizing reagent of the CUPRAC method, reacts with polyphenols [Ar (OH) n] in the manner:



2n Cu (Nc) + Ar (OH) n = 2n Cu (Nc) + Ar (=0) n + 2n H^{+}

The liberated protons may be buffered with the relatively concentrated ammonium acetate buffer solution. In this reaction, the reactive Ar-OH groups of polyphenols are oxidized to the corresponding quinones and Cu (II)-Nc is reduced to the highly colored Cu (I)-Nc chelate showing maximum absorption at 450 nm.

The cupric reducing antioxidant capacity as determined by Neocuproine method (REF), is reported as mg Trolox equivalent/g of the extract with reference to the standard curve ($y = 0.0418x + 0.4342$, $r^2 = 0.941$). The Cupric reducing antioxidant capacity varied in the different fractions tested. The cupric reducing antioxidant capacity of the leaves samples ranged from 29.70±0.016 to 49.0±0.04 mg TE/g of the extract while for the bark, it ranged from 55.3±0.00 to 55.8±0.00 mg TE/g of the extract with ethyl acetate fraction of the bark having the highest cupric reducing capacity. The N-hexane fractions of both the leaves and bark extracts tested negative for this particular assay. The bark of *A. leiocarpa* gave higher CUPRAC values when compared with the leaves fractions.



(b)

Figure 4: CUPRAC of the methanol extracts and fractions of the leaf and stem bark of *Anogeissus leiocarpa*

Metal chelating assay results

A complex with a red colour can be produced by Ferrozine by forming chelates with Fe^{2+}. This reaction is restricted in the presence of other chelating agents and results in a decrease of the red colour of the ferrozine-Fe^{2+} complexes. Measurement of the colour reduction determines the chelating activity to compete with Ferrozine for the ferrous ions (Soler-Rivas *et al.*, 2000). Dose

dependent metal chelating capacity of the crude methanol, ethyl acetate and N-hexane fractions of the stem bark and leaves extracts of *A. leiocarpa* was observed. The chelating activity was expressed as a percentage of the ratio of the decrease in absorbance of the test samples to that of the $FeSO_4$ (Iron II Sulphate) and Ferrozine solutions without the plant extracts. The IC_{50} values (Concentration of the plant extracts that can chelate half of the metal ions (Ferrozine)) are presented in Figure 3. Most of the tested extracts showed strong chelating activity above 50% even at very low concentrations with the IC_{50} values ranging from 1.34±1.86 to 133.99±1.66 µg/mL. The lowest IC_{50} value was obtained from the crude methanol extract of the bark, while the highest was obtained from the N-hexane fraction of the bark. Comparing chelating activity of the tested extracts with EDTA at 3.91µg/mL, the crude methanol extract of the bark showed relatively higher chelating activity than the standard drug having the value of 79.39±2.44% while EDTA had 78.4±3.62% and ethyl acetate fraction of the bark gave 50.99 ± 0.88%. The crude methanol and ethyl acetate fractions of the leaves at higher concentration also showed reasonable metal chelating activities giving 42.90±0.63% and 62.34±0.79% respectively at 100µg/mL. The crude methanol extract of the bark showed highest metal chelating activity.

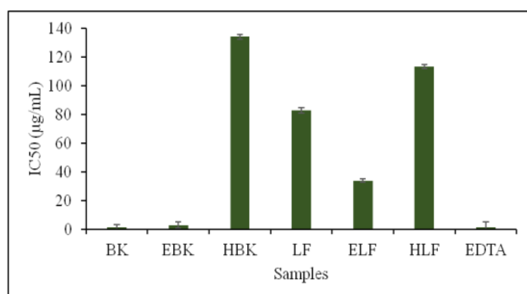


Figure 5: Metal chelating activity of *A. leiocarpa*

Total antioxidant capacity assay

Total antioxidant capacity (TAC) is reported as mg ascorbic acid equivalent/g of extract with reference to standard curve ($y = 0.0028x - 0.0028$, $r^2 = 0.984$). The Total antioxidant capacity varied in different fractions of both the leaves and bark extracts of *A. leiocarpa*. The TAC of the leaves of *A. leiocarpa* ranged from 227.99±4.63 to 379.86±9.97mg AAE/g while that of the bark ranged from 192.07±24.73 to 596.80±5.05 with the ethyl acetate fraction of the bark having the highest TAC

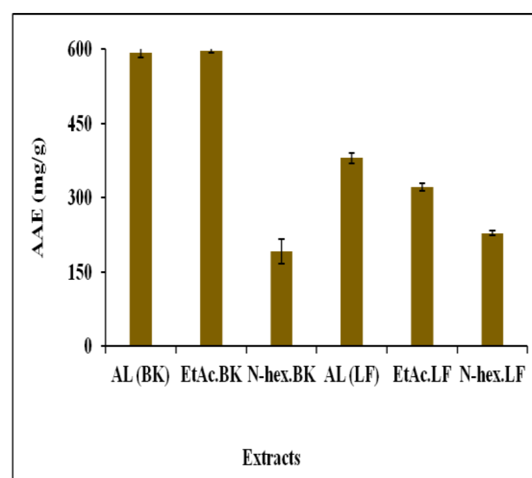


Figure 6: Total antioxidant capacity of *A. leiocarpa* leaf and stem bark extracts

Total phenolic content (TPC)

Total phenolic content (TPC) as determined by Folin Ciocalteu method, is reported as mg Gallic acid equivalent/g of the extract with reference to standard curve ($y = 0.0038x + 0.0156$, $r^2 = 0.965$). The total phenolic content varied in different fractions Figure 7. The TPC of the leaves ranged from 78.38±4.04 to 324.29±10.56mg GAE/g of extract while that of the bark ranged from 103.71±7.19 to 448.21mg GAE/g of extract. The bark of *A. leiocarpa* have higher phenolic contents than the leaves with Ethyl acetate fraction of the bark having the highest TPC followed by the crude methanol extract of the bark.

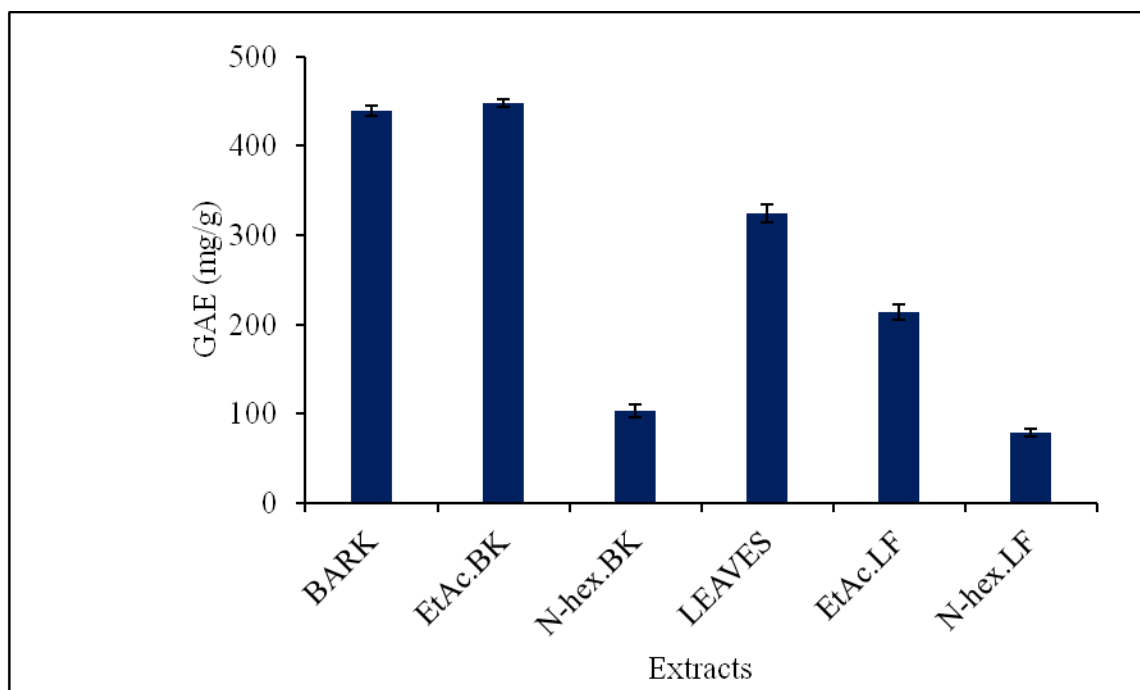


Figure 7: Total Phenolic content of *A. leiocarpa* leaf and stem bark extracts

Phenolic compounds undergo a complex redox reaction with phosphotungstic and phosphomolybdic acids present in the Folin-Ciocalteu reagent (Amin *et al.,* 2007) and phenolic compounds have received considerable attention because of their potential antioxident activities (Pham-Huy *et al*., 2008).

Total flavonoid content (TFC)

The total flavonoid content (TFC) is reported as mg Quercetin equivalent/g of extract with reference to standard curve ($y = 0.018x + 0.2801$, $r^2 = 0.945$).

The total flavonoid content also varied in different fractions Figure 8. The total flavonoid content of the leaves ranged from 36.27± 6.23 to 89.29±8.97mg QE/g of extract while that of the bark ranged from 37.24±1.05 to 103.82±8.87mg QE/g of extract with the ethyl acetate fraction of the bark having the highest TFC followed by ethyl acetate fraction of the leaves.

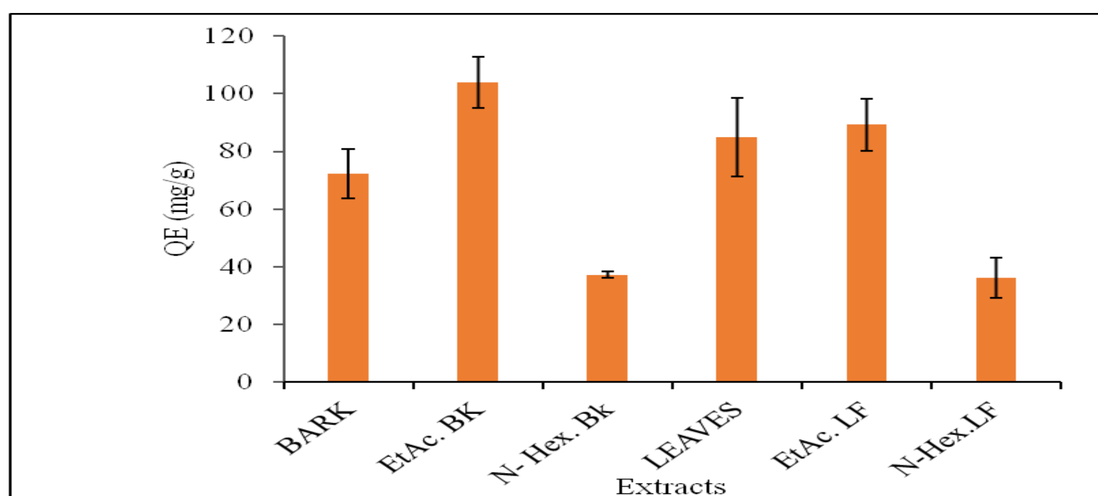


Figure 8: Total Flavonoid content of *A. leiocarpa* leaf and stem bark extracts

Flavonoids, one of the most diverse and widespread group of natural secondary metabolites are probably the most important natural phenolic. They exhibit a broad spectrum of chemical and

biological activities including radical scavenging (Prasad *et al.,* 2009) and have thus found application in the prevention and management of several disease conditions.

Proanthocynin content (pAC)

In this study., pro-anthocyanin content was reported as percentage of the ratio of the decrease in absorbance of test solution after 90 minutes of incubation at 85°C to that of the extract incubated at room temperature for 90 minutes (without heat). Crude methanol extract of the leaves showed highest pro-anthocyanin content having 56.71±0.00 mg [PA]/mL of extract while ethyl acetate fraction of the leaves contained 46.62±2.81 mg [PA]/mL of extract. Pro-anthocyanin content of the crude methanol and ethyl acetate fraction of the extract of the bark were 50.67±0.25 and 52.90±0.00, respectively.

Table 2: Percentage pro-anthocyanin content in crude methanol and ethyl acetate fractions of the leaves and bark extracts of *A. leiocarpa*

S/N	EXTRACTS	mg [PA] /mL
1.	Bark	50.67± 0.02
2.	Et.Ac. BKF	52.90± 0.12
3.	Leaves	56.71± 0.00
4.	Et.Ac. LF	46.62± 0.28

Total anthocyanin content (tAC)

The total-anthocyanin content is expressed as mg Cyanidin-3-glucoside Equivalent/L. TAC of the leaves is higher than in the bark. Crude methanol extract gave 1.63 mg CGE/L and ethyl acetate fraction of the leaves gave 1.59 mg CGE/L while the crude methanol and ethyl acetate fraction of the bark gave 0.17 mg CGE/L and 0.33, respectively.

Table 3: Percentage Total-anthocyanin present in crude Methanolic and Ethyl acetate fractions of the leaves and Bark extracts of *A. leiocarpa*

S/N	SAMPLES	[An]mg/L (CGE)
1.	Bark	0.17
2.	Et.Ac. BKF	0.33
3.	Leaves	1.63
4.	Et.Ac. LF	1.59

EtAc BKF: Ethyl acetate fraction of the Bark; EtAc LF: Ethyl acetate fraction of the leaves; CGE: Cyanidin-3-glucoside Equivalent. Values are expressed as mean percentage in mg of pro-anthocyanin/ mL± SEM (Standard error of mean) n = 3

CONCLUSION

The antioxidant potential of an extract can be determined by a number of tests. In order to evaluate the ability of *A. leiocarpa* extract as an antioxidant, its DPPH radical scavenging, Ferric reducing antioxidant power, ABTS radical decolourisation activity, Cupric reducing antioxidant capacity, Metal chelating ability and total antioxidant capacity were measured. Generally, the extracts and fractions of both the leaves and the stem bark showed good antioxidant potential. Comparing the antioxidant effects and the phenolic content however, it was observed that the total antioxidant capacity (TAC) total phenolic contents (TPC) of the bark extracts were higher than the leaves extracts while the total flavonoid content (TFC) of the crude methanol extract of the leaves was higher than that of the bark. Plants usually produce phenolics and flavonoids through their defence mechanism to develop resistance to stress (Chintalwar *et al*., 2003).

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