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POLYPHENOLIC CONTENT AND *IN VITRO* ANTIOXIDANT EVALUATION OF THE STEM BARK EXTRACT OF *PELTOPHORUM AFRICANUM* SOND (FABACEAE)

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Abstract

Background: *Peltophorum africanum* has been traditionally used to relief stress induced diseases. The study was aimed to evaluate the antioxidant activities of ethyl acetate extract.

Material and methods: The *in vitro* antioxidant activities of *Peltophorum africanum* stem bark extract was examined in this study by means of +radical scavenging and ferric reducing power analysis using 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2`-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS) kit, hydrogen peroxide (H_2O_2), iron (iii) chloride (Fe^{3+}) and nitric oxide (NO). In assessing the likely effects of secondary metabolites on the activities observed; total proanthocyanidins, phenolics, flavonols, and flavonoids were determined using standard phytochemical methods. Data was analyzed by ANOVA test and the *p*-value < 0.05 was considered significant.

Results: Extract scavenging activity of 88.73± 6.69 % (25 µg mL^{-1}), 53.93±1.09 % (25 µg mL^{-1}), 87.293±6.64 % (25 µg mL^{-1}), 10.55±2.16 mM (0.42 mM) and 3.8115±0.06 (25 µg mL^{-1}) were recorded for H_2O_2, NO, DPPH, ABTS and Fe^{3+} reducing power respectively. These values were comparable to the standard compounds; DBPC*BHT, L (+) - Ascorbic acid and Trolox™ ($p < 0.05$). Proanthocyanidins (92.18±4.68 mg/g) occurred more ($p < 0.05$) in the extract when compared to all other compounds tested: phenolics (60.53±1.46 mg/g) > flavonoids (18.37± 2.11 mg/g), > flavonols (11.20±3.90 mg/g). However the difference between flavonols and flavonoids was not significant ($p > 0.05$) at 95% confidence interval.

Conclusion: The results of this study validated the folkloric use of *P. africanum* which could be exploited as an easily available and a cheaper source of natural antioxidants.

Key words: *Peltophorum africanum*, antioxidant, phytochemicals, polyphenolics, radicals.

Introduction

Antioxidants are molecules capable of neutralizing the harmful effects of reactive oxygen species (ROS) through an enzymatic (superoxide dismutase, catalase and glutathione peroxidase) and non enzymatic defense system (vitamin E, vitamin C and glutathione) (Paz´dzioch-Czochra and Widen). However, with the growing damages in environmental factors such as smoke, radiation and toxic chemicals; the natural defense system is often weakened (Valko et al., 2006). The ROS consist of free radicals (OH^-, $O_2^{\cdot-}$, $^{\cdot}NO$, RO_2^- and LOO^-) and non-free radical species (H_2O_2, O_2^{-1}, O_3, and LOOH). Radicals are chemically unstable atoms that cause oxidative damage to various bio-molecules including lipid cells, proteins and DNA. They are known to be the underlying cause of oxidative stress which is grossly implicated in the pathogenesis of various diseases such as cancer, diabetes, cardiovascular diseases, aging and metabolic syndrome (Mandal et al., 2009).

Some synthetic antioxidant agents including butylated hydroxyanisole (BHA) and the related compound, butylated hydroxytoluene (BHT) are phenolics compounds that are often added to foods to preserve fats. These are commercially available; on the other hand, they have been considered to be toxic to living cells (Mbaebie et al., 2012). In recent years, there is increasing interest in the discovery of natural antioxidants from medicinal plants. These antioxidants act as free radical scavengers by preventing and repairing damages caused by ROS, and therefore can enhance the immune defense mechanism and significantly reduce the risks of cancer and other degenerative diseases. This activity is mainly due to the presence of compounds such as flavonoids, phenols, flavonols, carotenoids and proanthocyanidins (Ebrahimzadeh et al., 2010).

Peltophorum africanum (Fabaceae), also known as Weeping Wattle, is a semi-deciduous to deciduous tree, widespread in South Africa. Leaves and bark have been conventionally used to clear intestinal parasites and relieve stomach problems; stem bark used in treating colic, diarrhoea, human immunodeficiency virus/ acquired immune deficiency syndrome (HIV/AIDS), venereal diseases and infertility; whereas roots are used to cure sore throat and toothache (Bizimenyera et al., 2005a; Theo et al., 2009). It has also been reported to be active against *E. coli, S. aureus, C. jejuni*, HIV-1 (Betulinic acid) and the acetone, hexane, ethanol and dichloromethane extract to contain scavenging property (Bizimenyera et al., 2005a; Theo et al., 2009; Samie et al., 2005; Bizimenyera et al., 2005b). Other species of this plant common in other countries such as *Peltophorum pterocarpum* (DC) Backer ex K. Heyne (Leguminosae) has been reported to contain phytochemicals (Nathan et al., 2012; Elufioye et al., 2013), high antioxidant activity (ethyl acetate and methanol) with known compounds (Jain et al., 2012).

There has not been any scientific report on the antioxidant potential of the ethyl acetate extract of this plant despite the marked susceptibility reported in our previous study against *Helicobacter pylori*, an organism implicated in gastritis, ulcer and gastric cancer (Okeleye et al., 2010; Tanih et al., 2010). Therefore, the present study was aimed at assessing the qualitative and quantitative compositions of the phytochemicals and antioxidant potential of the ethyl acetate stem bark extract of *P. africanum* in order to provide scientific basis to justify its therapeutic usage.

Materials and Methods

Preparation of plant extract

Peltophorum africanum (stem bark) (voucher number, BP01) was selected based on ethno-botanical survey. The plant was collected and identified in Limpopo Province, South Africa in partnership with a botanist at the University of Venda (with voucher specimen number BP01 assigned). The extraction and the sterility test were done as previously reported (Okeleye et al., 2013).

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Total polyphenolics content
Phenolics

Folin-Ciocalteu method was used to determine the total phenolics content of the extract (Wolfe et al., 2003). One millilitre of the extract (10 mg mL^{-1}) was mixed with 5 mL of Folin-Ciocalteu reagent (10% v/v) and 4 mL of Na_2CO_3 (7.5% w/v). Samples of extract were kept at a final concentration of 1 mg mL^{-1}. The mixture was vortexed and allowed to stand for 30 min at 40°C for color development. Absorbance was measured at 765 nm (Helios Epsilon Thermo Spectronic; USA) and expressed as Gallic acid equivalent (mg/ g of dry mass) using the calibration curve equation: Y = 0.1216x, R^2 = 0.936512, where; x was the absorbance and Y was the Gallic acid equivalent (mg/g). The experiment was conducted in triplicate and the results reported as mean ± SD values.

Flavonols

Total flavonols was estimated using the method of Kumaran & Karunakaran (2007). To 2 mL of a sample (3.5 mg mL^{-1}), 2 mL of $AlCl_3$ ethanol (2% w/v) and 3 mL $NaNO_2$ (5% w/v) solutions were added. Samples were maintained at a final concentration of 1 mg mL^{-1}. After 2.5 h at 20°C, absorbance was read at 440 nm. Total flavonols were calculated as quercetin (mg/g) using the equation: Y= 0.0255x, R^2 = 0.9812, where; x was the absorbance and Y the quercetin equivalent (mg/g).

Proanthocyanidins

The procedure reported by Mbaebie *et al.* (2012) was used to determine the total proanthocyanidins. A volume of 0.5 mL of 10 mg mL^{-1} extract solution was mixed with 3 mL of vanillin-methanol (4% w/v) solution and 1.5 mL HCl. Extracts were at a final concentration of 1 mg mL^{-1}. The mixture was allowed to stand for 15 minutes and then measured at 500 nm (Helios Epsilon Thermo Spectronic; USA). The total proanthocyanidins was expressed as catechin equivalents (mg/g) based on the following curve equation: Y = 0.5825x, R^2 = 0.9277, where; x was the absorbance and Y the catechin equivalent (mg/g).

Flavonoids

Total flavonoid was determined using the method of Ordonez *et al*. (2006). A volume of 0.5 mL of $AlCl_3$ ethanol solution (2% w/v) was added to 0.5 mL of sample solution (2 mg mL^{-1}). Extracts were evaluated in triplicate at a final concentration of 1 mg mL^{-1}. The resultant mixture was incubated for 1 hr for yellow color development which indicated the presence of flavonoid. The absorbance was measured at 420 nm using Helios Epsilon Thermo Spectronic (USA). Total flavonoid was calculated as quercetin equivalent (mg/g) and was quantified on the basis of the following equation: Y = 0.255x, R^2 = 0.9812, where x was the absorbance and Y the quercetin equivalent (mg/g).

***In vitro* antioxidant capacities**
Ferric-reducing power (FRAP) assay

The reducing power of the extract was evaluated according to the method of Kumar and Hemalatha (2011). Exactly 1.0 mL of the extract prepared in distilled water (25 – 500 µg mL^{-1}) was added to the mixture containing 2.5 mL of phosphate buffer (0.2 M; pH 6.6) and 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1% w/v). Standard compounds used were L (+) - Ascorbic acid ($C_6H_8O_6$) and 2, 6 – Di – *tert* – butyl – 4 – methyl phenol [$(CH_3)_3C]_2C_6H_2(CH_3)OH$]. The resulting mixture was incubated at 50°C for 20 min, followed by the addition of 2.5 mL of CCl_3COOH (10% w/v) and then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1 %, w/v). The experiment was conducted in duplicate and absorbance measured at 700 nm (Helios Epsilon Thermo Spectronic, USA) against a blank sample of only phosphate buffer. Higher reducing power of the extract was indicated by the increased absorbance.

Hydrogen peroxide scavenging activity

Scavenging activity of hydrogen peroxide by the plant extract was estimated using the method of Nabavi *et al*. (2009). Four millilitres of extract prepared in distilled water (25 – 500 µg mL^{-1}) was mixed with 0.6 mL of H_2O_2 solution (4 mM) prepared in phosphate buffer (0.1 M; pH 7.4) and incubated for 10 min. A negative control containing H_2O_2 and methanol was included in the experiment. The absorbance of the solution was taken at 230 nm (Helios Epsilon Thermo Spectronic, USA) against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenged by the extract and standard compounds (DBPC*BHT and L (+) - Ascorbic acid) were calculated as follows: % Scavenged [H_2O_2] = [(Ao − A_1)/Ao] × 100 where Ao was the absorbance of the control and A_1 was the absorbance in the presence of the sample of extract and standard.

Scavenging activity of nitric oxide

The method of Ebrahimzadeh *et al*. (2010) was used with some modifications. A volume of 2 mL of sodium nitroprusside (10 mM) prepared in phosphate buffer saline (0.5 mM; pH 7.4) was mixed with 0.5 mL of plant extract and standard compounds; $C_6H_8O_6$ and $[(CH_3)_3C]_2C_6H_2(CH_3)OH$ at different concentrations (25 – 500 µg mL^{-1}). Sodium nitroprusside mixed with methanol was used in the procedure as a negative control. The mixture was incubated at 25°C for 2 $^1/_2$ hrs and 0.5 mL of incubation solution was withdrawn and mixed with 0.5 mL of Griess reagent [1.0 mL sulfanilic acid reagent (0.33 % dissolved in 20 % glacial acetic acid) mixed with 1.0 mL of naphthylethylenediamine dichloride (0.1% w/v)]. The absorbance was measured at 540 nm (Analytical & Diagnostic Product Gen™ BioTek, USA) after incubation at room temperature for 30 min. The percentage of nitric oxide scavenged was calculated as follows: % Scavenged [NO] = [(Ao − A_1)/Ao] × 100 where Ao was the absorbance of the control and A_1 was the absorbance in the presence of extract and standards.

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DPPH radical scavenging assay

The total antioxidant capacity of the extract was carried out using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical as previously described by Shen *et al*. (2010). A solution of 0.135 mM DPPH in methanol was prepared and 1.0 mL of this solution mixed with 1.0 mL of extract (25 – 500 µg mL^{-1}) dissolved in methanol. DPPH in methanol was used as a negative control. The combination was thoroughly mixed and left in the dark at room temperature for 30 min before the absorbance was measured at 517 nm (Helios Epsilon Thermo Spectronic, USA). L (+) - Ascorbic acid and DBPC*BHT were used as the reference. All the tests were run in duplicate. The ability to scavenge DPPH radical was calculated using the following equation: % Scavenged [DPPH] = $[(Ao - A_1)/Ao] \times 100$ where Ao was the absorbance of the control and A_1 was the absorbance in the presence of extract and standards.

ABTS radical scavenging assay

ABTS (2, 2`-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging assay was carried out using antioxidant assay kit (Sigma, USA) and manufacturer's instructions were followed. Briefly, 10µL of each sample (0 – 0.42 Mm); ethyl acetate extract and (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox™); a water-soluble vitamin E analog, served as a standard or control antioxidant), was added to 20µL of myoglobin (from horse heart) in 96 well plate. Exactly 150µL of ABTS was added and the mixture incubated at room temperature for 5 minutes. Stop solution (100µL) was added to each well and the endpoint absorbance read at 405 nm using Analytical & Diagnostic Product Gen™ BioTek, USA. Concentration was varied at 0 to 0.42 mM and assay buffer (500 µL) without the samples (0 mM) was included as negative control. ABTS scavenging capacity was expressed as total antioxidant concentration in relation to the Trolox™ standard concentration calculated using the equation: X (mM) = $[y(A_{405}) - \text{Intercept/Slope}] \times$ dilution factor; obtained from the linear regression of the standard curve ($y = -1.3066x + 1.3235$, $R^2 = 0.9477$), where $y(A_{405})$ was the average absorbance of the test sample. The experiment was conducted in duplicate and the results reported as mean ± SD values.

Statistical analysis

Statistical analysis was performed using MINITAB, version 12 for windows and the SPSS Version 17.0 (Illinois USA, 2011). Regression analysis was used to determine effective concentration ($EC_{50;}$ MINITAB). The one way ANOVA test was used to determine if there was any statistical difference in the activity of the ethyl acetate extract and the standard compounds (DBPC*BHT, L (+) - Ascorbic acid and Trolox™) against DPPH, ABTS, NO, H_2O_2, ferric reducing agent and polyphenolics (SPSS). *P*-values < 0.05 were considered significant.

Results

Total polyphenolics content

Polyphenolic compounds may contribute directly to anti-oxidative activities. Higher quantity of proanthocyanidins (92.18±4.68 mg/g of catechin equivalent) was observed to be present in the extract compared to all other compounds tested ($p < 0.05$), followed by phenolics (60.53± 1.46 mg/g Gallic acid equivalent); flavonoids (18.37± 2.11 mg/g of quercetin equivalent) and flavonols (11.20±3.90 mg/g quercetin equivalent) respectively as represented in Figure 1. However the difference between flavonols and flavonoids was not significant ($p > 0.05$) at 95% confidence interval.

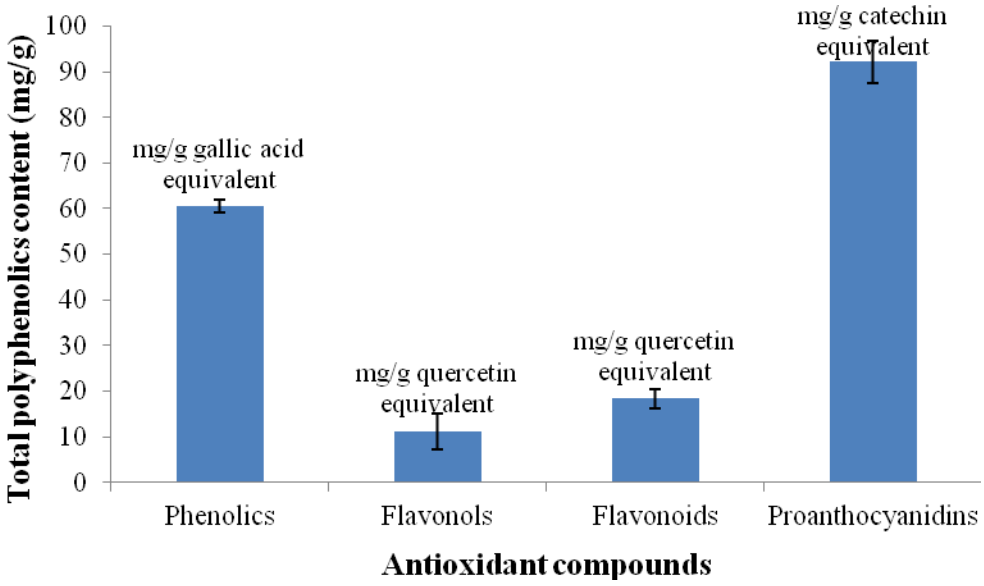

Figure 1: Total polyphenolic content of ethyl acetate extract of *P. africanum*.

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Antioxidant capacities
Ferric-reducing power activity

An important mechanism of phenolic antioxidant action is Fe^{3+} reduction which is often used as an indicator of electron donating activity. The antioxidant potentials of the plant extract was estimated from their ability to reduce Fe^{3+} to Fe^{2+} with observable colouration from lemon to green after the addition of ferrous chloride. Ferric reducing activity of 3.8115±0.06 and 3.828±0.05 were observed at 25 µg mL^{-1} and 500 µg mL^{-1} concentration of the extract. The extract (3.828±0.051) and 2, 6 – Di – *tert* – butyl – 4 – methyl phenol (DBPC*BHT; 3.828± 0.040) were higher in reducing power activity than L (+) - Ascorbic acid (3.446±0.070; $p < 0.001$), however; there was no significant difference observed between the extract and DBPC*BHT ($p > 0.05$) as presented in Figure 2.

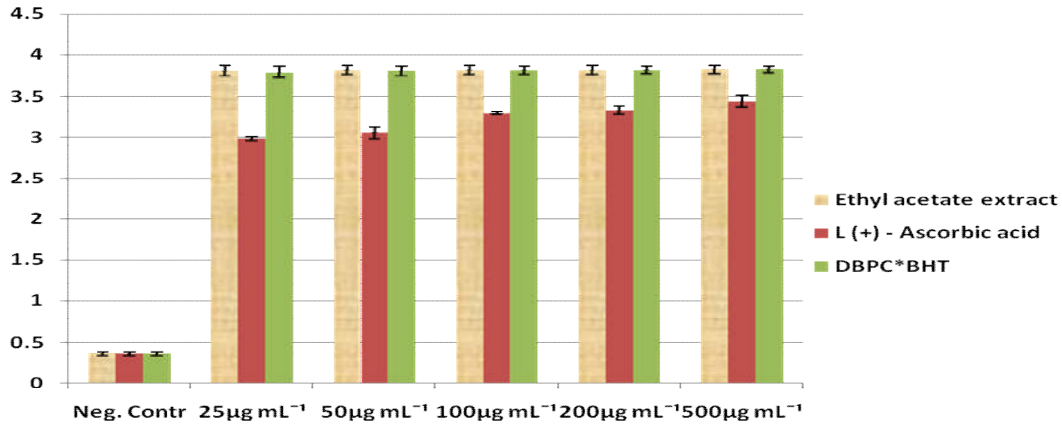

Figure 2: Reducing power of the ethyl acetate extract of *P. africanum* in comparison to L (+) - Ascorbic acid and DBPC*BHT.

Hydrogen peroxide scavenging activity

The measurement of H_2O_2 scavenging activity is one of the useful methods used to determine the ability of antioxidants to decrease the level of prooxidants. At concentrations of 25 µg mL^{-1} & 500 µg mL^{-1}, ethyl acetate extract exhibited 88.73± 6.69 and 92.86±2.39 percent hydrogen peroxide scavenging activity, respectively, while, at the same concentration, higher activity was observed for L (+) - Ascorbic acid (99.54±0.42 & 99.62±0.42 %) and DBPC*BHT (98.81±0.83 & 98.69±0.39 %) respectively (Figure 3). There was no statistically significant difference observed in activity of the extract and the standard compounds ($p > 0.05$).

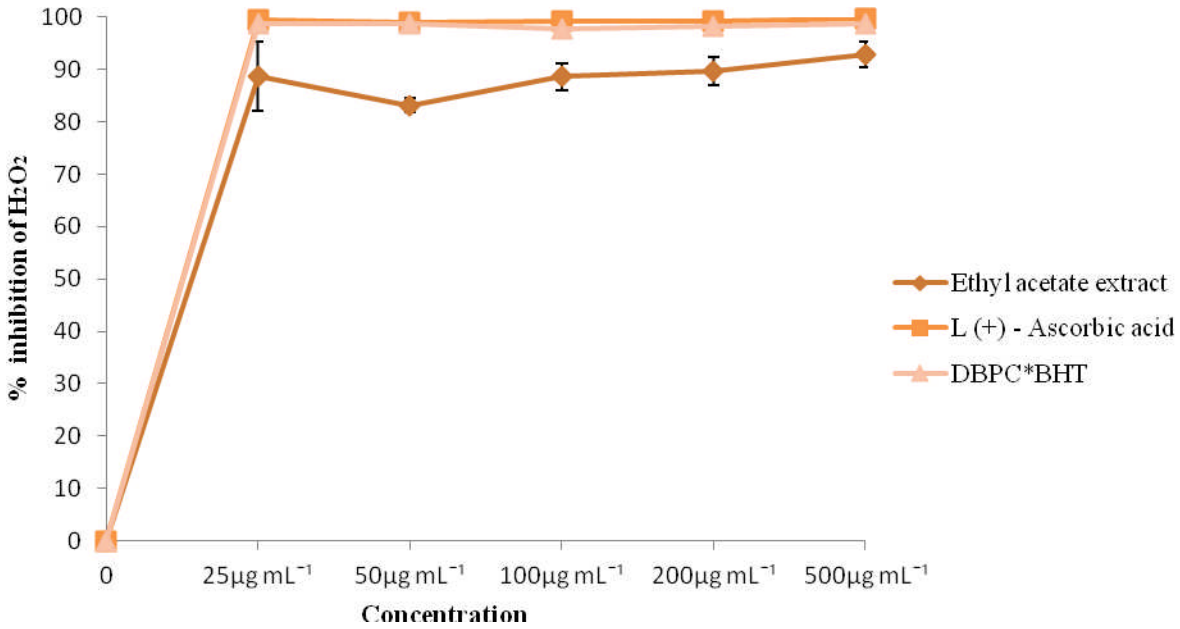

Figure 3: Percentage inhibition of hydrogen peroxide scavenging activity of ethyl acetate extract of *P. africanum*, L (+) - Ascorbic acid and DBPC*BHT.

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Scavenging activity of nitric oxide

The highest percentage inhibitory activity of the extract, L (+) - Ascorbic acid and DBPC*BHT against nitric oxide radical was 53.93±1.09 (25µg mL^{-1}), 51.37±9.85 (50µg mL^{-1}) and 49.97±0.49 (50µg mL^{-1}) percent (Figure 4). The inhibitory effect of the extract was relatively similar to the standard drugs, as no statistical difference was observed in their activities ($p > 0.05$).

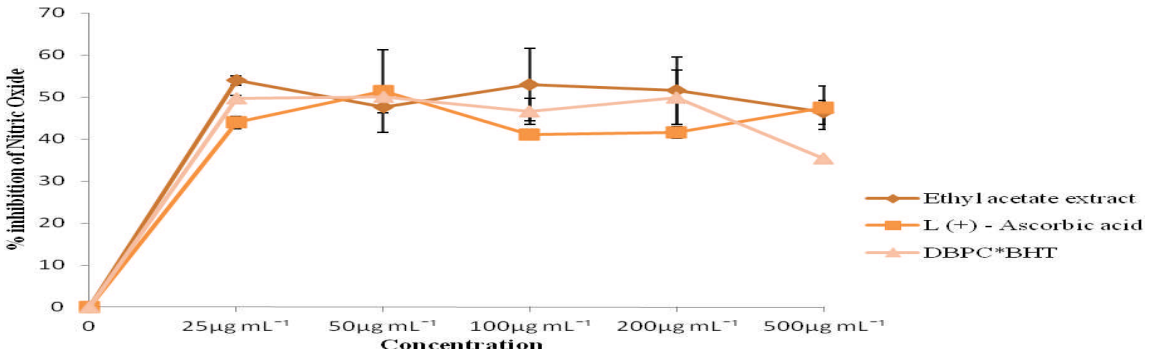

Figure 4: Nitric oxide scavenging activity of the ethyl acetate extract of *P. africanum* compared to L (+) - Ascorbic acid and DBPC*BHT.

DPPH radical scavenging assay

The stable DPPH radical is a commonly used substrate for relatively quick evaluation of free radical scavenging activity. Exactly 87.293±6.64 and 89.503±0.39% inhibition of DPPH was noted at 50 µg mL^{-1} and 25µg mL^{-1} of the extract respectively. The scavenging effect of DBPC*BHT increased distinctively with concentration. At the highest dose of 500 µg mL^{-1}, L (+) - Ascorbic acid showed high scavenging effect of 92.40±0.59 percent, followed by the extract (89.50±0.39 %) and DBPC*BHT (82.60±1.56 %) respectively as shown in Figure 5. The EC_{50} (effective concentration at 50 %) of Ethyl acetate extract, (+) - Ascorbic acid and DBPC*BHT that scavenged H_2O_2, NO and DPPH were analysed and presented in Table 1.

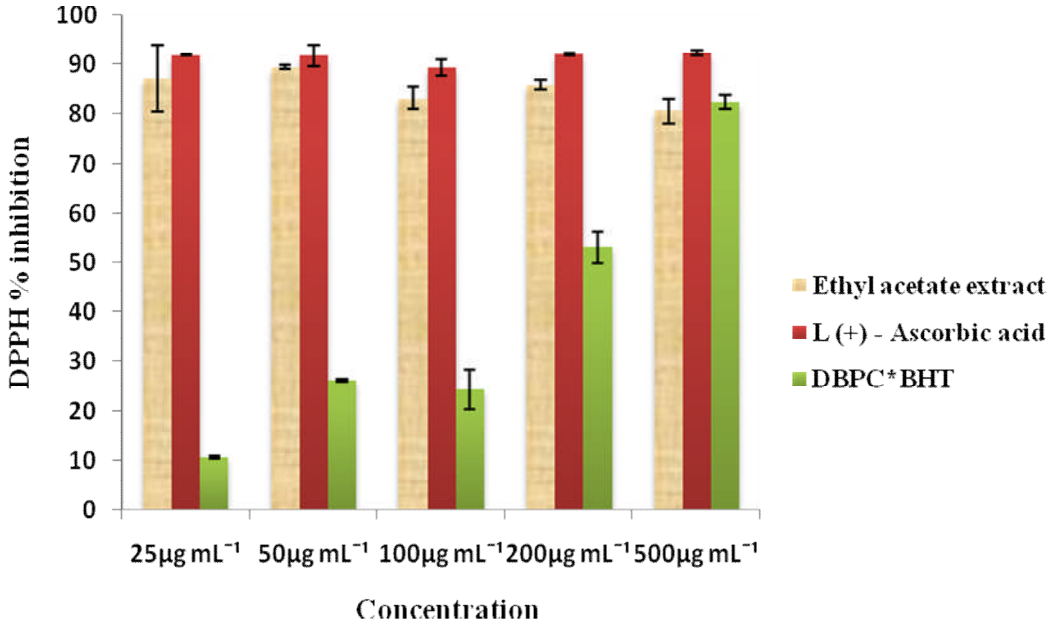

Figure 5: DPPH radical scavenging activity of the ethyl acetate extract of *P. africanum* in comparison to L (+) - Ascorbic acid and DBPC*BHT.

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Table 1: Effective concentration (EC) of the scavengers using regression analysis

	Ethyl acetate extract		(+) – Ascorbic acid		DBPC*BHT	
	R. Eq.*	EC_{50}* (µg mL^{-1})	R. Eq.	EC_{50} (µg mL^{-1})	R. Eq.	EC_{50} (µg mL^{-1})
H_2O_2	Y = 86.2 + 0.0138X	131.16±1.65	Y = 99.2 + 0.000790X	88.61±0.17	Y = 98.5 + 0.00010X	100±0.31
NO	Y = 52.4 – 0.0110X	204.55±1.89	Y = 44.6 + 0.0025X	276±3.15	Y = 51.5 – 0.0296X	298.99±1.84
DPPH	Y = 87.9 – 0.0142X	193.66±1.46	Y = 91.2 + 0.00228X	109.65±0.80	Y = 14.4 + 0.143X	225.80±5.27

*R. Eq., Regression Equation; *EC_{50}, Effective Concentration at which 50% of radicals were scavenged.

ABTS radical scavenging assay

ABTS radical assay, can be used in both organic and aqueous solvent systems. Our result showed the scavenging activity of *P. africanum* stem bark extract against ABTS radical in a concentration dependent manner. At 0.42 mM, higher antioxidant activity was observed for the extract (10.55±0.45 mM) than in Trolox™ (6.07±0.62 mM; Figure 6). Comparison was made based on a calibration graph generated using Trolox™ and it was noted that the ABTS scavenging activities between the extract and the Trolox™ were statistically insignificant ($p > 0.05$).

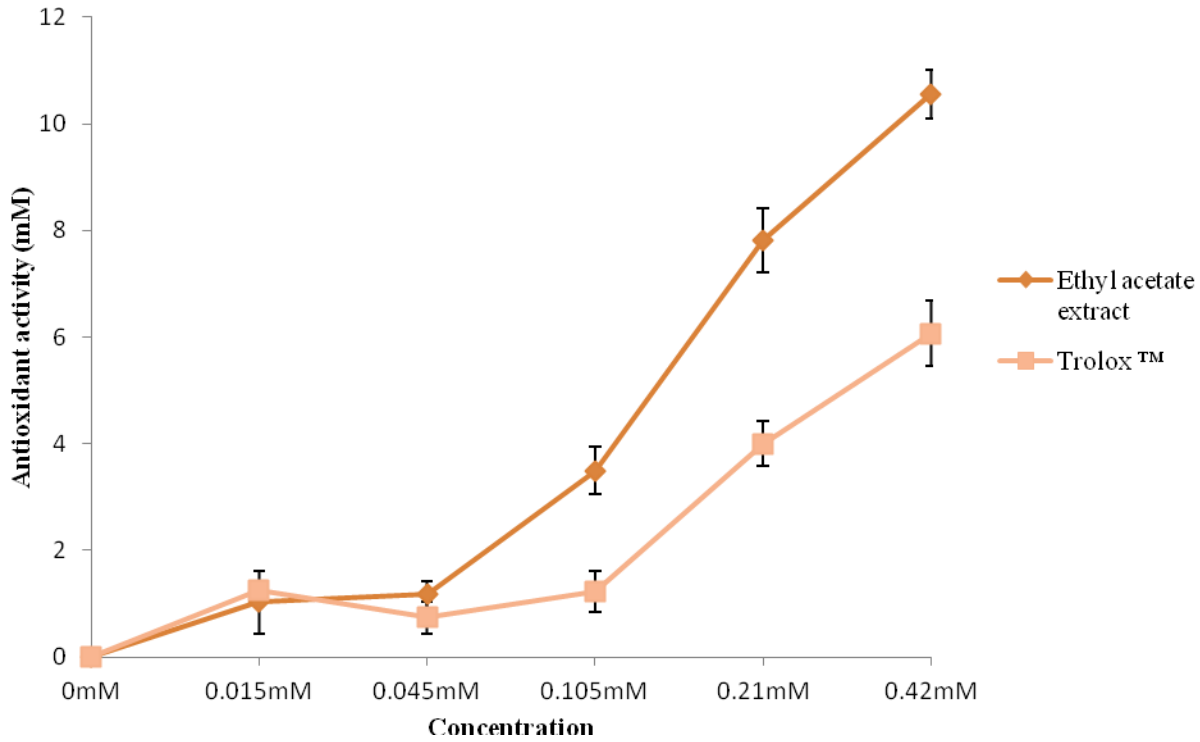

Figure 6: ABTS radical scavenging antioxidant activity in relation to the Trolox™ standard concentration.

Discussion

Free radicals were constantly generated in living system and can cause extensive damage to the tissues and biomolecules leading to various disease conditions, especially degenerative diseases. Many synthetic drugs protect against oxidative damage but most often with adverse side effects (Bizimenyera et al., 2005b; Ndip et al., 2009). An alternative solution to the problem is to consume natural antioxidants from food supplements and traditional medicines (Yazdanparast et al., 2008). In this study we established the total proanthocyanidins, phenolics, flavonols, flavonoids and high antioxidant activity in the ethyl acetate extract of *P. africanum*. Exactly 89.503±0.39 % inhibition of DPPH was noted at 50 µg mL^{-1}, this with known compounds such as; Hexadecanoic acid, β-Sitosterol, Cholesta-4,6-dien-3-ol,benzoate and other several monoterpenes and sesquiterpenes components identified in our previous study justify its valuable pharmacological activities (Okeleye et al., 2013). This is in line with other study on *Peltophorum pterocarpum* (DC) Backer ex K. Heyne (family Leguminosae) reported to contain phytochemicals and antioxidant activity of 96.70 ± 0.22 % DPPH inhibition at 80 µg mL^{-1} with similar identified compounds (Nathan et al., 2012; Jain et al., 2012; Elufioye et al., 2013).

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Proanthocyanidins (92.18±4.68 mg/g) occurred more ($p < 0.05$) in the extract compared to all other compounds tested: phenolics (60.53±1.46 mg/g) > flavonoids (18.37± 2.11 mg/g), > flavonols (11.20±3.90 mg/g). However the difference between flavonols and flavonoids was not significant ($p > 0.05$) at 95% confidence interval. The differences in polarity of the antioxidant components are notable reasons why phenolics compounds and antioxidant activity of the extract differ (Benzie and Strain 1996; Meyer et al., 1997; Chang et al., 2002). Proanthocyanidins help to defend the body from tissue damage and to improve blood distribution by reinforcing the blood vessels, while flavonoids in the human diet reduce the risk of different cancers and have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A2 (Benzie and Strain 1996; Meyer et al., 1997; Mbaebie et al., 2012). The reducing capacity of the compounds in the extract (3.828±0.051) serve as a significant indicator of its potential antioxidant activity. Antioxidant activities have been ascribed to various mechanisms such as prevention of chain initiation, disintegration of peroxides, reducing capacity and radical scavenging (Benzie and Strain 1996; Yildirim et al., 2000). The reducing power of the plant extract (3.828±0.051) was compared with the standard DBPC*BHT (3.828±0.040) and no significant difference was observed in their potency ($p > 0.05$), while for L (+) - Ascorbic acid it was 3.446± 0.070 ($p < 0.001$) which is similar to the findings reported by Jain *et al*. (2012).

Scavenging of H_2O_2 by extract (EC_{50} = 131.16±1.65µg mL^{-1}) is credited to it's phenolics (60.53±1.46), which can offer electrons to H_2O_2, thus neutralizing it to H_2O. Even though H_2O_2 itself is not very reactive, it can occasionally cause cytotoxicity by formation of hydroxyl radicals in the cell (Ebrahimzadeh et al., 2010). This demonstrated similar results with the standard compounds; L (+) - Ascorbic acid (EC_{50} = 88.61±0.17µg mL^{-1}), and DBPC*BHT (EC_{50} = 100±0.31µg mL^{-1}). Fe3+ was reduced to Fe2+ in the presence of extract and the reference compounds which corroborates significant effect on the activities against nitric oxide (NO) observed [NO: extract (EC_{50} = 204.55±1.89µg mL^{-1}) in comparison to L (+) - Ascorbic acid (EC_{50} = 276±3.15µg mL^{-1}) and DBPC*BHT (EC_{50} = 298.99±1.84µg mL^{-1})], a finding which is similar to the result of other reports (Balakrishnan et al., 2009; Ebrahimzadeh et al., 2010). NO is lipophilic and produced by phagocytes and endothelial cells from the terminal guanido nitrogen atom of L-arginine by various NADPH-dependent enzymes called NO synthases. It is a strong pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling and can significantly be inhibited through direct competition with plant extract for oxygen (Jagetia et al., 2004; Balakrishnan et al., 2009).

Mineral nutritional status and physical properties of the soil greatly influenced the presence of secondary metabolites in different parts of a plant (Mandal et al., 2009). Plant phenolics constitute one of the major groups of compounds acting as primary antioxidant or free radical terminators (Igbinosa et al., 2011). High antioxidant activities observed (ABTS, 10.55±0.45 mM; DPPH, EC_{50} = 193.66±1.46µg mL^{-1}) have been contributed by the combined effect of phytochemicals and polyphenolic compounds. As the reaction between antioxidant molecules and radical progressed, there was a decrease in absorbance of DPPH radical (reverse is the case in ABTS radical) with an increase in concentration. This leads to the scavenging of the radical through hydrogen donation; hence, a noticeable change in colour from purple to yellow (Elmastas et al., 2006). High antioxidant activity of 89.503±0.39 % DPPH inhibition at 50 µg mL^{-1}; 53.93±1.09 %, 88.73± 6.69 % inhibition of NO and H_2O_2 respectively at 25 µg mL^{-1} observed in this study is similar to other investigators (Bizimenyera et al., 2005b; Balakrishnan et al., 2009; Jain et al., 2012; Sridharamurthy et al., 2012), therefore adding to its credibility as a likely template or adjunct of an antioxidant compound.

The findings of this study demonstrated high *in vitro* antioxidant activity of the ethyl acetate extract of *P. africanum* which is considerably aided by the high quantity of secondary metabolites; polyhenolics, and phytochemicals. It can therefore be useful in preventing or slowing down the progress of various oxidative stress induced diseases. Furthemore it could be exploited as an easily available source of natural antioxidant or as a food supplement.

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