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A study of the antioxidative potentials of acetone and aqueous extracts of *Parkia biglobosa* and *Tetracarpidium conophorum* stem barks *in vitro*

Olabinri, B. M.[1]*, Adetutu, A.[1], Olaleye, M. T.[2], Oluwafunsho, B. O.[1] and Oyeniyi, O. O.[1]

[1]Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.
[2]Department of Biochemistry, Federal University of Technology, Akure, Nigeria.

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A study of *in vitro* antioxidative potentials of acetone and aqueous extracts of *Parkia biglobosa* and *Tetracarpidium conophorum* stem barks were investigated. At all concentrations investigated (50 to 250 µg/ml), the acetone extract of *P. biglobosa* and *T. conophorum* stem barks failed to scavenge hydroxyl radical *in vitro*. Polyphenol extract of *T. conophorum* stem bark failed to exhibit antioxidant activity *in vitro* at all concentrations except at 250 µg/ml, where it exhibited weak antioxidant activity (19.6%). *P. biglobosa* extract showed concentration dependent increase in antioxidant activity with increase extraction time (10 to 30 min). The maximum antioxidant activity of the aqueous stem bark extracts of *P. biglobosa* and *T. conophorum* were 87.68% at 50 min and 76.16% at 10 min, respectively.

Key words: Bioassay, clinical medicine, natural products, active principle and bioactive compound.

INTRODUCTION

Free radicals are known as major contributors to several clinical disorders, such as diabetes, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defense mechanism (Parr and Bowell, 2000). Natural products have the potential to be developed into new drugs for the treatment of various diseases (Chen et al., 2009). Current research is now directed towards natural antioxidants originated from plants due to safe therapeutics (Lobo et al., 2010). It is believed that medicinal plants are a potential source of reactive oxygen species scavenger molecules (Anandjiwala et al., 2008). Plant extract could be utilized as a source of nutritional phenolics (Kuate et al., 2011). There is a growing interest in natural anti-oxidants present in medicinal and food plants that might attenuate oxidative stress (Silva et al., 2007).

Parkia biglobosa is popularly known as the African locust bean (Osundina, 1995). It is a perennial tree of legume, belonging to the family Leguminosae (Campbell-Platt, 1980). The seeds of the plant are embedded in a yellowish, mealy, sweet tasting edible pulp (Aliero et al., 2001). It is a plant recognized to be very rich in phenolic compounds (Millogo-Kono et al., 2008). The bark of the plant contained epigallocatechin, epicatechin 3-*O*-gallate and epigallocatechin 3-*O*-gallate (Alabi et al., 2005). The leaf extract contains cardiac and saponin glycosides (Ajaiyeoba, 2002). The fruit pulp and seeds are rich in proteins and lactose (Alabi et al., 2005). The seeds

*Corresponding author. E-mail: olabinriexcellent@yahoo.com

contain antinutritional factors (oxalate, hydrogen cyanide, tannin and phytate) (Mohan and Jonardhaman, 1995).The *P. biglobosa* extract possesses antibacterial (Millogo-Kone et al., 2008), antidiabetic (Odetola et al., 2006), antifungal (Fawole and Abioye, 2002), anti-inflammatory (Kouadio et al., 2000), anti-diarrhoeal (Agunu et al., 2005) and anti-hypertensive (Mohammed et al., 2005) properties. The leaves, barks and root are used in treating leprosy, eye sores, tooth ache and fever (Ajaiyeoba, 2002). The leaves, barks and pods are used to treat new and old wounds (Inngjerdingen et al., 2004). The bark is chewed by men for lack of virility (Irvine, 1961). The seeds are used as food seasoning (Ajaiyeoba, 2002). The leaves of *P. biglobosa* contained more flavonoid than the bark (Miollogo-Kone et al., 2009). Also, the total phenolics content of the leaves of the plant varied significantly year round (Miollogo-Kone et al., 2009).

Tetracarpidium conophorum belongs to the family Euphorbiaceae, and is known as African walnut (Dalziel, 1937). The plant is known as adala in Yoruba and ukpo in Ibo. It is used as a male fertility agent and the leaves of the plant are used for the treatment of dysentery (Ajaiyeoba and Fadare, 2006). *T. conophorum* extract possesses cardioprotective (Landbo and Meyer, 2001) and antioxidative (Fukuda et al., 2004) and antimicrobial properties (Ajaiyeoba and Fadare, 2006). The plant is used in hair dye (D'Amelio, 1999). The shell flour is used as a carrier for insecticides (Ensminger, 1993). It is a very low glycemic food (Liu et al., 2000). *T. conophorum* contained oxalates, phytates, tannin as well as proteins, fibres, oil and carbohydrates (Enujuigha, 2003). The seed extract of *T. conophorum* possesses agglutin I and agglutin II (Animashaun et al., 1998). The seed flour oil is used as a vulcanized oil for rubber (Oke and Fafunso, 1975). The aqueous extract of the nut is used to relieve pain, increase sperm count and enhance sexual performance (Alade and Umukoro, 2011).

The *in vitro* antioxidant activity, chelating ability and total phenolic content of the aqueous seed extract of *T. conophorum* have been reported (Olabinri et al., 2010a). The *in vitro* antioxidant activity of the stem bark of *P. biglobosa* has been studied (Molliogo-Kone et al., 2009). To the best of our knowledge, the influence of extraction time on antioxidant activity of both plant parts has not been addressed. Also, there is no scientific paper which investigates the *in vitro* hydroxyl radical scavenging potentials of the acetone fractions and aqueous extracts from the stem barks of *P. biglobosa* and *T. conophorum*. Therefore, the study was carried out to assess: (i) *in vitro* hydroxyl radicals scavenging potentials, (ii) the influence of extraction time on the total phenolics concentration of the aqueous extracts of both stem barks of the two plants, and (iii) the relationship between the total phenolics concentration and the antioxidant activity of the aqueous extracts of the stem barks of the two plants.

MATERIALS AND METHOD

Reagents

Tannic acid, iron (ii) sulphate and 1,10-phenanthroline were products of British Drug House (BDH), UK. 2,2-diphenyl-1-picrylhyrazyl was purchased from Sigma-Aldrich, USA. Folin-Ciocalteu was a product of Merck, Germany. Hydrogen peroxide used was a product of Sigma-Aldrich, Switzerland.

Preparation of extracts

Collection of plant materials and preparation of aqueous extracts

The stem barks of *P. biglobosa* and *T. conophorum* were obtained in November, 2009 from Ogbomoso North Local Government area of Oyo State, Nigeria. The stem barks were washed with distilled water and dried at room temperature for four days and pounded using pestle and mortar into powder. Aqueous extracts of the plants were prepared by adding 5 ml of distilled water to 0.05 g (0.1% w/v) of the powder and centrifuged (5,000 rpm) at different time intervals (10, 20, 30, 40 and 50) min for each 5 replicates.

Extraction of polyphenol from P. biglobosa and T. conophorum stem barks

The acetone extracts of *P. biglobosa* and *T. conophorum* stem barks were prepared by soaking 25 g of the *P. biglobosa* stem bark and 25 g of *T. conophorum* stem bark powder in 75 and 100 ml of acetone, respectively for 24 h and then filtered. The filterates were allowed to evaporate and the final residues were the acetone extracts of the two plants. They were weighed and found to be 1.5 g for *P. biglobosa* and 1.2 g for *T. conophorum*. Therefore, the yield was 6 and 4.8% respectively. A quantity (0.2 g) of the acetone extracts were weighed and mixed with 20 ml of 70% ethanol for each. One milliliter (1 ml) of these stocks were taken, mixed with 9 ml of 70% ethanol to obtain 1000 µg/ml stock from which different concentrations (50 to 250 µg/ml) were made for the two acetone extracts with 5 replicates for each concentration.

In vitro assays

Total phenolic concentration estimation

The total phenolic concentration of the samples was estimated according to the method of Hung et al. (2002). The phenolic group present in plant extract interacts with Folin-Ciocalteu reagent in alkaline medium using Na_2CO_3 solution, giving a blue colour which has maximum absorption at 765 nm. The extracts reduce Folin-Ciocalteu reagent (yellow solution of polyphosphotungstate and molbydate) in mild base medium to form deep blue colour. Briefly, five hundred microlitre of Folin-Ciocalteu reagent (10% w/v, aqueous) was added to 0.1 ml of samples of different concentrations (50 to 250 µg/ml) and of 0.1% (w/v) concentration followed by the addition of 0.4 ml of aqueous Na_2CO_3 (7.5%, w/v). The mixture was allowed to stand in the dark for 30 min.The absorbance of the blue colour solution was read at 765 nm on a

spectrophotometer (Genesy 10vis, Thermoelectronic Incorportion, USA) against blank (distilled water). Total phenolic concentration (mg/ml) of the sample was extrapolated from a standard curve constructed using tannic acid as a standard.

Antioxidant potential estimation assay

The antioxidant activity of the aqueous and acetone extracts was determined according to the method of Blois (1958). This is based on the ability of the extracts to inhibit stable diphenyl picryl hyhdrazyl radical (DPPH). In the presence of an antioxidant, DPPH radical obtains one or more electrons and the absorbance decreases (Koleva et al., 2002). Five hundred microlitre (0.5 ml) of 0.1 mM 70% methanolic DPPH solution was added to 50 µl of the samples of different concentrations (50 to 250 µg/ml) and of 0.1% (w/v) concentration in a test tube. The mixtures were allowed to stand in the dark at room temperature for 30 min. The absorbance of the yellow color solution was read at 517 nm on a UV/visible spectrophotometer after 30 min against blank (distilled water). Antioxidant activity was expressed in terms of inhibition of DPPH free radical:

$$\text{Antioxidant activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where $A_{control}$ = Absorbance of the methanol DPPH solution (0.1mM) in the absence of sample. A_{sample} = Absorbance of the reaction mixture in the presence of sample.

Hydroxyl radical scavenging activity assay

The hydroxyl radical scavenging activity of samples of different concentrations was determined according to the method of Yu et al. (2004). Hydroxyl radical is generated *in vitro* by mixing $FeSO_4$ which generates ferrous ion (Fe^{2+}) with H_2O_2 and phenanthroline. The 1,10–phenanthroline was used since phenathroline-Fe^{2+} is a commonly used indicator of redox reaction. The H_2O_2/Fe^{2+} system produces hydroxyl radical through the Fenton reaction with the phenanthroline-Fe^{2+} complex oxidized to Fe^{3+}. The hydroxyl radical produced was then determined due to change in absorbance at 560 nm. 60µl of aqueous $FeSO_4.7H_2O$ (1 mM) was added to 90 µl of aqueous 1,10 phenanthroline, after which 2.4 ml of 0.2 M Na_2HPO_4 (pH 7.8) was added to the mixture, followed by the addition of 150 µl of H_2O_2 (0.17 M), and 1.5 ml of different concentrations of different concentrations (50 to 250 µg/ml), and 0.1% (w/v) concentration. The mixture was incubated for 5 min at room temperature. The absorbance of the mixture was read at 560 nm on a UV/visible spectrophotometer with distilled water as blank.

$$\text{Hydroxyl radical scavenging ability (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where $A_{control}$ = Absorbance of the control reaction mixture in the absence of the extract. A_{sample} = absorbance of sample in the presence of the other reagents in the reaction mixture.

RESULTS AND DISCUSSION

Our past research revealed that the aqueous seed extract of *T. conophorum* exhibited antioxidative potential *in vitro* (Olabinri et al., 2010a), while the aqueous extract of *T. conophorum* stem bark in the present study also displayed antioxidative capability *in vitro*. Regrettably, the acetone extract of *T. conophorum* stem bark failed to exhibit antioxidative potential *in vitro* at all concentrations investigated, except at 250 µg/ml with weak *in vitro* antioxidant activity (19.63%).

The aqueous extract of *P. biglobosa* stem bark showed increasing antioxidant activity with increasing extraction time except at 40 min extraction time. The order of increasing antioxidant activity with extraction time for the extract of the plant part was 10 < 20 < 40 < 30 < 50 min. On the other hand, antioxidant activity of the aqueous extract of *T. conophorum* showed increased antioxidant decreasing order with increasing extraction time except at 30 and 50 min. The aqueous extract of *T. conophorum* stem bark displayed maximum *in vitro* antioxidant activity (76.2%) at 10 min extraction time. The minimum *in vitro* antioxidant activity of the extract was 70.6% at 40 min extraction time. Moreover, at 50 min extraction time, the maximum total phenolic (26.78 mg/ml) of the aqueous extract of *T. conophorum* stem bark extract did not correspond to the maximum antioxidant activity (Table 1). Also, the maximum total phenolic of the aqueous extract of *P. biglobosa* (29.58 mg/ml) did not correspond to its maximum antioxidant activity (Table 1).

The order of increasing antioxidant activity with time of aqueous *T. conophorum* stem bark extract was 40 < 20 < 30 < 50 <10 min. The antioxidant activity values were 70.64 ± 0.03, 73.75 ± 0.04, 73.75 ± 0.04, 75.42 ± 0.05, 78.36 ± 0.01%. The aqueous extract of *T. conophorum* stem bark had the highest antioxidant activity at 10 min with 78.36 ± 0.01% and the lowest antioxidant activity at 40 min with 70.64 ± 0.03%.

The acetone stem barks extracts of *P. biglobosa* and *T. conphorum* did not exhibit any hydroxyl radical scavenging activity at all concentrations (Table 2). We have demonstrated clearly in our past research work that the polyphenolic extract of mango (*Mangifera indica*) leaves failed to scavenge hydroxyl radical *in vitro* (Olabinri et al., 2010b) at all concentrations (50 to 250 µg/ml), which was consistent with the results obtained in the present study for the acetone extracts of *T. conophorum* and *P. biglobosa* stem bark extracts. The acetone stem bark extract of *P. biglobosa* showed concentration dependent decrease in antioxidant activity between 50 to 150 µg/ml, while at 200 and 250 µg/ml it failed to exhibit antioxidant activity. At all concentrations, the acetone stem bark extract of *T. conophorum* did not exhibit any antioxidant activity, except at 250 µg/ml.

In our present research work, we observed moderate positive non significant correlation between total phenolic content and antioxidant activity for the aqueous extract of *P. biglobosa* stem bark at 30 min extraction time (r = 0.5;

Table 1. Changes in the levels of antioxidant activities and total phenolic contents of aqueous extracts of *P. biglobosa* and *T. conophorum* stem barks.

Extraction time (min)	*P. biglobosa* stem bark		*T. conophorum* stem bark	
	Antioxidant activity (%)	Total phenolic (mg/ml)	Antioxidant activity (%)	Total phenolic (mg/ml)
10	81.49±0.05	17.46±0.83	76.16±0.01	23.90±2.63
20	84.67±0.02	16.64±1.80	73.75±0.04	25.68±3.14
30	86.52±0.81	26.52±2.61	73.99±0.04	25.68±3.14
40	84.71±1.68	29.58±6.63	70.64±0.03	22.34±2.70
50	87.68±1.77	21.82±3.33	75.42±0.05	26.78±6.65

Values are mean ± SD of 5 analyses.

Table 2. Changes in the levels of hydroxyl radical scavenging and antioxidant activities of acetone extracts (50 to 250 µg/ml) of *P. biglobosa* and *T. conophorum* stem barks.

Concentration (µg/ml)	*P. biglobosa* stem bark		*T. conophorum* stem bark	
	Hydroxyl radical scavenging (%)	Antioxidant activity (%)	Hydroxyl radical scavenging (%)	Antioxidant activity (%)
50	-9.96±5.84	55.26±1.30	-24.11±3.36	-5.67±1.47
100	-1.65±5.29	49.88±1.72	-21.07±2.26	-14.89±1.77
150	-10.12±5.43	27.18±6.66	-16.96±1.24	-9.31±2.21
200	-19.50±8.35	-5.87±1.61	-26.17±4.15	-26.98±9.63
250	-29.60±7.95	-11.06±2.03	-26.66±6.37	19.63±1.73

Values are mean ± SD of 5 analyses.

P = 0.1, 0.05, 0.01, 0.001). The total phenolic content showed non-significant negative correlation with antioxidant activity in the aqueous extract of *P. biglobosa* at 10 min extraction time (r = -0.15; P = 0.05, 0.01, 0.001). At 50 min extraction time, a weak non-significant positive correlation was observed between total phenolic content and antioxidant activity for the aqueous extract of *P. biglobosa* stem bark (0.14; P = 0.05, 0.01, 0.001).

Moreover, non-significant positive correlations were observed between these two parameters at 30 to 50 min.

In our past research work (Olabinri et al., 2010a), we have shown weak negative non-significant correlation between total phenolics and antioxidant activity for the aqueous seed extract of *Tetracarpidium conophorum* at all concentrations. However, weak positive non-significant correlations were observed between total phenolic content and *in vitro* antioxidant activity for the aqueous extract of *T. conophorum* stem bark in the present study between extraction time intervals (20 to 50 min) (r = 0.31, 0.61, 0.35 and 0.49, respectively; P = 0.05, 0.01, 0.001). At 10 min extract time, a moderate negative non-significant correlation was observed between these two parameters in aqueous extract of *T. conophorum* stem bark.

A strong relationship was observed between total phenolic content and antioxidant activity in selected plants extracts by some researchers (Javanmardi et al., 2003; Velioglu et al., 1998). In addition, some reports revealed that there was no relationship between the total phenolic content and the antioxidant activity (Hinneburg et al., 2006; Motalleb et al., 2005) even though it was demonstrated that the phenolic substances are responsible for the antioxidant activity of plant materials (Rice-Evans et al., 1996).

Plant natural antioxidant sources are primarily plant phenolics which may occur in virtually all plants (Kahkonen et al, 1999; Pratt and Hudson, 1992). Plant-derived phenolic compounds are well known to exhibit antioxidant activity through a variety of mechanisms, including free radical scavenging, lipid peroxidation and chelating of metal ions (Shahidi et al., 1997). *P. biglobosa* and *T. conophorum* are plant recognized to be very rich in phenolic compounds (Kouadio et al., 2000; Tringali et al., 2000; De and Ifeoma, 2002; Enujiugha, 2003). The phenolic composition and content depends on different factors such as infusion time, the water to-part of plants

ratio (leaf, stem bark) and the amount of agitation (Astill et al., 2001). The molecular conformation of phenolic compounds could be one of the factors affecting their antioxidant activity which is intrinsically related to DPPH (Silva et al., 2007).

Hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology (Wang et al., 2006).

Conclusion

Both polyphenol extracts of *P. biglobosa* and *T. conophorum* stem barks failed to scavenge hydroxyl radical *in vitro* while the maximum antioxidant activity of both aqueous and polyphenol extract of *P. biglobosa* stem barks was significantly higher than that of the aqueous and polyphenol extract of *T. conophorum* ($P < 0.05$). The present study revealed that *P. biglobosa* is a better source of natural antioxidants than *T. conophorum.*

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