Int J Pharm Bio Sci 2012 July; 3(3): (P) 312 - 321

Research Article **Natural Chemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ANTIOXIDANT ACTIVITY OF METHANOL AND ETHANOL/WATER EXTRACTS OF *TETRAPLEURA TETRAPTERA* AND *PARKIA BIGLOBOSA*

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ABSTRACT

Antioxidants present in natural sources help to scavenge free radicals and thus provide health benefits. This study reports *in vitro* radical scavenging and antioxidant capacity of crude methanol and ethanol-water extracts of the fruits of *Tetrapleura tetraptera* and *Parkia biglobosa*. Total phenolic contents in *Tetrapleura tetraptera* were 147.82±1.36 and 130.33±1.04 mg GAE/g dry weight while that of *Parkia biglobosa* were 128.32± 0.49 and 127.23±0.11 mg GAE/g dry weight respectively. The total antioxidant capacity of the extracts ranges from175.52±4.66 (methanol) to 172.87±2.15 mg/g (ethanol/water) for *Tetrapleura tetraptera* and from 160.44±2.26 (methanol) to 157.31±1.90 mg/g (ethanol/water) for *Parkia biglobosa*. The antioxidant activities of both fruits determined by the 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) and the reducing power (RPA) assays produced concentration-dependent values comparable to that of ascorbic acid control. The results of the study showed that fruits of *Tetrapleura tetraptera* and *Parkia biglobosa* have strong radical scavenging and reducing capacities.

KEYWORDS : Antioxidant activity, *Tetrapleura tetraptera, Parkia biglobosa*, free radicals, scavenging activity, antioxidants

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INTRODUCTION

Reactive oxygen species and free radicals formed during oxidation have been reported to contribute to diseases such as cancer, diabetes, cardiovascular diseases and ageing [1]. Anitoxidants have the ability to protect the body from oxidative damage [2] by scavenging the free radicals and inhibiting peroxidation and other radical mediated processes. In recent years, significant attention has been directed towards exploring plant-based natural antioxidants, especially the phenolics and tocopherols [3,4,5]. Such natural antioxidants are not only reported to have anti-carcinogenic potential that protects the foods from oxidative deterioration but also, these are associated with other health beneficial effects such as, lowering the incidence of aging, inflammation, cardiovascular diseases and certain cancers [6,4,7,8]. Various antioxidant activity methods have been used to monitor and compare the antioxidant activity of food [9]. They may be free radicals which possess an unpaired electron in their outermost shell and are capable of independent existence [9]. Their half-lives vary from a few nanoseconds for the most reactive compounds to seconds and hours for rather stable radicals. They trigger chain reactions resulting in the oxidation of macromolecules in order to reach a steady state [10].

Polyphenolic compounds constitute a crucial category of antioxidant metabolites. They are plant secondary metabolites which have at least one aromatic ring in their molecule and usually exist in the form of glycosides. They protect plants against harmful environmental conditions and the attack of microorganisms and contribute to the development of several characteristics such as color [11].

Tetrapleura tetraptera and *Parkia biglobosa* are two widely utilized plants in West Africa for their perceived nutritional and medicinal value [12].The fruits of both plants are used in foods for flavor and seasoning of different traditional dishes. Ethnomedical practices throughout West Africa use these plants for the treatment of several ailments including arthritis, asthma, diabetes mellitus, hypertension, epilepsy, schistomiasis, and even prevention of post-partum contraction [13] and gastro-intestinal disorders are attributable to the bioactivity of phytochemicals in the fruits of *Tetrapleura tetraptera* [12]. The phyto constituents of the fruits of *Parkia biglobosa* are also used in West African ethnomedicine to treat leprosy and hypertension. Taken together, anecdotal evidence from nutritional supplementation and ethnomedicinal practices suggests that the biologically active molecules in the fruits of *Tetrapleura tetraptera* and *Parkia biglobosa a*re present a potential source of drug leads.

Prior studies have confirmed the presence of glycosides, flavonoids, saponins, essential oils, saponosides, triterpenes, coumarins, tannins, sugars, steroids, tritepene glycosides, tannins, and polyphenolic compounds as phyto constituents in the fruits of *Tetrapleura tetraptera* and *Parkia biglobosa*. The fruit of *Tetrapleura tetraptera* shows an additional presence of alkaloids. Since tannins, flavonoids and polyphenols represent molecules generally known for antioxidant bioactivities [12,14], their collective presence in both fruits suggests the possibility that one or more constituents of the chemically diverse compounds in these fruits will exhibit antioxidant properties [14]. However, neither the fruits of *Tetrapleura tetraptera* nor those of *Parkia biglobosa* have been screened for antioxidant activities in vitro. In view of the potential that exists for developing an efficacious antioxidant from one or more phytoconstituents of both fruits, an examination of the antioxidant activities of the polar extracts of the fruits of *Tetrapleura tetraptera* and *Parkia biglobosa* were conducted.

This study characterized the phytochemical composition of the methanol and water/ethanol extracts of *Tetrapleura tetraptera* and *Parkia biglobosa* fruits, quantified the total

polyphenol content and total anti-oxidant capacity of the methanol and ethanol/water extracts of *Tetrapleura tetraptera* and *Parkia biglobosa* fruits and assessed the antioxidant bioactivities of the methanol and ethanol/water extracts of *Tetrapleura tetraptera* and *Parkia biglobosa* fruits using the DPPH and the RPA assays.

MATERIALS AND METHODS

Collection and Preparation of Fruits

Fruits of *Tetrapleura tetraptera* and *Parkia biglobosa* were obtained from the Central market of Kumasi, Ghana. Purchased fruits were identified by a botanist at the College of Agriculture of the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. Voucher specimen was deposited at the School of Botany of the KNUST. Prior to extraction, fruits were initially air dried, and the dried fruits were pulverized and stored in an air tight bottle.

Chemicals

2,2-Diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide, potassium Sodium phosphate, gallic acid, sodium carbonate, ascorbic acid and ammonium molybdate were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Ferric chloride, Methanol, Folin-Ciocalteu phenol reagent and other chemicals were obtained from Merck Chemical Supplies (Damstadt, Germany). All reagents used were of analytical grade.

Plant Material Extraction

To 50 g of each pulverized dried fruits was added 500 ml methanol (99%) in the Soxhlet apparatus. Extraction of phyto constituents was then performed for 10 hours. After the extraction, the solvent was removed under vacuum at 45^{o}C using a rotary evaporator and the resulting residue stored at -4^{o}C until further used for analysis. To obtain the ethanol/water extract of both fruits, 500 ml of 1:1 mixture of distilled water: 98% ethanol was added to 50 g of each pulverized dried fruits in a Soxhlet apparatus. Extraction and drying of samples were performed as earlier described.

Basic Phytochemical Screening

To determine the broad classes of phytoconstituents of both fruits, samples of the methanol and water/ethanol extracts of both fruits were assayed for the presence Tanins, Flavonoids, Saponins, Glycosides, Anthraquinone Glycosides, alkaloids, carotenoids and cumarins of basic phytochemicals as described by Trease & Evans, [14].

Determination of Total Phenolic Content

The total polyphenolic compounds in the extracts were quantitatively determined by slight modifications of the colorimetric assay using Folin-Ciocalteu's reagent. Gallic acid concentrations of 0.01, 0.05, 0.10, and 0.15 mg/ml were used as a standard phenolic compound [15]. A 10 mL of extract solution containing 1.0 g of extract in acetone was placed in a 100 mL volumetric flask and diluted with 45 mL of methanol. The solution was mixed thoroughly with 1.0 mL of Folin-Ciocalteu's reagent for 5 mins. A 3.0 mL of 2 % sodium carbonate solution was then added to the mixture. The reaction mixture was then allowed to stand for 3 hours with intermittent shaking. The absorbance of the solution was measured at 760 nm using the UV-Visible spectrophotometer (UV mini 1240, Shimadzu, Kyoto, Japan). All measurements were done in triplicate. The content of total phenolic compounds was expressed as mg/g of dry extract in Gallic acid equivalents (GAE) [16].

Total Antioxidant Capacity (TAC) Assay

The total antioxidant capacity was evaluated using the method described by Prieto et al., [17] with slight modifications. Ascorbic acid of concentrations 0.01, 0.05, 0.10 and 0.15 mg/ml was used as the standard antioxidant drug. A 3 ml solution of the extract in acetone was placed

in a test tube. A 0.3 ml of the reagent solution [(0.6 M Sulphuric acid, 28 mM Sodium phosphate and 4 mM Ammonium molybdate)] was then added to the solution of the extract and the resulting mixture was incubated at 95^{o}C for 90 minutes. After the mixture has cooled to room temperature, the absorbance of each solution was measured in triplicates against a blank in a UV-Visible spectrophotometer (UV mini 1240, Shimadzu, Kyoto, Japan) at 695 nm. The total antioxidant capacity was expressed as Ascorbic Acid Equivalents (AAE).

DPPH radical scavenging activity

A 10 ml solution of the fruit extract containing 0.1, 0.3, 1, 3 mg/ml in methanol and ethanol/water were added to 3 ml of 0.002% DPPH solution. After 30 minutes incubation at room temperature in the dark, the absorbance was read against blank at 517 nm. The blank used was methanol and the control was solution of 5 ml methanol and 5 ml DPPH solution (0.002%).

Reducing Potential Assay (RPA)

Solutions containing 0.1, 0.3, 1 and 3 mg/ml each of methanol extract in methanol and ethanol/water extracts in ethanol were prepared. A solution containing 1 mL distilled water, 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1 % potassium ferricyanide solution in a test tube was added each solution of the extracts. The mixture was incubated at 50 ^{o}C for 20 mins. A 2.5 ml of 10 % trichloroacetic acid solution was then added to the incubated mixture and centrifuged at 3000 rpm for 10 mins. A portion of the supernatant (2.5 ml) was taken and mixed with 2.5 ml distilled water and 0.5 ml of 0.1% ferric chloride solution in a test tube. The absorbance of the final solution was then measured at 700 nm using the UV-Visible spectrophotometer (UV mini 1240, Shimadzu, Kyoto). Increase in absorbance of the reaction mixture indicated increased reducing power [18].

Statistical Analysis

All experiments were performed in triplicates. The descriptive statistics (mean and standard deviation) were conducted using Microsoft Excel software. A one-way ANOVA statistical procedure was employed in the assessment of variation in polyphenol concentrations using different extracts.

RESULTS AND DISCUSSIONS

Phytochemical Screening

Table 1
***Phytochemical screening results on the extracts of* Tetrapleura tetraptera *and* Parkia biglobosa**

TEST	*TETRAPLEURA TETRAPTERA*	*PARKIA BIGLOBOSA*
Tannin test	Present	present
Anthraquinone Glycosides	present	present
Carotenoids	absent	absent
Saponin	present	present
Alkaloids	present	absent
Flavonoids	present	present
General Glycosides	present	present
Coumarin	absent	absent

The fruits of *Tetrapleura tetraptera* and *Parkia biglobosa* contain a mixture of phytochemicals used in nutritional supplementation and in diverse ethnomedicinal practices throughout West Africa [13]. Both *Tetrapleura tetraptera* and *Parkia biglobosa* fruits contain multiple compounds as demonstrated by the phytochemical screen of the methanol and ethanol/water extract. The screen confirmed literature reports of the presence of glycosides, flavonoids, saponins and tannins in both plants and the additional presence of alkaloids in *Tetrapleura tetraptera*. Report by Eze et al, (2010) [19] on Phyto-chemical analysis of *Tetrapleura tetraptera* showed that, saponin, alkaloid, flavonoid, glycoside and reducing sugars were present.

Total polyphenol content

Plant phenolics are gaining continuing interest as ingredients for functional foods and nutriceutical applications due to their potential health benefits [5]. Phenolic compounds from plants are known to act as very good natural antioxidants [20]. Many studies reported that total phenols and flovonoids contribute significantly to the antioxidant activity of fruits and vegetables [4]. Estimated total polyphenolic compositions of the methanol extracts were marginally higher than that of the ethanol/water extracts for both fruits. The amount of total phenolics (TP) in the fruits of *Tetrapleura tetraptera and Parkia biglobosa* ranging from 127.23±0.11 to 147.82±1.36 are presented in table 2. Below.

Table 2

Total polyphenol content (mgGAE/g dry weight) of* Tetrapleura tetraptera *and* Parkia Biglobosa*.

Extract (Dawadawa)	*Tetrapleura tetraptera* (Prekese)	*Parkia Biglobosa*
Methanol	147.82±1.36	128.32± 0.49
Ethanol/Water	130.33±1.04	127.23±0.11

The amount of phenolic compounds extracted from the fruits using the two different solvents varied significantly ($p < 0.05$) with regard to the extraction solvent and material used. Ethanol is usually preferred for the extraction of antioxidant compounds from plant matrices mainly due to its toxicity and good extraction efficacy [21,22] .

Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities [23]. The correlation between total phenol contents and antioxidant activity has been widely studied in different foodstuffs such as fruit and vegetables [24-27].

Phenolic compounds are a class of antioxidant agents which act as free radical terminators [28,29]. They are considered as a major group of compounds that contribute to the antioxidant activities of plant materials, because of their scavenging ability on free radicals due to their hydroxyl groups [30]. Flavonoids and tannins are also a group of polyphenolic compounds which possess diverse biological activities such as anti-inflamatory, anti-carcinogenic and anti atherosclerotic activities. These activities may be related to their antioxidant activity [31-34]. It has been established that phenolic compounds contribute to the quality and nutritional value in terms of modifying color, taste, flavor and aroma. They are also used in plant defense mechanisms to counteract reactive oxygen species in order to prevent molecular damage and damage by microorganisms, insects and herbivores [35].

Antioxidant activity

It is established that the function of reactive species is highly related to their concentration. The detrimental effects of oxidative stress are linked to the disruption of normal signaling pathways, damage of macromolecules, and disruption of homeostasis. It should be noted that oxygen is not evenly distributed in tissues

and cells; therefore, there are sites that are under physiological oxidative stress [10].

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples [36]. DPPH is very stable. It was found that the radical- scavenging activities of all the extracts increased with increasing concentration. Organic free radical with deep violet colour gives absorption maxima within 515 and 528 nm. Upon receiving proton from any hydrogen donor, mainly from phenolics, it loses its chromophore and becomes yellow. It is widely accepted that as the concentration of the phenolic compounds increases, DPPH radical scavenging activity and hence antioxidant activity of a plant or a related compound also increases [37].

The measurement of the scavenging of DPPH radical allows one to determine exclusively the intrinsic ability of substance to donate hydrogen atom or electrons to this reactive species in a homogenous system. The method is based on the reduction of methanolic-DPPH solution because of the presence of antioxidant substances having hydrogen donating groups (RH) such as phenolic and flavonoid compounds due to the formation of a non-radical DPPH-H form [38]. The phytochemicals which might be responsible for the scavenging activity in this species are the phenolic and flavonoid constituents [39].

The antioxidant activity of the methanolic and ethanolic/water extracts of *Tetrapleura tetraptera* and *Parkia biglobosa*, were observed in the present study by in vitro assays such as DPPH, and the Total Antioxidant Capacity and Reducing Power Assays to evaluate the free radical scavenging activity and antioxidant capacity of the extracts.

Substantial antioxidant capacities were observed for both fruits with the methanol extracts exhibiting relatively higher values than the ethanol/water extracts (Table 3 and 4).

Table 3
***DPPH Scavenging Activity (I%) of Methanol and Ethanol/Water Extracts of* Tetrapleura tetraptera**

Concentration (mg/ml)	Methanol (I%)	Ethanol/Water (I%)	Ascobic Acid (I%)
0.01	30.70±0.37	32.98±0.13	66.87±0.82
0.03	36.58±0.05	39.89±1.29	69.56±0.14
0.1	68.35±1.45	69.49±0.00	75.23±0.00
0.3	74.62±0.07	77.78±0.80	78.62±0.03

Table 4
***DPPH Scavenging Activity of Methanol and Ethanol/Water Extracts of* ParkiaBiglobosa**

Concentration (mg/ml)	Methanol (I%)	Ethanol/Water (I%)	Ascobic Acid (I%)
0.01	30.25±0.42	31.72±1.92	66.87±0.82
0.03	31.67±0.28	31.70±0.09	69.56±0.14
0.1	56.83±0.65	58.17±0.13	75.23±0.00
0.3	68.27±0.01	69.23±0.00	78.62±0.03

Antioxidant capacities generally correlated with total polyphenol content with *Tetrapleura tetraptera* exhibiting higher antioxidant potential than *Parkia biglobosa*.

Estimated antioxidant activities using the DPPH assay shows the inhibition of free radical (I%) for both *Tetrapleura tetraptera* (Table 3) and *Parkia biglobosa* (Table 4) to be concentration dependent. Ethanol/water extracts of both fruits exhibited higher I% than the methanol extracts for all examined concentrations. Since the methanol extracts of both fruits possess higher polyphenol contents and higher antioxidant capacities, this observation suggests that other phytoconstituents, in addition to polyphenols, contributed to the disappearance of the DPPH radical absorption and, thus, to the overall antioxidant activities of both fruit extracts. In all cases, the I% of ascorbic acid control was higher than that of both extracts of both fruits with minimal I% differences occurring at higher sample concentration (0.3 mg/ml). In comparing the scavenging activity of the extracts of methanol and ethanol/water solvent systems, there was a high correlation ($P<0.05$) which suggest that both solvent systems have similar predictive capacity for free radical scavenging for both *Tetrapleura tetraptera* and *Parkia biglobosa*.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing ability of a compound generally depends on the presence of reductones, which have exhibited antioxidative potential by breaking the free radical chain and donating a hydrogen atom [31]. Increase in absorbance values correlated with increase in concentration for both extracts of *Tetrapleura tetraptera* and *Parkia biglobosa* fruits. The highest reduction capabilities for both fruits were displayed at 0.3 mg/ml with the methanol extract of *Tetrapleura tetraptera* exhibiting the highest reductive potential. In all cases, absorbance values correlated with polyphenol content and antioxidant capacities. For all samples, the methanol extracts exhibited higher electron-donating ability than for the ethanol/water extracts. Absorbance values for ascorbic acid controls at all concentrations were higher than those for both extracts of both fruits with higher absolute absorbance differences observed at higher sample concentrations (0.3 mg/ml).The reducing power of the various samples for both fruits followed this decreasing order: ascorbic acid >methanol extract >ethanol/water extracts (Fig 1 and Fig 2).

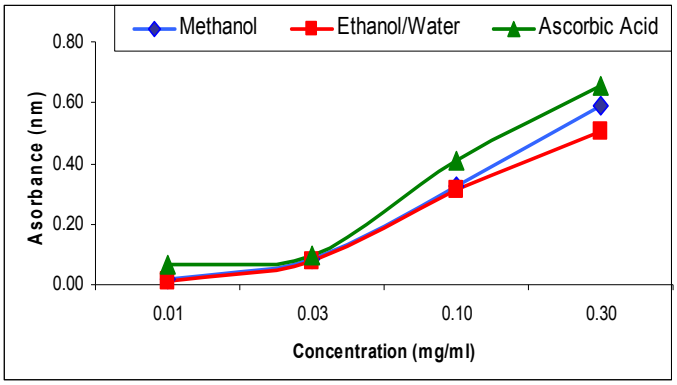

Figure 1
Reducing Power of The Methanol And Ethanol/Water* Tetrapleura tetraptera *Extracts And TheStandard Ascobic Acid

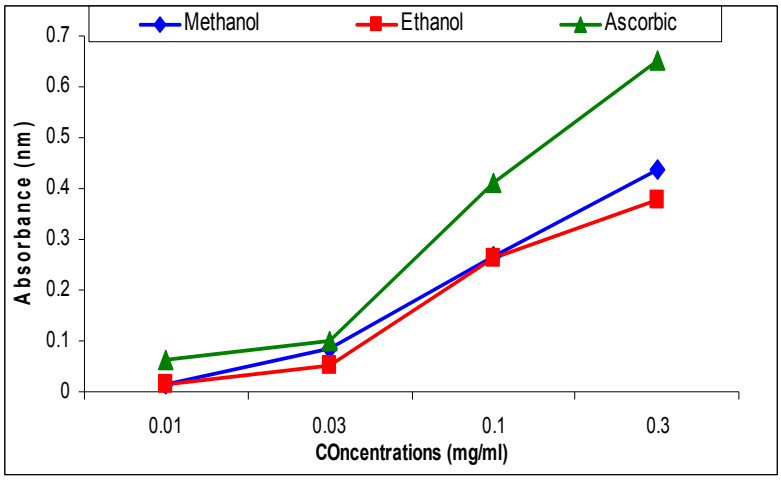

Figure 2
Reducing Power of The Methanol And Ethanol/Water* Parkia biglobosa *Extracts And The Standard Ascobic Acid

The demonstrated presence of diverse biologically active molecules including glycosides, flavonoids, saponins and tannins in the fruits of both plants confirms literature reports [12,23] and suggests that both fruits show promise as sources of natural products antioxidant drugs leads.

CONCLUSION

The fruits of *Tetrapleura tetraptera* and *Parkia biglobosa* have strong radical scavenging and reducing activities. Both fruits display remarkable chemodiversity with the notable presence of polyphenols that accounts largely for the observed antioxidant activities. The extracts of both fruits showed antioxidant activities that are quantitatively comparable to that of ascorbic acid. The extracts of *Tetrapleura tetraptera* showed relatively higher antioxidant activities than that of *Parkia biglobosa*. This study provides valuable scientific information for the ethnomedicinal and dietary use of fruits of *Tetrapleura tetraptera* and *Parkia biglobosa* by the local people of the Ashanti and Northern Regions of Ghana.

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