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Free Radical Scavenging Activity of Different Parts of *Tetrapleura tetraptera* (Schumach. and Thonn.) Taub.

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Abstract: This study evaluated the free radical scavenging activity of fruit wing, seed, leaf and stem bark of *Tetrapleura tetraptera* (Fabaceae) with a view to providing scientific information on its potential in the management of diseases. The antioxidant activity was evaluated against 1, 1 diphenyl-2-picrylhydrazyl (DPPH) radicals. The phytochemical and proximate components of the plant were determined using standard laboratory techniques. The mature seeds (68.7%) had the highest % inhibition against DPPH radicals ($DPPH^+$), followed by the leaf (64.6%) and the stem bark (46.2%). The flavonoids (766.7 mg/100g) and phenolics (59.3 mg GAE/g) contents were highest in mature fruits, followed by the leaf with 318.33 mg/100g of flavonoids and 56.0 mg GAE/g of phenolics. In addition to phytochemicals, the leaf had the highest quantity of ash (11.2%); crude fibre (20.1%); crude protein (18.9%) and dry matter (92.7%) compared to other plant parts. There was correlation between phenolics content and antioxidant activity of the different parts of the plant; the higher the phenolics content the more the antioxidant activity. The findings of this study confirmed the varied antioxidant activity of different parts of *T. tetraptera*. Although the fruit is commonly used in ethnomedicine, the leaf is also regenerative and could be used for therapeutic purpose based on its valuable phytochemical components and antioxidant activity, especially when the fruit is not in season. Furthermore, the fruit and leaf of the plants could have therapeutic effect in the management of metabolic and degenerative diseases.

Key words: Tetrapleura tetraptera · Antioxidant activity · Phenolic content · Nutritional value · Fabaceae

INTRODUCTION

Tetrapleura tetraptera is a species of flowering plant in the pea family (Fabaceae) and native to West Africa [1] and generally found in low lands of tropical Africa. In Nigeria, it is referred to as “Aridan” in the west, “Oshosho” in the east and “Dawo” in the north. *T. tetraptera* is an economic plant, the fruit as food is very rich in sugar, oil and vitamins, the reddish hard and heavy wood of the plant is used for firewood, building poles, pestles, tool handles and carvings [1]. Its fruits and leaves are widely used medicinally in Nigeria and West Africa in general. The tannin content of the fruit is used as dye, oil, pomade, ointment; perfume and soap produced from the fruit are used as cosmetics. As food additive, the fruit is used as spice and condiment in traditional soups in Southern and Eastern Nigeria. The wastes from fruits and wood are carbonised to produce charcoal, the wood as fire wood and biodiesel could be produced from the fruit and leaf oil. Overall, the fruit of *T. tetraptera* is sold in markets as item of commercial [1-4].

Medicinally, an infusion of the whole fruit is usually taken by convalescents to bathe in order to get relief from feverish conditions. It is used as an enema and emetic. It is valuable in the management of constipation [5]. An infusion of the whole fruit is taken as a recuperative tonic [6]. Also the fruits have been reported to have nutritional, antiparasitic, antidiabetic, analgesic and anti-inflammatory properties [4, 7, 8]. The decoction of *Tetrapleura tetraptera* bark is used to treat tummy-ache, vomiting, fever and headache. It is also used as an anthelmintic and purgative agent at low doses. People also use this plant around food crops to protect them against pests [9]. *T. tetraptera* forms part of traditional recipe used in the management of neonatal jaundice [1, 10]. Its potency in the treatment of skin

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infections has been reported [11]. The pods of the plant have an interesting gastronomic use for mothers from the first day of delivery to post parturition and as a lactation aid [12].

In humans, oxidative stress could be caused by smoking, environmental pollution, consumption of fast food, inadequate intake of fruits and vegetables, lack of good nutrition, inadequate amounts of physical activity and excessive exercise. Oxidative stress causes production of free radicals. Free radicals are molecules with unpaired electron on their outer orbit. They are formed naturally in the body and play an important role in many cellular processes. Over production of free radicals can cause oxidative damage to biomolecules (lipids, protein and DNA) eventually leading to many chronic diseases such as atherosclerosis, cancer, diabetes, rheumatoid arthritis, myocardial infarction, stroke, aging and other degenerative diseases in humans [13-15].

An antioxidant is a molecule stable enough to donate electron to a rampaging free radical and neutralise it, thus reducing its ability to damage cells. Antioxidants are classified as exogenous (natural or synthetic) or endogenous compounds, both responsible for removal of free radicals, scavenging ROS (Reactive Oxygen Species) or their precursors, inhibiting formation of ROS and binding metal ions needed for catalysis of ROS generation [16]. Antioxidants have been reported in epidemiological studies as compounds that have anti-inflammatory, anti-atherosclerotic, anti-tumour, anti-carcinogenic, antibacterial and anti-viral activity [15]. Functional foods such as fruits, vegetables and medicinal "herbs" are promising antioxidant compounds.

In view of the above, this study investigated the free radical scavenging activity, phytochemical profile and nutritional components of fruit, leaf and stem bark of *T. tetraptera*. This was with a view to providing more information on the sustainable use and therapeutic potential of the plant in management of metabolic and degenerative diseases.

MATERIALS AND METHODS

Collection and Identification of Plant: *Tetrapleura tetraptera* was collected from the University of Ibadan botanical garden and identified at the University of Ibadan Herbarium (UIH). A sample of the plant was deposited at UIH. The plant parts were dried, powdered and stored at 4°C for experiments.

Free Radical Scavenging Activity of Samples: The extracts (100mg/ml) of samples were prepared in methanol and 0.2ml of each extract was added to 2.8ml of freshly prepared 20mg/dm^3 DPPH in methanol. The mixture was incubated for 20min in the dark at room temperature and the absorbance was measured at 517nm using UV/VIS SHIMADZU brand UVmin1240 spectrophotometer. Methanol only was used as the blank to adjust the spectrophotometer to zero absorbance and DPPH only in methanol was used as the control. The scavenging activity of each extract was calculated [17] as follows:

$$\% \text{ Inhibition of DPPH}^+ = [1 - (A_{sample} / A_{control})] \times 100$$

$A_{control}$ is Absorbance for control and A_{sample} is Absorbance for test sample

Phytochemical Screening of Plant Samples: The samples were screened for the presence of alkaloids, cardiac glycosides, carotenoids, flavonoids, phenolics, saponins and tannins using standard techniques [18-21].

Proximate Analysis of Plant Samples: The carbohydrate, crude fibre, crude protein, fat, moisture and total ash contents of samples were analyzed using AOAC protocols [21] in the Laboratory of the Department of Animal Science, Faculty of Agriculture and Forestry, University of Ibadan, Nigeria.

Data Analysis: Data were expressed as mean ± standard deviation (SD) using analysis of variance (ANOVA). Values were expressed as mean ± standard deviation and considered significant at $P < 0.05$.

RESULTS

The plant parts showed varied antioxidant activity against DPPH radicals (Table 1). The mature seed (68.7%) had the highest % inhibition, followed by the leaf (64.6%) and mature wing (57.4%), whereas the stem bark (46.2%) had the least. There was significant difference in the scavenging ability of the juvenile wing (47.3%) and the stem bark (46.2%). Overall, the seeds (mature and juvenile) and leaf had significantly high antioxidant activity.

The stem bark (840.0 ± 31.2 mg/100g) had highest content of alkaloids (Table 2); followed by the juvenile fruit (648.3±23.6 mg/100g) and the leaf (190.0±5.00 mg/100g) had the least. The mature fruit (766.7±12.58 mg/100g) was very rich in flavonoids.

Table 1: Antioxidant activity of different parts *Tetrapleura tetraptera* against DPPH radicals

Plant parts	Bleaching of DPPH solution	% Inhibition of $DPPH^+$	Phenolics (mg GAE/g)
Leaf	+++	64.6^b±0.32	56.5±0.20
Juvenile wing	++	47.3^c±0.25	35.1±0.15
Juvenile seed	++	64.3^b±0.15	
Mature wing	+++	57.4^c±0.21	59.3±0.31
Mature seed	+++	68.7^a±0.47	
Stem bark	++	46.2^d±0.40	31.4±0.27

Legend: +++=High, ++=Moderate, +=Low; Values are mean ± SD; n=3; GAE = Gallic Acid Equivalent

Table 2: Phytochemical components of different parts *Tetrapleura tetraptera*

	Phytochemical Components						
Plant parts	Alkaloids (mg/100g)	Cardiac glycosides (mg/100g)	Carotenoids (μg/100g)	Flavonoids (mg/100g)	Phenolics (mg GAE/g)	Saponins (mg/100g)	Tannins (mg/100g)
Leaf	190.0^d ±5.00	95.0^c ±10.0	1283.3^b ±22.5	318.33^b ±2.89	56.5^b ±0.20	253.3^a ±12.6	235.0^d ±8.66
Juvenile fruit	648.3^b ±23.6	218.3^a ±10.4	891.7^c ±2.89	86.7^c ±2.89	35.1^c ±0.27	25.0^d ±5.00	1245.0^a ±15.0
Mature fruit	323.3^c ±10.4	76.7^c ±7.64	1642.0^a±7.64	766.7^a ±12.58	59.3^a ±0.31	60.0^b ±5.00	403.3^c ±18.9
Stem Bark	840.0^a ±31.2	255.0^b ±22.9	231.7^d ±22.5	61.7^d ±5.77	31.4^d ±0.27	41.7^c ±2.89	578.3^b ±11.5

Legend: Values are mean ± SD; n=3; GAE = Gallic Acid Equivalent

Table 3: Proximate components of different parts *Tetrapleura tetraptera*

	Parameter (%)						
Plant parts	Ash	Carbohydrate	Crude fibre	Crude protein	Dry matter	Ether extract	Moisture content
Leaf	11.0^a±2.00	44.0^d±4.00	20.1^a±0.20	18.9^a±0.05	92.7^a±0.05	6.0^a±3.00	7.4^d±0.25
Juvenile fruit	4.0^b±1.00	76.7^b±0.20	3.0^c±0.02	14.3^b±0.20	90.2^c±0.02	2.0^c±0.10	9.8^b±0.04
Mature fruit	5.0^b±0.10	80.8^a±0.20	3.33^c±0.10	8.8^d±0.15	68.5^d±0.15	2.2^{bc}±0.20	31.5^a±0.20
Stem bark	4.0^b±1.00	66.8^c±0.15	12.0^b±2.00	12.3^c±0.05	91.0^b±0.04	5.0^{ab}±1.00	9.0^c±0.02

Legend: Values are mean ± SD; n=3.

The phenolics content was in the order: mature fruit (59.3±0.31 mg GEA/g) > leaf (56.5±0.20 mg GEA/g) > stem bark (31.4±0.27 mg GEA/g). The plant parts differed significantly ($P < 0.05$) in phytochemical composition.

The leaf had the highest values for ash content (11.0±2.00 %), crude fibre (20.1±0.20%), crude protein (18.9±0.05 %), dry matter (92.7±0.05%) and ether extract (6.0±3.00 %) (Table 3). The mature fruit was very rich in carbohydrate (80.8±0.20%) and moisture (31.5±0.20%) contents. Other parts of the plant had varied proximate constituents.

DISCUSSION

The free radical scavenging activity observed in the present study is in line with previous reports on antioxidant activity of *T. tetraptera* [22, 23]. The relatively high % inhibition of DPPH radicals by the mature seed (68.7%) supports the use of the mature fruit in ethno-medicine. The seed could be harvested, powdered and stored in airtight bottle for future use to ensure the availability of the plant throughout the season. Of importance is the fact that the leaf (64.6%) could be used as substitute to fruit based on its antioxidant activity. The observed antioxidant activity against $DPPH^+$ was in the order: Mature seed (68.7%) > Leaf (64.6%) > Juvenile seed (64.3%) > Mature wing (57.4%) > Juvenile wing (47.3%)> Stem bark (46.2%). The antioxidant effect of the fruit could be responsible for its reported bioactivities by previous authors [4, 8]. *T. tetraptera* fruit has antidiabetic, analgesic, anticonvulsant and anti-inflammatory properties. The aqueous extract of the fruit could be effective in the management of arthritis [4, 8, 24]. Furthermore, synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been reported as promoters of carcinogenesis, hence, plant derived antioxidant agents are valuable alternatives to synthetic antioxidant because they are readily available and have no side effects in regimen.

The literature is replete with reports on antioxidant activities of phytochemicals. Flavonoids have scavenging and chelating abilities. They have been reported to have antiviral, antiallergic, antiplatelet and anti-inflammatory, antitumor and antioxidant activities. Of importance is the role of flavonoids in chemoprevention of cancer [25, 26, 27]. In the present study, the flavonoids content of *T. tetraptera* was in the order: Mature fruit (766.7 mg/100g) > Leaf (318.33 mg/100g) > juvenile fruit (86.7 mg/100g) > Stem bark (61.7 mg/100g). Carotenoids could have protective effect against cancer and cardiovascular diseases [28]. The carotenoids content of different parts of *T. tetraptera* was as follows: Mature fruit (1642.0 μg/100g) > Leaf (1283.3 μg/100g) > Juvenile fruit (891.7 μg/100g) > Stem bark (231.7 μg/100g). Phenols manifest their activity by decreasing reactive oxygen concentration preventing formation of free radicals [29]. Phenolics content of *T. tetraptera* occurred in the order: Mature fruit (59.3 mg GAE/g) > Leaf (56.5 mg GAE/g) > Juvenile fruit (35.1 mg GAE/g) > Stem bark (31.4 mg GAE/g). Generally, the highest antioxidant activity displayed by the mature seeds could be attributed to major carotenoids, flavonoids and phenolics composition of the mature fruit.

The proximate constituents could complement the antioxidant activity of the phytochemicals especially in the leaf due to high content of ash (11.0%), crude fibre (20.1%), crude protein (18.9%) and fat (6.0%). Information on antioxidant activity of plant derived protein is available in literature. Ajibola *et al*. [30] reported the antioxidant activity of protein hydrolysate fractions of African yam bean seed (*Sphenostylis stenocarpa*). Also, soy protein hydrolysate obtained from *Glyxine max* after 48 h germination had best antioxidant potency and could be used as natural antioxidant in food system [31]. The high moisture and carbohydrate contents of the mature fruit could be responsible for its short shelf life [32].

CONCLUSION

The fruit, leaf and stem bark of *T. tetraptera* showed varied antioxidant activity against DPPH radicals with the mature seed being significantly active. The observed antioxidant activity could be due to the phytochemical components of various plant parts supplemented with nutrients. The therapeutic potential of the mature fruit could be a function of the significant antioxidant activity of mature seed. Furthermore, this study justifies the use of *T. tetraptera* fruits in traditional medicine and validates the potency of the leaf as an alternative to its fruit in regimen. Overall, the confirmed antioxidant activity of *T. tetraptera* validates its potential in the management of metabolic and degenerative diseases.

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