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Evaluation of antioxidant activity and phytochemical composition of *Tectona grandis* stem bark

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ABSTRACT

Oxidative stress is involved in the commencement and advancement of many human diseases such as inflammatory, ischemic, respiratory and some degenerative disorders. The aim of the present study was to investigate the antioxidant activity of *Tectona grandis* bark extracted with ethanol. Standard methods were used for the identification and quantification of the phytochemical composition of the plant. The antioxidant activity of the sample was evaluated using Ferric Reducing Antioxidant Power (FRAP), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-3-ethylbenzothiaziline-6- sulfonate (ABTS) radical scavenging assay and linoleic acid method. Phytochemical studies of all the crude extracts showed the presence of secondary metabolites such as tannin, phenol, flavonoid, glycoside, steroid, terpenoid and alkaloid in the concentration of 0.441±0.007, 0.486±0.006, 0.196±0.009, 0.357±0.020, 0.131±0.004, 0.644±0.01 and 0. 323±0.004 mg/100g respectively. The antioxidants results were expressed as IC_{50} (where 50 % concentration of the extract scavenged free radical). The results showed that DPPHA, FRAP, ABT and Linoleic acid had IC_{50} values of 0.126±0.05, 2.486±0.028, 0.0741±0.001 and 0.845±0.02, mg/mL respectively which indicate that the extract possess strong antioxidant activity.

Keywords: Antioxidant activity, ABTS, DPPH, FRAP, oxidative stress, phytochemicals and *Tectona grandis*

INTRODUCTION

The chemical reaction of donating or accepting an electron from biological molecules is termed oxidation or reduction and it is this process that gives rise to generation of free radicals in humans (Soni and Chauhan, 2015). The most important oxygen-containing free radicals in many disease states include hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite and nitric oxide radicals (Young et al., 2001). Free radicals occur naturally inside the body, and frequently, the body's natural antioxidants can take care of their detoxification but certain external factors such as pollution, smoking, unhealthy diet, excessive exercise and certain medications can provoke the generation of these disastrous free radicals. Oxidative stress mechanism deals with an enhanced production of Reactive Oxygen Species (ROS) and impaired antioxidant defense mechanisms (Patel *et al.*, 2012). Unrestrained reactive oxygen species (ROS) production can subdue cellular antioxidant fortification giving rise to pernicious conditions. Oxidative stress is involved in the commencement and advancement of many human ruinous diseases such inflammatory diseases, ischemic diseases, respiratory diseases and some degenerative disorders (Pham-Huy, 2006), which may occur through deleterious modification of DNA, protein and/or lipids (Finkel and Holbrook, 2000; Valko *et al.*, 2007). A great number of aromatic chemical compounds are used as antioxidants. Antioxidants are substances that show at low concentrations the ability to hinder or slow substrate oxidation in comparison with oxidizable substrates (Niki, 2010). It has been suggested that antioxidants such as flavonoids, tannins, coumarins, curcumanoids, xanthones, phenols, lignans and terpenoids found in various plant products as fruit, leaves, seeds and oils (Roby *et al.*, 2013) could inhibit cellular damage by scavenging the free radicals.

The present work aimed to determine the antioxidant activity of *Tectona grandis* bark extracted with ethanol using DPPH, FRAP, ABTS and linoleic acid assay. *Tectona grandis* is a tropical large, deciduous tree that is dominant in mixed hardwood forests in

the family *Lamiaceae*. It has small, fragrant white flowers and papery leaves that are often hairy on the lower surface. The plant has been traditionally used as medicines in southeast Asia, mainly India, Indonesia, Malaysia, Thailand and Burma as well as in Africa for destroying parasitic worms, treating and healing wounds, treating asthma attacks, promoting hair growth and as anti-inflammatory agent for reducing the inflammation of the skin as well as for preparing herbal remedies for increasing the passage of urine (Goswami et al., 2010). The high medicinal value of this plant prompted the investigation of their antioxidant properties.

MATERIALS

Plant material

Tectona grandis barks were sourced from Mushin, Ogun State, Nigeria. The plant material was identified and authenticated in the Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria, by Mr. Nwankwo.

Extraction

The fresh barks of *Tectona grandis* were cut into small pieces and air-dried at room temperature for six weeks. The dried pieces were reduced to powder by grinding and then weighed to obtain 4 portions of 100 g each. Each of 3 portions of the milled plant was macerated with 1500 mL of 95 % ethanol. Maceration was done for 3 h mechanically with the speed set at 400 rpm. After maceration with stirring for 3 h, filtration was carried out using Whatman filter paper (150 mm). Residues were re-macerated in ethanol also for 3 h with mechanical stirring (×2) to achieve exhaustive extraction.

METHODS

Phytochemical analysis

The phytochemical analysis was determined spectrophotometrically using standard methods.

Test for Steroids

Two *millilitres* of chloroform was added to 0.2 g of the extract and then 2 mL of concentrated tetraoxosulphate (vi) acid was added. The mixture was shaken for 5 min and allowed to stand for 30 min. Appearance of red colour at the lower layer indicated the presence of steroids (Rodney *et al.*, 2012

Test for Tannins

Ten *millilitres* of distilled water was added to 5 g of the extract, stirred and filtered. Then Ferric chloride reagent was added to the filtrate. The formation of a blue-green precipitate indicated the presence of tannin (Singh *et al.*, 1996).

Test for Glycosides

Five *millilitres* of dilute tetraoxosulphate (vi) acid was added to 0.2 g of the extract. The mixture was heated for 15 min. Fehling solution A and B was then added and the resulting mixture was heated for 15 min. The formation of a brick red precipitate indicated the presence of glycosides (Khaomek *et al.*, 2008).

Test for Saponins

Five *millilitres* of distilled water was added to 0.2 g of the extractand then shaken for 30 min. The formation of frothing, appearance of creamy mist of small bubbles indicated the presence of saponin

Test for Phenols

One percent of Ferric (iii) chloride in methanol/water (1:1) was added to 0.5 g of extract. A dirty green precipitate indicated the presence of phenols.

Test for Alkaloids

Five *millilitres* of 1 % hydrochloric acid solution was added to 0.5 g of the extract. The mixture was stirred on water bath and then filtered. Then Mayer's reagent was added to the filtrate. The formation of a white precipitate indicated the presence of alkaloids.

Test for Terpenoids

Acetic anhydride (0.5 mL) was added to 1 g of the extract and then 0.5 mL of concentrated tetraoxosulphate (vi) acid was added. The formation of a bluish green precipitate indicated the presence of terpenoids.

Test for Flavonoids

Two *millilitres* of dilute sodium hydroxide solution was added to 0.2 g of the extract, then 2 mL of concentrated Hydrochloric acid was added. A yellow colour was formed which turned colourless. This indicated the presence of flavonoids (Khaomek *et al.*, 2008). Antioxidant properties determination

Ferric Reducing Antioxidant Power (FRAP) assay

The ability of the extracts to reduce iron was determined according to the modified method of Benzie and Strain (1996) using trolox as standard

DPPH assay

DPPH radical scavenging capacity of the ethanol bark extract was determined according to the modified method described by (Repon *et al.*, 2013).

Free Radical-Scavenging Activity Using ABTS (ABTS Assay)

The ABTS assay was used to measures the relative antioxidant ability of extracts to scavenge the radical-cation ABTS + produced by the oxidation of 2,2′-azinobis-3-ethylbenzothiazoline-6-sulphonate. ABTS radical scavenging assay was determined using the method described by Oyaizu, 1988.

Linoleic acid bleaching

Linoleic acid test evaluates the inhibitory effect of a compound or a mixture on the oxidation of β-carotene in the presence of molecular oxygen (O2). Assay of the remained β-carotene gives an estimation of the antioxidant potential of the sample. The method described by Miraliakbari and Shahidi, 2008.

STATISTICAL ANALYSIS

The IC50 values were calculated from linear regression analysis using Microsoft Excel (2010) and values were expressed as means ± standard deviations.

RESULTS

Antioxidant analysis of T. grandis

Table 1 shows the IC50 data of the different antioxidant parameters used for determination of the antioxidant properties of T. grandis. The IC50 values were calculated to determine the concentration of the sample required to inhibit 50 % of the free radicals present in the system. The lower the IC50 value, the higher the antioxidant activity of samples. This study revealed that DPPHA, FRAP, ABT, Linoleic acid had IC50 values of 0.126, 2.486 and 0.0741, 0.845, mg/mL respectively

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Phytochemical analysis of *T. grandis*

Table 2 shows the result of the phytochemical analysis which indicate that tannin, phenol, flavonoid, glycoside, steroid, terpenoid and alkaloid were present in the following concentration: 0.441, 0.486, 0.196, 0.357, 0.131, 0.644 and 0. 323 mg/100g respectively .

Table 1: Antioxidant properties of *T. grandis*

ANTIOXIDANT PROPERTIES	**µg/Ml**
DPPHA	0.126±0.051
FRAP	2.486±0.027
ABTS	0.074±0.001
LINOLEIC	0.845±0.021

Table 2: Phytochemicals in *T. grandis*

PHYTOCHEMICALS	**mg/100g**
Tannin	0.441±0.007
Phenol	0.486±0.006
Flavonoid	0.196±0.009
Glycoside	0.357±0.009
Steroid	0.131±0.004
Terpenoid	0.644±0.01
Alkaloid	0.323±0.004

DISCUSSION

Deleterious free radical reactions play roles in many degenerative diseases that afflict humanity like neurodegeneration, diabetes, atherosclerosis, arthritis, ischemia, central nervous system injury, gastritis and cancer (Thagriki *et al.*, 2017; Igoli *et al.*, 2005). However, Antioxidants prevent oxidative stress, caused by free radicals, which damage cells and vital biomolecules. Antioxidants can guard the body by inhibiting the generation of free radicals through suspension of free radicals' attack, or by scavenging the reactive metabolites or converting them to less reactive molecules (Hedge, 2009). They also terminate chain reactions triggered by free radicals by removing free radical intermediates and inhibit other oxidation reactions (Sies, 1997). Antioxidant activity of the plant was assessed using DPPH radical scavenging activity, ferric reducing antioxidant power, ABTS radical scavenging activity, and Linoleic acid test. The results were expressed as IC_{50} value which is the concentration of the plant required to scavenge 50 % of the free radicals present in the system. IC50 value is inversely related to the antioxidant activity of the extracts, hence the lower the IC50 value the higher the percentage inhibition on free radicals. Extracts with IC50 value ranging between 10 and 50 μg/mL are considered to possess strong antioxidant activity (Phongpaichit, 2007). DPPH stable free radical method is a reliable way to measure the antioxidant activity of plant extracts (Koleva *et al.*, 2002; Suresh *et al.*, 2008). Natural antioxidants available in plants are in charge of inhibiting or preventing the detrimental effects of oxidative stress. The pronounced effect of *Tectona grandis* on DPPH radical scavenging is thought to be due to their hydrogen donating ability. Also, the result of the FRAP assay showed high potential of the extract in reducing ferric tripyridyltriazine (Fe3+-TPTZ) complex to ferrous tripyridyl triazine (Fe2+-TPTZ) which is evident in the IC50 value obtained. Similarly, the extract was highly able to scavenge the radical-cation ABTS + produced by the oxidation of 2,2′-azinobis-63-ethylbenzothiazoline-6-sulphonate which is a good indication of antioxidant activity. Linoleic acid test evaluates the inhibitory effect of a compound or a mixture on the oxidation of β-carotene in the presence of molecular oxygen (O2). Hence, in this study, the plant displayed high inhibitory effect. These antioxidant activities suggest that this plant may be very useful in the management and treatment of various maladies. Phytochemical components of plants act as primary antioxidants. It is believed that phytochemicals may be effective in combating or preventing disease due to their antioxidant effect (Halliwell and Gutteridge, 1992; Afolabi et al., 2007). The strong antioxidant property possessed *Tectona grandis* are mainly due to the presence of the identified phytochemicals such as flavonoid, alkaloids, phenol, tannins and glycoside. Similarly, terpenoids, play important role in controlling metabolism and also perform protective role as antioxidants (Soetan, 2008). Plant flavonoids and steroids show strong fondness for hydroxyl radicals and superoxides anion radicals and assist in ameliorating health (Thagriki *et al.*, 2017). Furthermore, phenolic compounds found in this plant are regarded as very important plant constituents and they account for most of the antioxidant activities of herbs. This may be due to their scavenging ability which result from their hydroxyl groups. Hence, phenolic compounds may have contributed directly to the antioxidant action of the plant owing to the rich phenolic content observed from the results of the phytochemical analysis (Fig. 2). It has been reported that polyphenolic compounds can inhibit effects on mutagenesis and carcinogenesis in humans, when ingested up to 1g daily from a fruits and vegetables rich diet (Rajeshwar, 2005).

CONCLUSION

In this work, T. *grandis* ethanolic stem bark extract showed strong antioxidant properties. Further work will need to be done *in vivo,* especially to establish its effect (if any) in some degenerative diseases, includingcancer.

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