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Research Article

DETERMINATION OF PHYTOCHEMICAL COMPOSITION OF THE STEM BARK OF *Triplochiton scleroxylon* K. Schum. (STERCULIACEAE)

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ABSTRACT: In the objectives to know the phytochemical constituents in *Triplochiton scleroxylon* aqueous, ethanol (50%) stem bark extracts and powdered stem bark were subjected to preliminary phytochemical examinations. The phytochemical tests were conducted using standard methods of analysis. The results of analyzes reveal presence of the following phytochemicals: carbohydrate, saponins, tannins, steroids, flavonoids and phlobatannins. Quantitative analysis showed the highest yield of tannins (12.67%) and lowest yield of phenols (0.06%). Saponin was 2.23% and flavonoid 0.69%. The presence of these important phytochemicals in the stem bark of *Triplochiton scleroxylon* play very vital roles in the treatment of diabetes mellitus (ethnomedicine) in some parts of Nigeria.

Key words: Stem bark, *Triplochiton scleroxylon*, Phytochemicals.

INTRODUCTION

Growing of most plants and trees now goes beyond afforestation purposes to making them available and accessible for exploitation of their natural chemical compositions vis- a –vis their usefulness in the management and/or cure of both human and animal diseases. These chemical compounds known also as phytochemicals provide defences for the plants against diseases and other environmental factors. It is now evident that most of these plant chemicals that shield and protect them from microbial and other types of diseases could also be helpful in the treatment of both animal and human diseases. These phytochemicals which are also bioactive constituents are alkaloids, tannins, flavonoids and phenolic compounds classified as the most important (Edeoga et al., 2005). Most of these plants are eaten or used for their rich phytochemical constituents, which provide both preventive and curative properties to consumers against diseases most of which have had an age long existence. Diabetes mellitus is one of these diseases without cure and which is currently crippling the population with recklessness. It is envisaged that by 2025, about 300 million would have the disease with its attendant life threatening complications. *Triplochiton scleroxylon*, is one of the over 30 medicinal plants used in some parts of Nigeria in the treatment of diabetes mellitus (Prohp et al., 2006, 2008; Prohp and Onoagbe 2009a, b; Prohp et al, 2011; Prohp and Onoagbe 2012). *T. scleroxylon* which belongs in the kingdom: plantae, division: magnoliophyta, class: magnoliopsida, order: malvales, family: sterculiaceae (APG: Malvaceae), genus: triplochiton and species: T. scleroxylon, is a tropical tree of Africa (Ritcher and Dallwitz, 2000; Prohp and Onoagbe 2012). It is widely distributed in tropical West Africa along waterways and farms between humid evergreen and semi deciduous forests (Prohp et al., 2008).

This present study examined the qualitative and quantitative phytochemical analyses of the stem bark powder and extracts of *T. scleroxylon.* This is with the view of understanding and possibly explaining the roles of this plant in ethnomedicine (treatment of diabetes mellitus) in some parts of Nigeria.

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MATERIALS AND METHODS

Chemicals/reagents
All reagents/chemicals used were obtained from standard suppliers and were of analytical grade.
Plant material:

Triplochiton scleroxylon K. Schum., commonly known as tallow-tree, is a member of the Sterculiaceae family. Mature *T. scleroxylon* is a medium-sized tree species, about 30 m in height and 80 cm in diameter. It is a typical large canopy tree most common in Nigeria rainforest where it is among first timber tree species. Its areas of distribution are large. It spreads out from Guinea to Angola and Uganda (Centre Technique Forestier Tropical, 1989) and this species is locally abundant and usually found in dense patches. The bark of *T. scleroxylon* is locally used in the treatment of diabetes (Prohp and Onoagbe, 2009a, b).

Barks collection :

The barks of *T. scleroxylon* were obtained from the Forest of Uokha, Owan - East local government area, Edo State, Nigeria. Following identification by experts in the Department of Botany, University of Ibadan, Ibadan, Oyo State, Nigeria, as *Triplochiton scleroxylon* K. Schum, a voucher specimen (UIH – 22329) was deposited there (Prohp and Onoagbe 2012).

Extraction and preparation of plant extracts:

The barks of *T. scleroxylon* were washed with clean water, dried and cut into tiny strands. They were then pulverized into powder and 1000 g of powdered bark of this plant was then extracted separately in 7000 ml of aqueous (distilled water) and 50% ethanol in cold percolation by maceration technique under room temperature. This was followed by periodic stirring. The macerated samples were filtered with sintered glass funnel under suction to eliminate particles after 72 hours. The filtrates collected were then concentrated on a reduced pressure using the rotary evaporator to yield thick brown viscous pastes which were further dried under vacuum (Somchit et al., 2003). Dry concentrates obtained were kept in the desiccators until screened qualitatively and quantitatively for the phytochemical constituents utilizing standard methods of analyses (Vishnoi, 1979; Sofowora, 1993; Trease and Evans, 2002). The yield was 13.36% (w/w) and 10.94% (w/w) for aqueous and 50% ethanol dried concentrates, respectively (Prohp and Onoagbe 2012).

Qualitative phytochemical analysis of the stem bark powder, aqueous and ethanol (50%) extracts of *Triplochiton scleroxylon* K. Schum. (Sofowora, 1993).

General Test for Glycosides:

20 ml of water was added to 5 g powdered plant material in a beaker and boiled gently on a water bath for 10 minutes. The mixture was filtered and subsequently allowed to cool.

Molisch Test:

To 2 ml of the above filtrate was added 2 drops of 10% alcoholic solution of naphthol followed by 2 ml concentrated H_2S0_4, gently poured along the side of the test tube at an angle of 45^0. Formation of a purple ring at the interface of the two liquid layers confirmed a positive result for the presence of carbohydrates (Sofowora, 1993).

Fehling's Test:

To 2 ml of filtrate and 2 ml each of 0.1 g extracts were added 2 ml of Fehling's mixture A and B. They were boiled gently in a water bath for 5 minutes. Formation of a reddish brown precipitate confirmed a positive result indicative of the presence of reducing sugars (Sofowora, 1993).

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Test for Saponins:

To 2 ml of the water extract of the powdered plant and 2 ml of 0.1 g each of extracts were added 1ml distilled water in a test tube and shaken vigorously. Formation of foams/froths that persisted on warming confirmed a positive result (Sofowora, 1993).

Test for Tannins:

5 g of the powdered sample was extracted with water by heating on a water bath. It was cooled and filtered. 2 drops of 5% ferric chloride solution was added to 2 ml of the filtrate. 0.1 g of each extract was dissolved in 2 ml distilled water and 2 drops of 5% ferric chloride solution was also added. Formation of a bluish or greenish coloured solution with precipitate confirmed a positive result (Trease and Evans, 2002).

Test for Alkaloids:

20 g of the powdered plant material was moistened in water and mixed with 2 g $Ca(OH)_2$ to form a paste. It was allowed to stand for 5 minutes and evaporated to dryness on a water bath. 30 ml chloroform was added, mixed and heated gently on a water bath for 30 minutes. The extract was filtered and more chloroform added to the mark, mixed and filtered again. The combined filtrates were then evaporated to dryness and residue dissolved in 5 ml 1% sulphuric acid and filtered. Also 0.2 g of each extract was dissolved in 5 ml 1% sulphuric acid and filtered. 1 ml of filtrate each from powdered sample, aqueous and 50% ethanol extracts in different test tubes was tested for alkaloids using alkaloidal reagents (0.5 ml Dragendorff's, Wagner's, Hager's and Mayer's reagents respectively). Formation of reddish brown precipitate for Dragendorff's and Wagner's reagents, yellow precipitate for Hager's and cream precipitate for Mayer's confirmed a positive result (Sofowora, 1993; Trease and Evans, 2002).

Test for Anthracene Derivatives:

5 g of the powdered plant material was boiled with 20 ml 10% H_2SO_4 and filtered while hot. The filtrate was shaken with 10 ml chloroform. The chloroform layer was separated and half its own volume of 10% ammonium hydroxide solution was added. Also 0.1 g of each extract was dissolved in 10 ml chloroform and 5 ml 10% ammonium hydroxide solution was added. Formation of a pink colour in the aqueous layer confirmed the presence of anthracene derivatives (Sofowora, 1993; Trease and Evans, 2002).

Test for Cyanogenic Glycosides:

2 g of the powdered plant material was placed in three test tubes labelled A, B and C. The powdered sample in test tubes A and B were mixed with little quantity of water, test tube C had the dry powder and sodium picrate test paper was placed in each of the three test tubes. The test tubes were stoppered immediately. Test tube B was placed in a boiling water bath with test tubes A and C kept at room temperature. Also 0.1 g of each extract was prepared in a similar manner as above in three different test tubes. A change in colour from yellow to red of the sodium picrate test papers after 10 minutes confirmed a positive result (Sofowora, 1993; Trease and Evans, 2002).

Test for Steroids:

5 g of the powdered plant extract was extracted with 20 ml methanol, by heating on a water bath. It was filtered and the filtrate evaporated to dryness. A little quantity (0.5 g) of the residue obtained from the filtrate was dissolved in 2 ml chloroform. Also 0.1 g of each extract was dissolved in 2 ml chloroform and filtrate evaporated to dryness. Sulphuric acid was carefully added by the side of the test tubes to form a lower layer. Formation of a reddish brown ring at the interface of the two liquids confirmed a positive result (Sofowora, 1993; Trease and Evans, 2002).

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Test for Flavonoids:

5 g of the powdered plant material was extracted with water by heating on a water bath. It was cooled and filtered. To 5 ml of the filtrate, 1 ml NaOH was added followed by 1 ml of concentrated HCl. About 0.1 g of each extract was dissolved in 5 ml distilled water and also treated with 1 ml NaOH and HCl as for powdered plant filtrate. Intensification of the yellow colour of the filtrate which turned colourless upon addition of concentrated HCl confirmed a positive result (Trease and Evans, 2002).

Test for Phlobatannins:

To 5 ml of the aqueous powdered extract was added 5 ml 1% aqueous HCl and boiled in a water bath for 5 minutes. 0.1 g of each extract was dissolved in 5 ml distilled water and was treated as for the powdered extract. Formation of a red coloured precipitate confirmed a positive result (Sofowora, 1993; Trease and Evans, 2002)

Quantitative analysis of phytochemical compositions of aqueous and ethanol (50%) stem bark extracts of *Triplochiton scleroxylon* K. Schum.

Determination of total phenols by spectrophotometric method.

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 minutes. 5 ml of the extract was pipetted into a 50 ml flask, followed by the addition of 10 ml of distilled water. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amylalcohol were also added. The samples were made up to mark and colour developed was measured after 30 minutes at 505 nm under room temperature.

Determination of alkaloids using Harborne (1998) method.
5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol added. The beaker was covered and allowed to stand for 4 hours. It was then filtered and the extract concentrated on a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide (2M) and then filtered. The residue if available is the alkaloid which is then dried and weighed.
Determination of Tannin by Van – Burden and Robinson (1981) method.
500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipette out into a test tube and mixed with 2 ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 minutes.

Determination of saponin.
The method used was as described by Obadoni and Ochuko (2001). The samples were ground and 20 g of each put into a conical flask followed by the addition of 100 ml of 20% aqueous ethanol. They were then heated over a hot water bath for 4 hours with continuous stirring at about 55^0C. The mixture was filtered and the residue re – extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90^0C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n – butanol was added. The combined n – butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight. Saponin content was calculated as percentage.

Determination of flavonoids by the method of Boham and Kocipai-Abyazan (1994).
10 g of the stem bark extracts was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

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RESULTS

This study has revealed the presence of phytochemicals considered as active medicinal chemical constituents. The summary of the phytochemical characters of the powdered and stem bark aqueous and ethanol (50%) extracts of *Triplochiton* scleroxylon have been presented in Tables 1 and 2. Important medicinal phytochemicals such as tannins, saponins, flavonoids and phlobatannins were present in the samples screened (Table 1). Quantitative analysis showed highest yield of tannin (12.67%) and a higher yield of saponin (2.23%). While flavonoid was 0.69%, phenols recorded the least yield of 0.06%. Alkaloid gave negative indication for all tests administered and so was absent (Table 2).

Table 1: Qualitative phytochemical screening of powdered stem bark, aqueous and ethanol extracts of *Triplochiton scleroxylon*.

Phytochemicals	Powder	Aqueous Extract	Ethanol Extract
Carbohydrates	+	+	+
Reducing sugars	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Alkaloids			
Dragendorff's reagent	-	-	-
Wagner's reagent	-	-	-
Hager's reagent	-	-	-
Mayer's reagent	-	-	-
Anthracene derivatives	-	-	-
Cyanogenic glycosides	-	-	-
Steroids	+	+	+
Flavonoids	+	+	+
Phlobatannins	+	+	+

Key= + present; - absent.

Table 2: Quantitative analysis of the major phytochemical contents of stem bark extracts of *Triplochiton scleroxylon*.

Phytochemicals	**%**
Tannins	12.67
Flavonoids	0.69
Phenols	0.06
Saponins	2.23
Alkaloids	-

DISCUSSION

Plants that have biological activities usually contain secondary metabolites which are chemical substances responsible for such activities. Phytochemical screening of the stem bark powder and extracts of *Triplochiton scleroxylon* showed the presence of the following major secondary metabolites viz., saponins, tannins, steroids, flavonoids and phlobatannins (Table 1).

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These classes of compounds especially alkaloids, saponins, tannins, anthraquinones and flavonoids are known to have curative activity against several pathogens (Usman et al., 2009). Quantitative evaluation of extracts showed that tannin content was highest at 12.67%, saponins at 2.23%, flavonoids at 0.69% while 0.06% was obtained for phenols (Table 2). Tannins are phenolic compounds and so the level obtained quantitatively in this study may have been contributed by phenols as well as phlobatannins (phlobaphenes) (Table 2).

Saponins are steroid or triterpenoid glycosides characterised by their bitter or astringent taste, foaming properties and their haemolytic effect on red blood cells. Saponins possess both beneficial (cholesterol-lowering) and deleterious (cytotoxic permeabilization of the intestine) properties and also exhibit structure dependent biological activities (Osagie and Eka, 1998). Saponins cause a reduction of blood cholesterol by preventing its reabsorption

(http://www.phytochemicals.info/phytochemicals/saponins.php). It has also been documented that saponins have antitumor and anti-mutagenic activities and can lower the risk of human cancers, by preventing cancer cells from growing. Saponins are believed to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability (Roa et al, 1995). Plants produce saponins to fight infections by parasites and in humans saponins help the immune system and also protect against viruses and bacteria. The non-sugar part of saponins has a direct antioxidant activity which may result in reduced risk of cancer and heart diseases.

The detergent properties of saponins have led to their use in shampoos, facial cleansers and cosmetic creams (http://www.phytochemicals.info/phytochemicals/saponins.php). *Triplochiton scleroxylon* could be a source of saponins to serve the above purposes *albeit* in part due to the level of saponin present (Table 2). However, saponin content obtained in this study agrees with the values reported for the stems of *Cleome nutidosperma* (2.00%), *Emilia coccinea* (2.30%), and *Spigelia anthelmia* (2.26%) (Edeoga et al., 2005). The same authors have also reported 0.00% saponin content in *Euphorbia heterophylla*, *Scopania dulcis*, and *Sida acuta* with 3.10% in *Stachytarpheta cayennensis*. Tanko et al., (2007) reported the presence of saponins in the leaves of *Cissampelos mucronata (Menispermaceae)* known also to have hypoglycemic properties.

Flavonoids are water soluble polyphenolic molecules and therefore belong to the polyphenol family. Together with carotenes, flavonoids are also responsible for the coloring of fruits, vegetables and herbs (http://www.phytochemicals.info/phytochemicals/flavonoids.php). Flavonoids have antioxidant activities as well as much health promoting effects viz., anti-allergic, anti-cancer, anti-oxidant, anti-inflammatory, anti-thrombotic, vasoprotective, tumour inhibitory and anti-viral effects. These effects have been associated with the influence of flavonoids on arachidonic acid metabolism. Some flavonoid containing plants are diuretics (e.g. buchu), antispasmodic (e.g. liquorice) and others have antimicrobial properties (Trease and Evans, 2002). Epidemiological studies have shown that heart diseases are inversely related to flavonoid intake and that flavonoids prevent the oxidation of LDL therefore reducing the risk for the development of atherosclerosis.

It has also been reported that flavonoids lower the blood levels of cholesterol and triglycerides (http://www.phytochemicals.info/phytochemicals/flavonoids.php). Although, flavonoid content of stem bark extracts of *Triplochiton scleroxylon* was only 0.69%, this plant may be useful in serving some purposes as have been highlighted. Edeoga et al., (2005) also reported flavonoid contents in the stems of *Euphorbia heterophylla, Richardia bransilensis, Scopania dulcis, Spigelia anthelmia* and *Tridax procumbens* as 0.74%, 0.56%, 0.88%, 0.77% and 0.61% respectively. The presence of flavonoids in the leaves of *Cissampelos mucronata* (*Menispermaceae*) which have hypoglycaemic and anti-diabetic properties have also been documented (Tanko et al., 2007). Effects of flavonoids, quercetin and ferulic acid on pancreatic β-cells leading to their proliferation and secretion of more insulin have been proposed by Mahesh and Menon (2004) and Sri-Balasubashini et al., (2004) as the mechanism of their hypoglycaemic activity in streptozotocin-induced diabetic rats. These are justifications for the use of the extracts of *T. scleroxylon* in the treatment of diabetes mellitus in some parts of Nigeria.

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Tannins are usually found in large quantity in the bark of trees where they play a major protective role in the plant's defence against bacteria, fungi and insects (http://www.herbs2000.com/h_menu/tannins.htm). Tannins may decrease protein quality by decreasing digestibility, and palatability. Other nutritional effects which have been attributed to tannins include damage to the intestinal tract, toxicity of tannins absorbed from the gut, and interference with the absorption of iron, and a possible carcinogenic effect (Osagie and Eka, 1998). Phytochemical analysis showed that the stem bark extract of *T. scleroxylon* has tannin content as high as 12.67% (Table 2), as the highest of all the major phytochemical compounds determined. Edeoga et al., (2005), reported tannin contents of 15.25%, 12.46%, 12.13%, 15.05%, 6.08%, 6.23% and 7.45% in the stems of some Nigerian medicinal plants viz., *Cleome nutidosperma, Euphorbia heterophylla, Richardia bransilensis, Spigelia anthelmia, Sida acuta, Scopania dulcis* and *Tridax procumbens*. Tannin content recorded in this study correlates with most values documented for most medicinal plants in use today, indicative that the level obtained in the stem bark extracts of *T. scleroxylon* is sub-lethal and therefore tolerable. However, it has been reported that adding milk, lemon juice to tea or herbal extract or consuming food that is rich in vitamin C could help in reducing or neutralizing tannins' adverse actions on iron intake (http://www.herbs2000.com/h_menu/tannins.htm). Iwu, (1980 and 1983) have also reported that tannin containing drugs have been shown to posses anti – diabetic properties.

CONCLUSION

Our findings show that the stem bark powder and extracts of *Triplochiton scleroxylon* contain important and active phytochemical compounds also found in medicinal plants with hypoglycaemic and anti-diabetic properties.

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