

Full Length Research Paper

Importance of knowledge of chemical composition of stem bark of *Triplochiton scleroxylon* K. Schum. in traditional treatment of diabetes mellitus in Nigeria

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Triplochiton scleroxylon is a tropical forest-tree species that is valued for its timber and also stem barks, whose aqueous extract is commonly used as an anti-diabetic preparation. Stem bark extracts (aqueous and 50% ethanol) and powder of *T. scleroxylon* were investigated for elemental and proximate compositions. Atomic absorption spectroscopy (Buck Scientific VGP 210) was used for elemental analysis. Standard analytical methods were utilized for proximate investigation of the powdered stem bark of *T. scleroxylon*. Heavy metals identified were present at tolerable levels, namely, cadmium (<0.01 ppm), lead (<0.08 ppm) and nickel (<0.05 ppm) in the stem bark powder, aqueous and 50% ethanol extracts. Calcium content of stem bark samples was higher than all other elements determined at approximately 2.60% while potassium followed with 1.32%. Magnesium was highest in 50% ethanol extract at 0.26% while zinc, copper and manganese were 6.45, 2.30 and 14.50 ppm respectively. Sodium content was only 0.01%. Proximate analysis of stem bark powder showed carbohydrate content of 80.70% and nitrogen free extract of 78.9%. Crude protein, fat and fibre contents were 5.90, 6.80 and 0.80% respectively. Percentage ash content was 6.92 while moisture content of the stem bark powder was 0.68%. Stem bark samples of *T. scleroxylon* could be a good source of calcium and potassium to complement major sources. The use of the extracts in the treatment of diabetes mellitus in some parts of Nigeria is safe as heavy metals identified were present at sub-lethal levels. However, like all drugs, abuse of any form, is to be avoided.

Key words: Elemental, proximate, stem bark, *Triplochiton scleroxylon*.

INTRODUCTION

The exploitation of plants by humanity as the panacea for different diseases has been in practice for a very long time (Gill, 1992; Sofowora, 1984). To date more than 400 medicinal plants have so far been explored globally for the medication of diabetes mellitus, with investigations to ascertain their potency carried out in only a few (Satyavati et al., 1987; Bailey and Day, 1989; Onoagbe et

al., 1999a). The use of orthodox drugs such as sulphonylureas, biguanides and insulin result to severe hypoglycaemia and other complications in patients prompting the desire for alternative means of combating diabetes mellitus in Nigeria, Africa and the world-over (Onoagbe et al., 1999b). *Triplochiton scleroxylon* belongs to the family of tropical medicinal plants (Russel et al., 1997). The active ingredients are believed to be at the bark of this plant whose aqueous extract is commonly used as an anti – diabetic preparations in the rural communities and amongst most impoverished urban dwellers in the southern and western parts of Nigeria

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(Onoagbe et al., 1999b; Prohp et al., 2006, 2007). It is found in the humid ever green semi-deciduous forest along water ways in the tropical West Africa (Russel et al., 1997; Richter and Dallwitz, 2000). *T. scleroxylon* in the kingdom: plantae, division: magnoliophyta, class: magnoliopsida, order: malvales, family: sterculiaceae (APG: Malvaceae), genus: *triplochiton* and species: *T. scleroxylon*, K. Schum, is a tropical tree of Africa known also as Abachi under the Nigerian name Obeche whilst in Ghana, Cameroon and Ivory Coast it is called wawa, ayous and samba respectively. The Nigeria and Ghana names have been adopted as alternative British standard names. The trade name in Britain is Obeche (Richter and Dallwitz, 2000). The potency of medicinal plants is linked to their fundamental chemical compositions which to an extent help to understand the possible mechanism of pharmacological and biochemical actions of such plants in disease management. In this study the aim is to elucidate the elemental and proximate compositions of the stem bark of *T. scleroxylon* for the purpose of establishing any possible link to its anti-diabetogenesis.

MATERIALS AND METHODS

Chemicals/reagents

All reagents/chemicals used were obtained from standard suppliers and were of analytical grade.

Medicinal plant

The barks of *T. scleroxylon* (TS) were obtained from the forest of Uokha, Owan-East Local Government Area, Edo State, Nigeria. They were then identified by experts in the Department of Botany, University of Ibadan, Ibadan, Oyo State, Nigeria, as *T. scleroxylon* K. Schum where a voucher specimen (UIH – 22329) had been deposited.

Preparation of plant extracts

The barks of *T. scleroxylon* were washed with clean water, air – dried at room temperature for eight days and then reduced to smaller sizes by cutting. They were then pulverized into powder with the aid of a pulverizer and immediately stored in the freezer at -21°C to avoid any possible depletion of the phytochemicals through microbial growth. The powdered bark of this plant was then extracted in both aqueous (distilled water) and 50% ethanol (1 g/7 ml) in cold percolation by maceration technique under room temperature. This was followed by periodic stirring. The macerated samples were filtered under suction with the aid of the buchner funnel after 72 h. The filtrates collected were then concentrated on a reduced pressure using the rotary evaporator to yield brown pastes which were further dried under vacuum. Dry concentrates obtained were kept in the desiccators until used. The yield was 13.36% (w/w) and 10.94% (w/w) for aqueous and ethanol dried concentrate respectively. The powdered stem bark, aqueous and ethanol (50%) extracts were then screened for elemental composition. Also proximate analysis was carried out on powdered stem bark. Standard methods of analyses were utilized in this study (Pearson, 1976; AOAC, 1984). Quantitative evaluation of stem bark powder and extracts of *T. scleroxylon* (BP, 1988).

Moisture content determination

Two grams of the powdered stem bark of *T. scleroxylon* was introduced into clean crucibles. They were then heated for 1 h at 105°C, cooled in a dessicator and then weighed. The procedure was repeated until there was no further loss in weight. The average percentage weight loss in relation to the air–dried powdered stem bark was determined for three replicates.

Determination of total ash value

Two grams of the powdered stem bark of *T. scleroxylon* was placed in a tarred silica dish, previously ignited and weighed. The powdered sample was heated gently until it was charred, at a temperature not exceeding 450°C. Heating continued until all carbon was lost. The powder was cooled and reweighed. The percentage of ash with reference to the air–dried stem bark powder was calculated. Three replicates were used (Wallis, 1985).

Proximate and elemental analyses of powdered stem bark of *T. scleroxylon*

Proximate analysis of the powdered stem bark of *T. scleroxylon* was carried out, namely, protein, lipid, fibre, ash and carbohydrate contents. Gross energy value was also calculated. Each parameter was determined for three replicates (Pearson, 1976; AOAC, 1984).

Determination of protein content

Colorimetric or Kjeldahl method was used. The nitrogen percent values determined were multiplied by the factor 6.25 to obtain the percentage of crude protein in the powdered stem bark sample. About 0.1 g of dried pulverized (powdered) sample was put in a micro–Kjeldahl flask. To this, 2 ml of concentrated H₂SO₄ was introduced and then one tablet of selenium catalyst. The mixture was gently heated and then heated strongly until a clear solution was obtained. The filtrate was made up to a 100 ml in a volumetric flask after cooling and filtering. Aliquots of 2 ml were pipette into test tubes for analysis. To these were added 2.5 ml of alkali – phemate solution, 2 ml of sodium potassium tartrate and 1.5 ml of sodium hypochlorate and the total volume was made up to 25 ml using distilled water. The optical density (OD) values were then obtained from a spectrophotometer at 630 nm.

Determination of lipid content

About 10 g of powdered stem bark was kept in a thimble and placed in a soxhlet extractor unit for continuous extraction for a period of 8 h. The weight of the empty flask was pre–determined before the commencement of the process which involved filling the flask with n- hexane solvent to about half the mark, then placing the wrapped sample in the solvent extractor unit. The flask with n-hexane was in turn subjected to heat from a sand bath. The retort and the condensing system of the extractor unit were allowed for the intermittent recycling of the overflowing solvent back into the flask for the period of the extraction. The hexane was then allowed to escape from the flask by subjecting the open flask to a temperature of 100°C, after which the weights of the flask and oil were determined. The percentage of lipid obtained was then calculated as follows:

$$\text{Lipid \%} = \frac{(\text{Weight of flask + oil}) - (\text{Weight of flask})}{\text{Weight of sample used}} \times 100$$

Table 1. Elemental analysis of stem bark powder and extracts of *T. scleroxylon* K. Schum.

S/No.	Elements	Stem bark powder	Ethanol (50%) extract	Aqueous extract
1.	Na (%)	0.01	0.01	0.01
2.	Mg (%)	0.20	0.26	0.20
3.	Ca (%)	2.60	2.62	2.60
4.	Zn (ppm)	6.45	6.45	6.45
5.	Cu (ppm)	2.30	2.30	2.30
6.	Mn (ppm)	14.50	14.50	14.50
7.	K (%)	1.32	1.32	1.32
8.	Cd (ppm)	<0.01	<0.01	<0.01
9.	Ld (ppm)	<0.08	<0.08	<0.08
10.	Ni (ppm)	<0.05	<0.05	<0.05

Table 2. Proximate analysis of stem bark powder of *T. scleroxylon* K. Schum.

S/No.	Parameters investigated	Values obtained (%)
1.	Crude protein	5.90±0.40
2.	Crude fat	6.80±1.40
3.	Crude fibre	0.80±0.20
4.	Ash content	6.92±0.60
5.	Carbohydrate	80.70±0.70
6.	Moisture content	0.68 ±0.00
7.	Nitrogen free extract	78.90

Determination of fibre content

To about 2 g of each sample was added 200 ml of 5% concentrated H₂SO₄ in a 1000 ml conical flask. After boiling and digesting for 30 min on a sand bath, the solution was filtered through a Whatman filter paper and the washed residue was taken to neutrality by boiling in 200 ml of 5% KOH for 30 min and then rinsed with distilled water through another filter paper. Finally the residue was rinsed with alcohol into a previously weighed petri dish and transferred to a dessicator to dry before reweighing. The sample in the petri dish was taken to a muffle furnace for ashing at 500°C for 3 h and then to a dessicator to cool before being weighed again (Pearson, 1976). Percentage fibre was determined using the formula:

$$\text{Fibre \%} = \frac{(\text{Weight of digested sample}) - (\text{Weight of ash})}{\text{Weight of sample}} \times 100$$

Estimation of carbohydrate content

This is also known as the nitrogen free extract estimation. The value was determined by subtracting the summed percentage values for lipid, protein, fibre, moisture and ash from 100 (Muller and Tobin, 1980).

Estimation of gross energy

This was obtained by the procedure described by Osborne and Voogt (1978) as follows:

$$[\text{Gross energy} = (4 \times \text{protein \%}) + (4 \times \text{NFE \%}) + (9 \times \text{Lipid \%})].$$

Analysis of heavy metals and other elements

About 2 g of the powdered stem bark sample was put into a clean platinum crucible, ashed at 500°C and then cooled to room temperature in a dessicator. The ash was dissolved in 10 ml 20% nitric acid (polar solvent) and filtered into a 100 ml volumetric flask. The crucible was well rinsed with distilled water and transferred to the flask, shaken to mix well and made up to 100 ml with distilled water. Analysis of the sample for heavy metals and other elements was carried out in triplicate on the atomic absorption spectroscopy (AAS)(Buck Scientific VGP 210; Pearson, 1976).

RESULTS

Results have been presented in Tables 1 and 2. Elemental analysis showed calcium content was highest in the samples of stem bark of *T. scleroxylon* and this was closely followed by potassium. Other elements determined including the heavy metals were present in trace amounts (Table 1). Proximate analysis showed high carbohydrate content of stem bark powder of this plant with a very low moisture content. Crude protein, fat, fibre and ash content have also been presented (Table 2).

DISCUSSION

Stem bark powder of *T. scleroxylon* contains some amount of basic food nutrients such as proteins, fats,

carbohydrates and fibre. Dietary fibre enhances frequent waste elimination, promotes bowel regularity and has a physiological effect on the gastrointestinal function of promoting the reduction of tracolonic pressure and also a biochemical effect on the absorption and reabsorption of bile acids with the consequent lowering of the cholesterol pool (Okwu, 2006). Food fibre also aids absorption of trace elements in the gut (Kelsay, 1981). Proximate analysis of stem bark powder of *T. scleroxylon* gave crude protein, 5.90±0.40%; ash content, 6.92±0.60%; crude fibre, 0.80±0.20%; carbohydrate, 80.70±0.70% and crude fat, 6.80±1.40% (Table 2). Proximate content of *Irvingia gabonensis* seeds known to reduce fasting blood glucose levels and therefore used in traditional and modern medicine for the treatment of several illnesses have been well documented as follows: protein (8.33 to 8.71%), ash (2.06 to 3.80%), carbohydrate (15.71 to 55.00%) and oil (fat) (34.28 to 73.82%) (Nosiri et al., 2011). These values show higher oil and crude protein as well as lower ash and carbohydrate contents than were obtained in this study for *T. scleroxylon* K. Schum.

The moisture content of the stem bark powder of 0.68% (Table 2) obtained in this study, fell below the pharmacopoeia limits of water content for vegetable drugs, which is between 8 to 14% African Pharmacopoeia (AP, 1986). Excessive water in vegetable drugs, greater than the set limit will promote the growth of microbes and fungi. This would also lead to hydrolysis of constituents resulting ultimately to deterioration of the drug. Moisture content value obtained in this study was indicative that the material could be preserved over a long period of time without deterioration of the drug. Nitrogen free extract of 78.90% and gross energy of over 401.80 kcal/100 mg obtained were indicative of the high carbohydrate content and caloric value of this herbal powder. Nitrogen free extract of 62.00 and 50.00% documented for the leaf of *Vitex doniana* and Sorrel leaf (*Rumex acetosa*; (Ladeji and Okoye, 1993a, b) were lower than was obtained in this study. Energy values of 393.47, 405.45 and 398.26 kcal/100 g reported for fruit mesocarp of *Hyphaene thebaica*, fruits of *Ziziphus spina-Christi* and fruit pericarp of *Ziziphus mauritiana* respectively (Temple et al., 1990) agreed with the value of 401.80 kcal/100 mg obtained in this study.

Elemental analysis of stem bark powder and extracts of *T. scleroxylon* revealed the presence of the following elements: sodium (0.01%), magnesium (0.20%), calcium (2.62%), zinc (6.45 ppm), copper (2.30 ppm), manganese (14.50 ppm) and potassium (1.32%) (Table 1). Heavy metal toxicity is an excessive build-up of metals in the body. Oftentimes, the vague symptoms produced by heavy metal toxicity are mistakenly misdiagnosed as incurable chronic conditions. Lead is a cumulative poison and is accumulated in tissues over the years. It is not biodegradable. 90% of lead is seen in bones, 9% in blood and 1% in brain and kidneys. There is no safe level in blood; about 10 µg/dl can be tolerated. More than 10

µg/dl in children and more than 25 µg/dl in adults leads to toxic manifestations (Vasudevan and Sreekumari, 2007). In this study cadmium (<0.01 ppm), lead (<0.08 ppm) and nickel (<0.05 ppm) were found to be present in levels considered sublethal and therefore tolerable to both humans and animals. It is important to note that these three heavy metals are highly toxic at higher levels and even at low concentrations should not be ignored (Asaolu et al., 1997). The tolerable upper intake level for manganese is set at 11 mg for adults, zinc is 40 mg a day for adults and 10 mg per day for copper to protect against possible liver damage (IOM, 2001). The levels of zinc in aqueous and ethanol (1:1) bark extracts of *T. scleroxylon*, although low, were indicative of a possible valuable role of this plant in the management of diabetes as zinc is vital for the production of insulin (a hormone) and carbonic anhydrase in the body (Okwu, 2006).

Manganese ions function as cofactors for oxidoreductases, transferases and hydrolases amongst others and therefore serve as a required trace mineral for all known living organisms. Copper is essential for good health (ATSDR, 2000; 2004). The values of elements obtained in this study were lower than was reported for maize, sorghum, millet, rice, wheat, acha, pawpaw, banana fruits, raw and cooked full-fat pumpkin seeds (Osagie and Eka, 1998). Minerals serve as constituents of skeletal tissues, cofactors to enzymes, carrier proteins, protein hormones and electrolytes in the body fluids and cells (Osagie and Eka, 1998).

Conclusion

The stem bark powder and extracts of *T. scleroxylon* could be a good source of calcium and potassium but low in sodium, magnesium, copper, manganese and zinc. However, the values of these elements obtained in this study fall within the recommended 'not to exceed' daily maximum (tolerable upper intake limits) for minerals, as recommended for healthy adults by the Food and Nutrition Board of the National Academy. Stem bark extracts of *T. scleroxylon* K. Schum do not contain dangerous proportions of elements capable of large scale health risks, hence the continued use of this herb in some part of Nigeria to treat diabetes mellitus.

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