International Journal for Pharmaceutical Research Scholars (IJPRS)

V-2, I-4, 2013 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Phytochemical and Metallic Content Analyses of Gum Obtained from Pulp of *Treculia Africana* Fruit

Uzondu ALE*[1], Animam RN[1]

*[1]*Department of Pharmaceutical Technology and Industrial Pharmacy, Faculty of Pharmacy, Madonna University, Elele, Rivers State, Nigeria.*

Manuscript No: IJPRS/V2/I4/00189, Received On: 26/10/2013, Accepted On: 09/11/2013

ABSTRACT

The study was aimed at establishing the metallic and phytochemical contents of gum obtained from Treculia Africana Decne (African breadfruit) in order to assess its suitability for use as a pharmaceutical excipient. The pulp was obtained from the fruit after removing the skin and seeds, then dried, pulverised, and defatted using a mixture of chloroform and acetone at 2:1 ratio. Thereafter the resulting residue was air dried at room temperature (32^{o}C) for 72 h. The gum purification was effected by mixing 100 g of defatted gum with 500 ml of boiling water in a glass beaker and stirring using a stainless steel paddle for 10 min. The resultant mucilage, after cooling to 30^{o}C, was treated with acetone (ratio 1:3). Thereafter, the extracted gum was subjected to standard phytochemical and inorganic elemental screening protocols. Results revealed that phytate, oxalate and carbohydrates were present in the gum at concentrations of 0.137±0.008, 0.113±0.004 and 0.110±0.005 mg/100 g of gum, but alkaloids, flavonoids, tannins, saponins and cardiac glycosides were absent. The gum contains some heavy metals, but the quantities were found to be much lower than the limits permitted to be present in foods and pharmaceuticals. It is therefore worth suggesting that this gum is safe and may be a useful excipient for the formulation of various pharmaceutical dosage forms.

KEYWORDS

Treculia Pulp Gum, Phytochemical Analysis, Metal Content, Pharmaceutical Excipient

INTRODUCTION

Treculia africana Decne belongs to the family Moraceae and is native to Africa, hence the common name- African breadfruit. African bread fruit trees are deciduous and in Nigeria are found in the western and eastern regions[1]. The fruits are hard and fibrous, can be the size of a volleyball and weigh up to 8.5 kg each. The fruit of African breadfruit is a drupe which contains many small brownish-ash coloured seeds.

***Address for Correspondence:**
Uzondu Akueyinwa LE
Department of Pharmaceutical Technology,
Madonna University, Elele, Rivers State, Nigeria.
E-Mail Id: lovetuzondu@gmail.com

The pulp of the fruit is used as fodder for animals, while the seeds constitute a highly priced delicacy in South-Eastern Nigeria. Ethno-medicinally, *Treculia africana* is used as a vermifuge, febrifuge galactogogue and laxative[2]. Decoctions from different plant parts are used as an anti-inflammatory agent and in the treatment of whooping cough. The crushed leaves juice is applied on the tongue as a treatment for thrush in children; the latex is applied as an antibacterial agent in eardrops, and as chewing stick. The *T. africana* leaves decoctions were reported used in Trinidad and Bahamas to lower blood pressure[3], and is used also in some communities as an effective

treatment in stomach upset and other gastro intestinal infections. African breadfruit's seeds provide an important food item which is very popular and consumed as main dish especially in South-Eastern Nigeria.[4,5] The seeds are of particular interest because of their high nutrition value. The result of the proximate analysis of the species by [6] revealed that fresh seeds of the species has 38.3% carbohydrate 17.7% crude-protein, 3.8% moisture, 15.9% crude fibre, 4.0% ash and 15.9% ether extract (fat). African breadfruit is a good adjunct in brewing because it is a source of fermentable sugars[7]. They found that the yield in the production of ethanol is enhanced when its defatted seeds are used.

Natural gums are polysaccharides of natural origin, capable of causing a large viscosity increase in solution, even at small concentrations. In the food industry they are used as thickening agents, gelling agents, emulsifying agents, and stabilizers. In other industries, they are also used as adhesives, binding agents, crystan inhibitors, clarifying agents, encapsulating agents, flocculating agents, swelling agents, foam stabilizers, etc. Most often, these gums are found in the woody elements of plants or in seed coatings [8] Gums are considered to be pathological products formed following injury to the plant or owing to unfavourable conditions, such as drought, by a breakdown of cell walls (extra cellular formation; gummosis) while, mucilages are generally normal products of metabolism, formed within the cell (intracellular formation) and/or are produced without injury to the plant. Gums readily dissolve in water, whereas, mucilage form slimy masses.[9] Some natural gums are employed in the food and pharmaceutical industries as adjuvants in the production of various consumables. The gums from edible plant parts are in many cases generally regarded as safe, but it is also necessary to conduct some screening processes on them in order to ascertain the level of some phytochemicals and metallic elements in them and compare with official standards. This informs the quest for this study: phytochemical and elemental analyses of gum obtained from *Treculia africana* pulp.

MATERIALS AND METHODS

Materials

Fruits of *Treculia africana* were purchased from a local market in Elele Rivers State, Nigeria and authenticated by the Pharmacognosy Department, Madonna University Elele; chloroform, methanol and acetone (BDH Chem. Poole, England) and distilled water. Other reagents were of analytical grade.

Methods

Extraction of Gum from the Fruit Pulp of Treculia africana

The pulp was dried in a hot air oven (Ceword Medical Equipment, DHG-9101.ISA, England) at 60^{o}C and pulverised using a mortar and pestle. The powdered pulp was defatted using a mixture of chloroform and methanol at 2:1 ratio. One hundred grams (100 g) of the powder was defatted by passing 500 ml of the solvent mixture in a Soxhlet apparatus until the liquid dropping from the powder bed was colourless. Thereafter, the resulting residue was air dried at room temperature (32^{o}C) for 72 h. The gum was purified by mixing 20 g of defatted gum with 500 ml of boiling water in a beaker, and stirring using a stainless steel paddle for 10 min. The mixture was strained through a filter cloth and resulting mucilage precipitated with thrice its volume of acetone. Thereafter the gum was air dried for 48 h and heated in the hot air oven at 60^{o}C for 1 h to a moisture content of 0.5±0.1%, pulverized using kitchen blender and stored in air-tight container over silica gel.

Phytochemical Analysis of *Treculia africana* Gum

Determination of Oxalate Content

One gram (1.0 g) of the sample was placed in a 250 ml volumetric flask, 190 ml of distilled water and 10 ml of 6M HCl were added. The mixture was warmed in a water bath at 90°C for 5 h and the digested sample was centrifuged at a speed of 2500 rpm for 5 min. 50 ml aliquots of the supernatant was reduced by evaporation to

25 ml, the brown precipitate was filtered off and washed. The combined solution and washings was titrated with concentrated ammonia solution in drops until salmon pink colour of methyl orange changed to faint yellow. The solution was heated in a water bath to 90°C and the oxalate was precipitated with 10 ml of 5% calcium chloride ($CaCl_2$) solution. The solution was allowed to stand overnight and then centrifuged. The precipitate was washed into a beaker with hot 25% sulphuric acid (H_2SO_4) diluted with 125 ml distilled water and after warming to 90°C, it was titrated against 0.05 M $KMnO_4$. 1 ml 0.05 M $KMnO_4$ = 2.2 mg oxalate[10].

Determination of Alkaloid Content

5.0 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 2 h. This was filtered and the extract was concentrated on a water bath to one – quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract and the precipitate was recovered by filtration and washed with dilute ammonium hydroxide. The residue is the alkaloid, which was dried and weighed[11,12].

Determination of Flavonoid Content

The method reported by[13] was employed. Briefly, 10.0 g of the gum sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight.

Determination of Saponin Content

Twenty grams (20.0 g) of the gum was put into a conical flask and 100 ml of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at 55°C. The mixture was filtered and the residue re-extracted with 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated, before 60 ml of n-butanol was added to the extract. The combined n-butanol-extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated on a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponins content were calculated as percentage[11].

Determination of Cardiac Glycoside Content

Four hundred milligrams (400 mg) of the gum was macerated with 20 ml of 70% methanol for 24 h. Thereafter the mixture was filtered and the filtrate was made up to 20 ml with the methanol. Three millilitres of the extract and 3 ml of freshly prepared Kedde's reagent [14] were mixed together and the absorbance of mixture was measured after 2.5 min at 560 nm (Jenway, 6300). The amount of cardiac glycoside was calculated from calibration curve of digoxin using Kedde's reagent prepared as follows: twenty five mg of digoxin was dissolved in 25 ml spectroscopic methanol in a volumetric flask. The following volumes: (0.025, 0.05, 0.1, 0.2,1, 1. 4, 2, 3, 4, 5, 6 ml) were withdrawn from the volumetric flask and each one was completed to 10 ml with spectroscopic methanol. These were equivalent to the corresponding concentrations (0.025, 0.05, 0.1, 0.2, 1, 1.4, 2, 3, 4, 5, 6 mg/10ml). 10ml of freshly prepared Kedde's reagent was added to each one. The absorbance of the mixture was measured after 2.5 min at 560 nm against a blank: 10 ml of Kedde's reagent mixed with 10 ml of spectroscopic methanol[15]

Determination of Tannin Content

Tannin determination was done by the method of [16.] A portion (500 mg) of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. 5 ml of the filtrate was withdrawn and put into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in

0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

Determination of Phytate Content

Phytic acid was determined using the procedure described by Lucas[17]. A portion (2 g) of each sample was weighed into 250 ml conical flask; 100 ml of 2% concentrated hydrochloric acid was used to soak each sample for 3 h. The mixture was filtered, and 50 ml of each filtrate was placed in 250 ml beaker and 107 ml of distilled water was added in each case to give proper acidity. 10 ml of 0.3% ammonium thiocyanate solution was added to each solution as indicator and was titrated with standard ferric chloride solution which contained 0.00195 g Fe^{3+} per ml.

% Phytic acid = y × 1.19 ×100

Where y = titre value × 0.00195.

Determination of Carbohydrate and Glucose Content

One hundred milligrams (100 mg) of the gum was macerated with 40 ml of ethanol and left for 12 h and the mixture was centrifuged at 1200 rpm for 15 min. The resulting supernatant was removed and was concentrated on a water-bath. The volume of this aqueous concentrate was raised to 50 ml with distilled water, labelled Extract A, and processed further following the method of[18] for soluble sugars. The residue obtained by centrifugation was used for the estimation of starch as follows:

The above residue from test sample was suspended in a mixture of 5 ml of 52% perchloric acid solution and 6.5 ml of distilled water, shaken vigorously for 5 min and centrifuged at 2500 rpm for 15 min. This step was repeated three times and the supernatants of each sample were pooled and the volume was raised to 100 ml with distilled water and labelled as Extract B. out of Extract B, 1 ml aliquot was taken separately to estimate starch quantitatively.[19]

One millilitre (1 ml) each from Extract A and B were used to quantify the total level of carbohydrates using phenols-sulphuric acid method[20]. A regression curve for standard sugar (glucose) was prepared using a stock solution of glucose (100 mg/ml) in distilled water. From the stock solution 0.1 to 0.9 ml was transferred to test tubes and the volume made up to 1 ml with distilled water. To each of these, 1 ml of 5% aqueous phenol was added rapidly (having been kept in an ice chest) and shaken gently. Later 5 ml of Conc. H_2SO_4 was rapidly added and agitated gently during the addition of the acid. Subsequently, the tube was kept on a water-bath (26º – 30ºC) for 20 min, and the optical density of the yellow orange colours thus developed were taken at 490 nm in a Spectrophotometer after having set it for 100% transmission against the blank. Four replicates were run and the mean values were calculated. A regression was computed between its known concentrations and the respective optical densities which followed the Beer's Law. The concentration of the total soluble sugars was directly worked out from the regression curve of the standard glucose. The sugar content in terms of glucose equivalent was estimated using the conversion factor, 0.9, to convert the values of glucose to starch[21].

Metallic Content Analysis of Treculia africana Gum

Two grams (2.0 g) of the gum was digested by heating it with 20 ml of a mixture containing 650 ml conc. nitric, 80 ml perchloric acid, 20 ml conc. sulphuric acid. The heating was continued until a clear digest was obtained. The digest was then made up to 500 ml with distilled water. A Varian SpectrAA-640 flame atomic absorption spectrometer (Varian, Mulgrave, Australia) equipped with deuterium lamp background corrector was used. The instrumental spectrometer parameters for each element's determination were set according to the manufacturer's recommendations. In all experiments an air–acetylene flame, flowing at 13.5 and 2.0 l min^{-1}, respectively, was used. Hollow cathode lamps for the respective elements were used and each element was measured at its wavelength as shown in Table 2. Appropriate working standard solution was

prepared for each element and their calibration curves were generated.

It is noteworthy that all the determinations (including the phytochemical and metallic contents) were made in triplicates and mean results expressed.

RESULT

Phytochemical Composition of Gum obtained from *Treculia africana* Pulp

The amount of gum derived from the pulp of *Treculia africana* was about 8.57±1.1 % w/w. This amount is relatively encouraging for exploitation for pharmaceutical purposes. Since these pulps have been used as animal feeds for centuries, they may be regarded generally as a safe source of pharmaceutical excipients. The phytochemical contents of the gum derived from these seeds are shown in Table 1.

Table 1: Phytochemical contents of the gum extracted from *Treculia africana* pulp

Phytochemical	Content in gum (%w/w)
Alkakoids	0.000
Flavonoids	0.000
Saponins	0.000
Cardiac glycosides	0.000
Tannins	0.000
Phytate	0.137±0.008*
Oxalate	0.113±0.004*
Carbohydrate	0.110±0.005*

*Content is in mg/100 g.

The phytochemicals- alkaloids, flavonoids, saponins, cardiac glycosides and tannins were not present in the gum - an indication that the gum is a good candidate for use as a pharmaceutical excipient. Phytic acid known as inositol hexakis phosphate or phytate when in salt form is the principal storage form of phosphorous in many plant tissues, especially bran and seeds. Phytic acid is a strong chelator of important minerals, such as calcium, magnesium and iron, and can therefore contribute to mineral deficiencies. [22] The dianion known as oxalate is a reducing agent as well as a ligand in coordination chemistry. Many metal ions form insoluble precipitates with oxalate, a prominent example being Ca oxalate, which is the primary constituent of the most common kind of kidney stone.[23] Phytate and oxalate were present at concentrations of 0.137±0.008 mg/100 g and 0.113±0.004 mg/100 g of the gum respectively (Table 1). These values are much lower than those published for some herbal products [22], and since the gum is being advocated for use as pharmaceutical excipient, its use will be in small quantities which must be within acceptable limits for these phytochemicals. The carbohydrate content of the gum was 0.110±0.004 mg/100 g (Table 1). This value is low and is in agreement with earlier publications[24] and further re-emphasizes the fact that it will pose no threat to use by diabetic patients as earlier asserted[25].

The gum contains both heavy and non-heavy metals (Table 2). Among the category of inorganic impurities, metal impurities have long been monitored in food and drug articles intended for consumption by humans and other animals [26]. Major interest on metallic impurities is on the heavy metals, whose presence in food or drug beyond certain levels had given rise to documented toxicity manifestations in both humans and animals. This informs the need to establish limits for the amount of these metals in foods and pharmaceuticals. *Treculia africana* pulp gum contains some of these metals in amounts much lower than the accepted limits[26] (Table 2).

CONCLUSION

Treculia Africana gum has been shown, in this study, to contain no harmful phytochemicals, and the metal contents are within normal limits.

It is therefore very obvious that this gum which was harvested from a plant source that has been generally regarded as safe is probably safe for use pharmaceutically as adjuvant in formulations.

Metallic Content of *Treculia africana* Gum

Table 2: Metallic element content of gum extracted from *Treculia africana* pulp

Element	Lambda max (nm)	Content in gum (ppm)	Allowed Limit (ppm)
Potassium	766.5	1.036±0.009	
Cadmium	324.3	0.015±0.006	0.05
Silver	338.5	0.0000	
Chromium	357.9	.0310±0.002	0.15
Cobalt	240.7	0.0360±0.001	1.00
Calcium	422.7	0.850±0.004	
Aluminium	309.3	32.0000	500.00
Molybdenium	313.3	0.0000	2.50
Arsenic	193.7	0.095±0.002	0.15
Selenium	196.0	0.0000	2.50
Copper	324.7	0.233±0.155	25.00
Zinc	213.9	5.7814±1.230	150.00
Mercury	253.7	0.0000	0.15
Magnesium	285.2	34.0000±2.133	a
Iron	248.3	21.0000±1.913	150.00
Manganese	760.0	9.238±2.560	70.00
Lead	217.0	0.01230±0.0004	0.10
Nickel	232.0	1.399±0.012	2.50

a- Under deliberation

ACKNOWLEDGEMENT

I acknowledge with thanks, the contributions made by the following in this study.

Akukris Pharmacy Limited, Nsukka, Enugu State, Nigeria, through financial support.

Madonna University, Elele, Rivers State, Nigeria for making their facility available.

The Almighty God for the gift of life and health and all resources.

To Him be the Glory!

REFERENCES

1. Hutchinson, J, The Families of Flowering Plants. Oxford University Press, 1973, London.
2. Irvine FR, Woody plants of Ghana with special reference to their uses. Oxford University Press, London, 1961, 54-62.
3. Morton J, Breadfruit. In: Fruits of warm climates. Miami, USA, 1987, 50-58.
4. Okafor JC, Indigenous Trees of the Nigerian Rainforest. A Paper Presented in a

symposium on the Potentials for Domestication and Rebuilding of Forest Resources. Yaounde, Cameroon, 1990, 34-38.

5. Baiyeri KP, Mbah, BN, "Effect of Soiless and Soil-based Nursery Media on Seedling Emergency, Growth and Response to Water Stress of African Breadfruit (Treculia africana Decne)", African Journal of Biotechnology, 2006, 5(15), 1400-1405.
6. Onyekwelu, JC, Fayose, OJ, "Effect of Storage Methods on the Germination and Proximate Composition of Treculia africana Seeds", Paper Presented at the Conference on International Agricultural Research for Development. Tropentas, 2007, Germany.
7. Nwabueze TU and Uchendu CB, "African Breadfruit (Treculia africana) Seed as Adjunct in Ethanol Production", European Journal of Food Research and Review, 2011, 1(1), 15-22.
8. http://en.wikipedia.org/wiki/Natural_gum. Accessed on 02/10/13.
9. Umeshkumar MD, Vilas ND, Dinesh MS, Natural Gums and Mucilage's in NDDS: Applications and Recent approaches. International Journal of PharmTech Research, 2012, 4(2), 799-814.
10. Sarkiyayi S, Mohammed M and Yakubu A, "Comparative Analysis of Nutritional and Anti Nutritional Contents of Some Varieties of Mango (Mangifera indica) in Kaduna Metropolis-Nigeria", Research Journal of Applied Sciences, Engineering and Technology, 2013, 5(4), 387-391.
11. Obadoni BO and Ochuko PO, "Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria", Global Journal of Pure Applied Sciences, 2001, 86, 203 – 208.
12. Omale J, Adeyemi AR and Bamaiyi OJ, Phytoconstituents, proximate and nutrient investigations of Saba florida (Benth.) from Ibaji forest", International Journal of Nutrition and Metabolism, 2010, 2(5), 88-92.
13. Boham B.A. and Kocipai-Abyazan R, "Flavonoids and condensed tannins from leaves of Hawaiian Vaccinium vaticulatun and V. calycinium", Pacific Science, 1994, 48, 458 – 463.
14. Kedde DL, "Mechanism of the Raymond, Kedde and Baljet reactions", Pharm Weekbl., 1947, 82, 741.
15. Ahmed AE, Ezzat G, Safeyia MG, and Shams AR, "Regeneration,Cardenolide and Flavonoid Production from In Vitro Cultures of Cynanchum acutum L. (Asclepiadaceae)", Australian Journal of Basic and Applied Sciences, 2011, 5(6), 704-717.
16. Van–Burden TP and Robinson WC, "Formation of complexes between protein and tannin acid", Journal of Agricultural and Food Chemistry, 1981, 1, 77.
17. Markakas P and Lucas GM, "Phytic acid and other phosphorus compounds of bean (phase olus vulgaris)", Journal of Agricultural Education Chemistry, 1975, 23, 13 – 15.
18. Loomis and Shull, 1937.
19. McCready RM, Guggoiz J, Silviera V and Owens HS, "Determination of starch and amylase in vegetables", Analytical Chemistry, 1950, 22, 1156-1158.
20. Dubois M, Gilles K, Hamilton JK, Rebers P.A. and Smith F, "A colorimetric method for the determination of sugar", Nature, 1951, 168, 167.
21. Vijayvergia R and Kumar J, "Quantification of Primary Metabolites of Nerium indicum Mill", Asian Journal of Experimental Sciences, 2007, 21(1), 123-128.
22. Muneeb UK, Farhat AK, "Evaluation of inorganic profile and anti- nutritional values of Cocculus hirsutus", African

Journal of Pharmacy and Pharmacology, 2012, 6(3), 144-147.

23. Anita BS, Akpan EJ, Okon AA and Umoren IU, "Nutritive and anti-nutritive evaluation of sweet potato (Ipomoea batatas) leaves", Pakistan Journal of Nutrition, 2006, 5(2), 166-168.
24. Ogunleye AJ and Parakoyi DB, "Chemical compositions of the pulp and seedlings of Treculia africana and clinical trial of the extracts from pulp in treatment of worms", Nig. J. Pure & App. Sci, 1992, 7, 179 - 181.
25. Nuga OO and Ofodile EAU, "Potentials of Treculia Africana Decne – An Endangered Species of Southern Nigeria", Journal of Agriculture and Social Research, 2010, 10(2), 91- 99.
26. Zaidi K, "General Chapter on Inorganic Impurities: Heavy Metals. USP AdHoc Advisory Panel on Inorganic Impurities and Heavy Metals and USP Staff", 2006, 1-6.