Extraction and Assessment of Polymer from *Triculia Africana* Seeds, As a Pharmaceutical Excipient

Ordu J I* and Onichabor Serah

Department of Pharmaceutics/Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Nigeria
*Correspondence author**

Abstract: **The use of natural polymers appears attractive, as they seem to be economical, readily available, non-toxic, capable of chemical modifications, potentially biodegradable and biocompatible though with few exceptions. This study aims to evaluate the gum obtained from seeds of *Treculia africana* as a suspending agent. The pericarp of the plant seeds was, removed and the seeds cleaned and milled. The resulting powder was defatted using a 2:1 mixture of chloroform and acetone in a soxhlet apparatus and the pure gum recovered by several washing with acetone. The mean percentage yield of dried seed gum was 12.3%. Preliminary investigation studies involving solubility, swelling index, pH, and hydration capacity, elemental and proximate analysis were, undertaken on the extracted gum. The gum was characterized with pH 7.0 – 7.4, hydration capacity 7.97± 0.01 and swelling index 8.58±0.05. Elemental and proximate analyses reveal high potassium 61% and carbohydrate content 72% respectively.**

***Key words: Treculia africana seeds*, polymer, extraction, assessment, excipient**

I. INTRODUCTION

Polymers(synthetic and natural), have been investigated and employed in the formulation of solids, liquids and semi-solid dosage forms and are found useful in the design of novel drug delivery systems [1]. For a number of reasons there has been an increase interest in the development of new plant excipients because some drugs show incompatibilities with many of the current range of excipients especially the synthetic ones as some of them possess drawbacks of toxic effects and health challenges. To avoid these and reduce import expenses, alternative natural gums are being explored. The use of natural polymers for pharmaceutical applications is attractive because they are economical, readily available, non-toxic, capable of chemical modifications, biocompatible but potentially biodegradable although with few exceptions [2].

The usefulness of plant-based materials example natural gums, as pharmaceutical excipients have been explored as they can be modified to meet the requirements of drug delivery systems and thus compete with the synthetic ones available commercially [3]. The traditional use of excipients in drug formulation was to act as an inert substance, providing necessary weight, consistency, and volume for proper administration of the active pharmaceutical ingredient (API). In modern pharmaceutical formulation, the excipients could serve multifunctional roles as, modifying release, improvement of stability and bioavailability of API, enhancement of patient acceptability and ensuring ease of manufacture.

The interest on natural plant based material, stems from the fact that resources are renewable and if cultivated or harvested in a sustainable manner, can provide a consistent supply of needed materials. However, substances from plant origin also pose several potential challenges as, occurrence in small quantities and in structurally complex mixtures, which may differ according to the location of plants as well as influence of such variables as season and environmental conditions, which may result in slow and expensive isolation and purification process.

Mucilages/gums are often found in different parts of plants such as the epidermal cells of leaves (*C. olitorius*), seed coats (linseed, mucuna), roots (taro), barks (slippery elm) and middle lamella (aloe), marine algae and selected microorganisms. Mucilages are polysaccharide complexes formed from sugar and uronic acid units; they form slimy masses in water, and are typically heterogenous in composition. Upon hydrolysis, arabinose, galactose, glucose, mannose, xylose and various uronic acids are the most frequently observed components [5].

Chemically, nearly all of these plant gums are carbohydrates composed of repeating sugar (monosaccharide) units with equilibrium moisture content of 10% or more and are non-toxic.

During production, they are exposed to the external environment and so there is a chance of microbial contamination, however, this can be prevented by proper handling and the use of preservatives.

Due to differences in the collection of natural materials at different times, as well as differences in region, species, and climate conditions the percentage of chemical constituents present in a given material may vary. Therefore, there is a need to develop suitable monographs on available gums and mucilages as synthetic manufacturing is a controlled procedure with fixed quantities of ingredients, while the production of natural gums and mucilages are dependent on environmental and seasonal factors.

Gums and mucilages, because of their polysaccharide nature, produce an indefinite number of monosaccharides on

hydrolysis. Depending on the type of hydrolysed products obtained; they can be classified into pentosans (e.g. xylan) and hexosans (e.g. starch and cellulose).

Gums are otherwise, regarded as pathological products consisting of calcium, potassium and magnesium salts of complex substances often termed as 'polyuronides' while mucilages are physiological products related to gums, but are generally sulfate acid esters, with the ester group being a complex polysaccharide [6].

Both gums and mucilage's are closely related to hemicelluloses in composition, except that the sugars produced by hemicelluloses are glucose, mannose and xylose, whereas those form gums and mucilages are galactose and arabinose [7].

Although various kinds of gums and mucilages are used in the food industry and are regarded as safe for human consumption, there is growing concern over the safety of pharmaceutical excipients derived from natural sources, hence gums and exudates are now screened for their use as pharmaceutical adjuvants especially for their binding, stabilizing and humidifying effects in medicine. Newer uses of different gums and mucilages in cosmetics and textiles have increased in demand and thus screening of gums has become an important pharmaceutical area [8].

Gum and mucilage, known as hydrophilic polymers, could be used in medicines, for their demulcent and bulk laxative properties and also useful as, tablet binders, disintegrant, emulsifiers, suspending, gelling, stabilizing, thickening and as film forming agents for transdermal, periodontal use, and as sustaining and coating agents respectively in matrix tablets and microcapsules formulation including, those for protein delivery[8].

Treculia africana plant

Fig 1:*Treculia africana* tree

Fig 2:*Treculia africana* seeds

Fig 3: *Treculia africana* seeds

Treculia africana is a large tree species in the genus Treculia and family Moraceae often used as a food plant and for various traditional uses. It grows in wet areas and forests and the fruits contain polyphenols. The species can grow up to a height of 30 m (98 ft) and the bark is grey and discharges a cream latex while the leaves are large and dark green above and lighter below [9].

The tree is dioecious or sometimes monoecious and leaves occur in two ranks; the stipules are amplexicaul (enclosing the bud), inflorescences are unisexual but sometimes bisexual while the pistil late (female) flowers line the outer surface of a large receptacle termed the breadfruit.

The flowering period is from October until February. The fruit appears big, round, greenish yellow and the texture spongy when ripe and contains abundant dicotyledonous seeds, serving as the edible part of this fruit and under favorable environmental conditions, the yield from one tree could contain up to 200 kg of dried seeds.

Culinary use

African breadfruit is an edible traditional fruit, consumed as a main dish especially in some countries. The seeds are of particular interest because of their high nutritional value as the fresh seeds could have high content of carbohydrate, crude protein, and fat. *T. africana* can serve as alternative to rice and yam while the seeds can be ground to flour, pressed for oil, or used as flavoring agents in alcoholic drinks serving as a good adjunct in brewing because it is a source of fermentable sugars and can also be dry-roasted and eaten as a snack [10].

This tree helps to control erosion however, deforestation, higher demand for cultivated agricultural areas, and the increasing population has helped to reduce numbers of this important forest tree in the African tropics.

The aim of this study is to extract gum (polymer) from *Treculia africana* seed and assess its relevance as a potential pharmaceutical excipient.

II. MATERIALS

Chemicals used were of analytical grade and includes: Acetone (kermel,China), Chloroform (kermel China), propylene glycol (kermel, China),*Treculia africana* seeds (mile 3 market PortHarcourt). Pycnometer, water bath (HH-1, HH-4, HH-6, HH-8, (Techmel and Techmel, USA),oven (New lifemedical, England).

III. METHODS

Extraction of gum from the seeds of Treculia africana

The seeds were placed in hot air oven at 60^{o}C until dried but not brittle then the pericarp removed by gentle rubbing of the dried seeds in-between the palms. The cleaned seeds were milled using a kitchen blender and the resulting powder weighed. The powder was defatted using a 2:1 mixture of chloroform and acetone, 100g of the powder was defatted by passing 500 ml of the solvent mixture in a soxhlet apparatus until the liquid dropping from the powder pack was colorless. Thereafter the resulting residue was air dried at room temperature (25^{o}C) for 72hr. The procedure was repeated in defatting the remaining powder bulk while gum isolation was effected by immersing 100g of defatted gum in 500ml of boiling water in a bowl and stirring with a stainless steel paddle for 10 min. The mixture was then strained through a muslin cloth and resulting mucilage precipitated with twice its volume of acetone. The precipitate (gum) was harvested and soaked in fresh acetone for 6 hr to remove entrapped water and oil. Thereafter the gum was air dried for 48hr and in the hot air oven at 60^{o}C for 1h, pulverized using a blender, passed through a 1mm sieve aperture and stored in a desiccator.

IV. ORGANOLEPTIC STUDIES

The extracted *T. africana* gum was powdered and observed for color, texture and taste.

Phytochemical Examination of the Extracted Gum

Ruthenium red test: A 0.1g of dried and powdered gum was placed, on a slide then a drop of ruthenium red reagent added and observation made under a light microscope.

Molisch test: A 0.1g of the powdered gum was transferred into a clean test tube while two drops of freshly prepared Molisch reagent was introduced into the tube. Concentrated sulfuric acid (1ml) was gradually added on the side of the tube to form a layer below the aqueous solution and observations made.

Iodine test: 0.1g of dried powdered gum was mixed, with 1ml of 0.2N iodine solution in a test tube and observed for colour change.

*Determination of Moisture***:** Moisture content was determined by oven drying method. 1.5 g of powdered gum was accurately weighed into a clean, dried crucible (W). The crucible was placed in an oven at 100-105^{o}C for 6-12 h until a constant weight was obtained then removed and kept in a desiccator to cool. After cooling, it was weighed again and the weight recorded as (W_2). The percent moisture content was determined as:

$$\% \text{ moisture} = \frac{W - W_2 \times 100}{W} \quad \ldots\ldots\ldots\ldots (1)$$

Where W = initial weight of crucible + sample, W_2 = final weight of crucible + sample

Determination of Ash: Clean empty crucible was placed in a muffle furnace at 600^{o}C for an hour, cooled in a desiccator and then weight of empty crucible was noted (W). 1gram of powdered gum was placed in the crucible (W_2). The sample was ignited over a burner with the help of blowpipe, until it is charred. Then the crucible containing the charred gum was placed in a muffle furnace at 550^{o}C for 2-4 h. The appearance of gray white ash indicates complete oxidation of all organic matter in the sample. After ashing, the furnace was switched off, the crucible was removed, cooled and weighed (W_3). Percent ash was determined using the relation:

$$\% \text{ Ash} = \frac{W_2 - W_3 \times 100}{W_2} \quad \ldots\ldots\ldots\ldots (2)$$

$W_2 - W_3 =$ difference in wt. of ash

Determination of Crude Protein

Protein in the sample was determined by Kjeldahl method. The samples were digested by heating with concentrated sulphuric acid (H_2SO_4) in the presence 2- 4ml of digestion mixture. The mixture was made alkaline and ammonium sulphate formed. Released ammonia was collected in 2% boric acid solution and titrated against standard HCl. Total protein was calculated by multiplying the amount of nitrogen with appropriate factor (6.25). Percent crude protein content of the sample was calculated by using the relation:

$$\% \text{ Crude protein} = 6.25^{*} \times \%N \text{ ------ (3) (* Correction factor)}$$

$$\%N = \frac{(S - B) \times N \times 0.014 \times D \times 100}{\text{Wt of the sample} \times V} \quad \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Where S = sample titre vol, B = Blank titre vol, N = normality of HCl

D = dilution of sample after digestion, V = volume taken for distillation

0.014 = milli equivalent weight of nitrogen

Determination of Crude Fat

Crude fat was determined by ether extraction using Soxhlet apparatus. Approximately 1.0 g of moisture free powdered gum was wrapped in filter paper, placed in fat free thimble and then introduced into the extraction tube cleaned and dried. The receiving beaker was filled with petroleum ether and fitted into the apparatus, while water heater was turned on to start extraction. After 4-6 siphoning ether was allowed to evaporate and the beaker disconnected before last siphoning. The extract was transferred into clean glass dish with washing and evaporation of ether on water bath. The dish was placed in an oven at 105^{o}C for 2 hrs then cooled in a desiccator. Percent crude fat was determined as:

$$\% \text{ Crude fat} = \frac{\text{Wt. of ether extract} \times 100}{\text{Wt. of sample}} \quad \text{---------- (5)}$$

Determination of Crude Fiber

A moisture free and ether extracted sample containing crude fiber made of cellulose was first digested with dilute H_2SO_4 and then with dilute KOH 2-4ml solution. The undigested residue collected after digestion was ignited and loss in weight after ignition was registered as crude fiber.

The sample was dried in an oven at 150°C for 1 hr, then allowed to cool in a desiccator and weighed (W_1).The sample in the crucible was then placed in a muffle furnace at 550°C for 3-4 hr, later removed and cooled in a desiccator and weighed again (W_2).

Calculations were done using the formula

$$\%\text{Crude fiber} = \frac{W_1 - W_2 \times 100}{W_1} \quad \text{---------- (6)}$$

Elemental Analysis

Mineral contents of the extracted gum from *T. africana* seeds were determined by atomic absorption spectrometry with atoms of the elements (iron (Fe), calcium (Ca), Zinc (Zn) and Magnesium (Mg), absorbing, light at a characteristic wavelength and according to the methods of AOAC (2003) [11]. Sodium (Na) and potassium (k), were determined using flame photometer which measures the emission of radiant energy when 6 atoms of an element return to their ground state after their excitation by the high temperature of the flame. The degree of emission assumed to be equated to the concentration of the element in the solution.

Solubility Test: The powdered materials including the extracted gum were evaluated for solubility in water, ethanol and acetone.

A 1.0g quantity of each powdered material was weighed and transferred into clean test tubes containing 10ml of the solvents separately. The mixtures was shaken vigorously and observed for solubility and ease of dispersion each of the solvent.

Hydration Capacity: The hydration (water retention) capacity of the respective powders was determined following the method as explained. A 1.0g quantity of powder (y) was placed in a 15ml plastic centrifuge tube and 10ml of water was added. The tube was shaken intermittently for 2hrs then left undisturbed for 30mins. The mixture was centrifuged for 10mins at 3000rpm the supernatant was decanted and the weight of the powder after water uptake and centrifugation (x) was determined [12]. Triplicate readings were carried out and mean value used for the calculation.

$$\text{Hydration capacity} = \frac{x}{y} * 100 \quad \text{-------- (7)}$$

Where y = weight of dry powder, x = weight of moist powder after centrifugation.

Swelling Index

The swelling index was determined using the method of Ohwoavworhua and Adelakun [13]. The tapped volume occupied by 1.0g of the powder (Vo) was noted. The powder was then dispersed in 5ml of water and the volume made up to 10ml with water. After 24hrs of standing, the volume of sediment (V_1) was estimated. Triplicate determinations were carried out and the swelling capacity computed as:

$$\text{Swelling capacity} = \frac{V_0}{V_1} \quad \text{------- (8)}$$

Where V_0 is the volume of sediment and V_1 is the tapped volume occupied by powder.

pH determination

The pH of 1.0% w/v dispersion of the powders as the active pharmaceutical ingredient (API) was determined using the pH meter (Helmreasinn, PHS-25).

V. RESULTS

Table1: Organoleptic Properties OF *T. africana* gum

Parameters	Characteristic
Colour	Brown
Odour	Pleasant
Texture	Fine

Table 2: Preliminary confirmatory tests for dried *T. africana* gum

Test	Observation	Inference
Ruthenium test	Pink coloration	Gum Present
Molisch test	Violet color observed at the junction of the two layers	Carbohydrate Present
Iodine test	Blue-black coloration observed.	Starch present

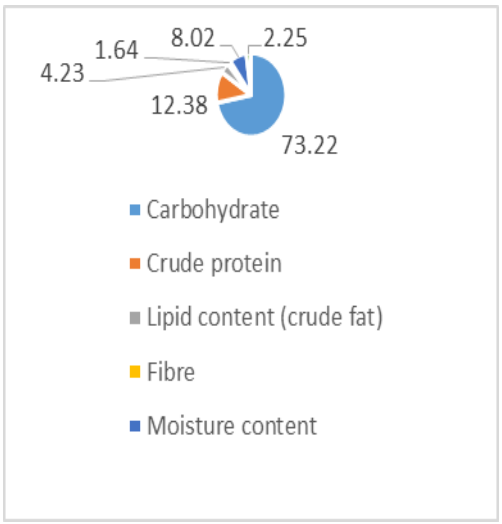

Fig.2: Proximate composition of *T. Africana* seed gum.

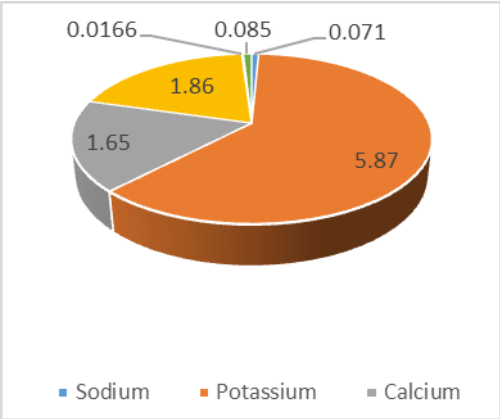

Fig 3: Elemental composition of *T. africana* gum.

Table 3: Physicochemical properties of extracted gum

Test	*Treculia africana*
Solubility Test Ethanol	Insoluble
Acetone	Insoluble
Water	Insoluble
Moisture content (%)	8.02±0.02
Hydration capacity (%)	7.97±0.01
Swelling index (in water)	8.56± 0.03

All values are expressed as the mean of triplicate values ± standard deviation

Table 4: pH of Various Concentrations (%w/v) of *Treculia africana* gum

Concentration (%)	Mean triplicate pH of *Treculia africana*
0.1	7.0
0.2	7.1
0.5	7.2
1.0	7.4

VI. DISCUSSION

Plant gums are associated with adhesive substances and consists of complex carbohydrate polymers which may contain hydrophilic, hydrophobic and poly rich proteins or other components and can be soluble, partly soluble or insoluble as observed in the gum from *T. africana* seed. The mean percentage yield of dried seed gum was 12.3%. Presence of gum, carbohydrate and starch were confirmed on the purified plant extract by treatment with ruthenium red, Molisch reagent and iodine respectively.

The result obtained following proximate analysis of the gum showed it contained carbohydrate 73.22%, protein 12.38%, fat 4.23%, fibre 1.64%, moisture 8.02% and ash 2.25%. Carbohydrates are rich source of energy and are found in foods like fruits, vegetables, and dairy products. The body uses these foods to make glucose and fructose (the body's main energy source), vitamins and minerals, hence with the resultant mineral constituents of the *T. africana* seed, the gum extracted can be effective in the formulation of pharmacological and therapeutic active drugs due to rationally distributed broad based biologically effective components.

The elemental analysis result reveal presence of sodium 0.0710mg/g, potassium 5.8700mg/g, calcium 1.6500mg/g, magnesium 1.8600mg/g, iron 0.0166mg/g and zinc 0.0850mg/g. From the analysis, the gum is seen to be rich in mineral contents especially potassium which is regarded as one of the most important minerals in the body as it helps in the regulation of, fluid balance, muscle contraction, nerve signals, blood pressure and water retention hence protecting against stroke, osteoporosis and kidney stones [14].

The gum analyzed for phytochemical and physicochemical characterization as shown in Table 3, reveals the extracted *T. africana* seed gum as, having appreciable water retention (swelling index) and absorption (hydration) capacity hence the gum could serve as a useful excipient in pharmaceutical, food, dairy industries. The gum, based on outcome of the analysis, could find application as; adhesives, crystallization inhibitors, emulsifying agents, encapsulating agents, film formers, foam stabilizers, suspending agents, stabilizers, syneresis inhibitors and other specific functions as intended for the formulations.

The solubility test showed that *Treculia african* gum was in dispersible in ethanol, acetone and water and the moisture content is 8.02%, hydration capacity 7.97% and swelling index 8.56 hence could have the ability to absorb, retain and swell in the presence of water.

Knowledge of the pH of an excipient is an important parameter and determines its suitability in pharmaceutical product formulations since the stability and physiological activity of most preparations depends on pH.

From the result, as seen in table 4, pH of *T. africana* gum, increased progressively with increased concentration of the gum and ranging between, 6.9 to 7.4. This thus indicates that the gum have a neutral pH and hence associated with inert property and therefore, could be suitable in the formulation of varied range of pharmaceutical, food and dairy products needed in the human physiological system.

VII. CONCLUSION

Natural gums are promising biodegradable polymeric materials rich in elemental and mineral contents with clear advantages over synthetic materials. However, there is need to search for newer natural sources as well as modify existing ones for the development and formulation of novel drug delivery, biotechnological application and other delivery system. Therefore, there should be continued interest in natural gums and their modifications with the aim of developing better materials for drug delivery system.

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