WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 5, Issue 2, 86-97. Research Article ISSN 2277– 7105

CHARACTERIZATION OF *RAPHIA HOOKERI* GUM FOR THE PURPOSE OF BEING USED AS A PHARMACEUTICAL EXCIPIENT

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Article Received on
26 Nov 2015,

Revised on 17 Dec 2015,
Accepted on 06 Jan 2016

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ABSTACT

Gums are common constituents of plants obtained as a result of physical injuries to the plants. This study elucidated the physical, thermal, sorption and functional properties of a gum obtained from the cut trunk of *Raphia hookeri* G. Mann & H. Wendl (Family Palmaceae). The physicochemical properties of the plant was characterized by Scanning electron microscopy (SEM), x-ray powder diffraction (XPRD), thermogravimetric analysis (TGA), differencial scanning colorimeter (DSC), fourier transmittance infra red (FTIR), compressibility and flowability including swelling index and elemental analysis were used to characterize the gum sample. The excellent swelling properties of raffia gum demonstrated suggested that the gum may perform well as binder as well as polymer in controlled release dosage forms. The low protein and lipid contents couple with absence of fibres confirm the emulsifying properties of raffia gum. Raphia gum was also found to possess antimicrobial properties. Raffia gum is pH sensitive and may therefore be useful in intestinal/colon drug delivery. The studied parameters indicate that this gum could be used as a pharmaceutical excipient.

KEYWORDS: *Raphia hookeri*, Gum, Binder, Mineral elements, Physico-chemical analysis.

1. INTRODUCTION

Gums are abnormal products resulting from pathological injury or by unfavorable condition of growth brought about by changes in existing cell wall. Gums are produced by the conversion of the cell of the tissue into gum, by mean of an enzyme of the origin of which nothing definite is known.[1] Gums are widely employed in the pharmacy as thickeners,

suspending agents, emulsifying agents, binders and film formers. With the increase in demand for natural gums, it has been necessary to explore the newer source of gums to meet the industrial demands.[2] Excipients such as gums are additives used to convert active pharmaceutical ingredient into dosage forms suitable for administration to patient. Excipients of natural origin such as gums are of particular interest to us for reasons of reliability, sustainability and avoiding reliance upon materials derived from fossil fuels. Plant products are therefore attractive alternatives to synthetic product because of biocompartibility, low toxicity, environmental friendliness and low price compared to synthetic products.[3]

Raphia hookeri G. Mann & H. Wendl (Raffia palm) (Figs. 1a & b) is the largest palm in Africa and it is restricted to the tropical rainforest occurring in western and central Africa from Liberia to Angola. *Raphia hookeri* is the one of the most economically useful plants in Africa; the leaves are used for shelter and the stem produces palm sap (palm wine), which is drank as beverage. The fermented sap could be distilled into alcohol or local gin or ogogoro in Nigeria.[4] The trunk could serve as firewood. The mesocarp of the ripe fruit yields edible oil.[5],[6] The leaf petiole yields the fibrous piassava. In their local environments, raffia fibres are used for ropes, sticks and supporting beams, and various roof coverings are made out of its fibrous branches and leaves. The membrane on the underside of each individual frond leaf is taken off to create a long thin fibre which can be dyed and woven as a textile into products ranging from hats to shoes to decorate mats and coffins.

Figs. 1a & b: *Raphia hookeri* growing wild in the bush along Itu-Calabar high way at Itu Local Government Area of Akwa-Ibom State, Nigeria.

Although previous authors have reported works on the gum from Raphia palm because hardly do people know that the cut stem produces exudates. *Raphia hookeri* is tapped once in its lifetime because it flowers once and dies after fruit maturity[7],[8] that is after seven years of vegetative growth.[9] The spent *Raphia hookeri* is cut and its gum oozes out slowly until it covers the whole surface of the cut stem within a short time. But there is however scanty reports on the characterization and the pharmaceutical value as excipient of the Raffia palm.

2. MATERIALS AND METHOD

2.1 Materials

Raffia palm used in this work was obtained from Itu Road suburb, Uyo, Akwa-ibom State, Nigeria (Fig. 1). The gum obtained was as a result of the cut stem after the inflorescent part of the raffia palm was removed during tapping. The identification of voucher specimens was confirmed by Mr. Etafia, a botanical taxonomist of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria. Gum acacia (GA) which has severally been used as pharmaceutical excipient was used as the standard for our study. Ethanol, methanol, acetone and chloroform were of analytical grade.

2.1.1 Collection, extraction and purification of gum

The trunk of the tree *Raphia hookeri* was cut and the gum exudates were allowed to dry and then hand-picked from the tree. The dried gum was washed and dried in hot air oven at 40^{o}C for 48 hr. The gum was then crushed to break up the gum and then hydrated in double strength chloroform water for 5 days with intermittent stirring. The mucilage obtained was strained through a clean calico cloth and the gum was precipitated with 95% $^{w}/_{v}$ ethanol. The precipitated gum was filtered, washed with diethyl ether and then dried at 50^{o}C in hot air oven. The dried gum was pulverized and passed through sieve size No 60 (250 µm) Fig. 2.

Fig. 2: Photo of *Raphia hookeri* gum powder.

2.2 Methods

2.2.1 Phytochemical examination

Preliminary tests were performed to confirm the nature of gum obtained. The chemical tests conducted were: Ruthenium red test, Mollusch tests, test for reducing sugars and Ninhydrin test.[10]

2.2.2 Physicochemical characterization of the gum

Solubility test: The gum was evaluated in water, acetone, chloroform and ethanol in accordance with the B.P specifications (B.P., 2004).

2.2.3 Origin and Purification of the gum sample

Raffia palm used in this work was obtained from Itu Road suburb, Uyo, Akwa-ibom State, Nigeria. The gum obtained was as a result of the cut stem after the inflorescent part of the raffia palm was removed during tapping. The identification of voucher specimens was confirmed by Mr. Etafia, a botanical taxonomist of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.

2.2.4 Determination of nature of gum

Preliminary tests which were performed to confirm the nature of gum obtained were Ruthenium red test, Molisch tests and reducing sugars and Ninhydrin.[10]

2.2.5 Determination of vitamins, phytochemicals and antinutrients

The phytochemical constituents of the gum (alkaloid, saponin, phenols and flavonoids) and its vitamins contents were determined by the methods reported by.[11]

2.2.6 Solubility test

The gum was evaluated for solubility in water, acetone, chloroform and ethanol in accordance with the B.P specification.[12]

2.2.7 Loss on drying

Gum sample (1.0 g was transferred into each of several Petri dishes and then dried in an oven at 105^{o}C until a constant weight was obtained. The moisture content was then determined as the ratio of weight of moisture to weight of sample expressed as a percentage.

2.2.8 Determination of total ash and acid insoluble ash

Ash was estimated by the measurement of the residue left after combustion in a furnace at 450^{o}C. The ash obtained from the determination of the ash was boiled with 25 mL of 2M hydrochloric acid solution for 5 min and the insoluble matter was filtered and washed with hot water and ignited and the subsequent weight was determined. The percent acid insoluble ash was calculated.

2.2.9 Determination of pH

This was done by shaking 1% $^{w}/_{v}$ dispersion of the sample in water for 5 min and then the pH determined using a pH meter.

2.2.10 Determination of refractive index of the gum

An electron refractometer was used to determine the refractive index of the gum.

2.2.11 Determination of crude lipid content

The lipid content was determined using the method recommended by AOAC.[13]

2.2.12 Swelling index

Gum (1g) was placed in a plastic centrifuge tube and the volume occupied was noted. Distilled water (10 mL) was added from a 10 mL measuring cylinder and stoppered. The content was shaken for 2 min. The mixture was allowed to stand for 10 min and immediately centrifuged at 1000 rpm for 10 min. the supernatant was carefully measured. The swelling index was computed using the equation:

$$S = V_2 / V_1$$

Where, S = Swelling index

V_1 = Volume occupied by the gum prior to hydration.

V_2 = Volume occupied by the gum after to hydration.

2.2.13 Determination of viscosity

The intrinsic viscosity of raffia gum sample was determined in distilled water using a Canon Ubbelohde capillary viscometer (Cannon Instrument, model 1-17) which was immersed in a precision water bath maintained at 25^{o}C. The apparent viscosity of the mucilage was measured using a digital Brookfield DV I prime viscometer while shear rate was measured using a Schott Iberica, S.A 18549 rotational viscometer.

2.2.14 Fourier Transform Infra Red (FT-IR) analysis

FT-IR analysis of the gum was carried out using a Spectrum BX Perkin Elmer Fourier transform infra-red spectrophotometer. The sample was prepared in KBr and the analysis was carried out by scanning the sample through a wave number range of 400 to 4000 cm-1.

2.2.15 Determination of concentration of metals

Concentration of metals was determined using a Perkin Elmer atomic absorption spectrophotometer AAS Analyst 400 Perkin Elmer. A flame photometer, Jenway, PEP7 Flame photometer was used for potassium. A calibration curve for each metal was prepared and the concentration of the metal in the analyte was estimated by extrapolation.

2.2.16 Determination of vitamins, phytochemicals and anti-nutrients

The phytochemical constituents of the gum (alkaloid, saponin, phenols and flavonoids) and its contents were determined by the methods reported by Okwu and Nnamdi.[11] Anti-nutritional contents of the gum were determined using the methods reported by Akpabio *et al.*[14]

2.2.17 Angle of repose

The static angle of repose was measured according to the fixed funnel and free standing cone method. A funnel was clamped with its tip 2 cm above a graph paper placed on a flat horizontal surface. The powder was carefully poured through the funnel until the apex of the cone thus formed just reached the tip of the funnel. The mean diameter of the base of the powder cone was determined and the tangent of the angle of repose calculated using the equation:

$$\text{Tan } a = 2h/D$$

2.2.18 Determination of elemental analysis

Elemental analysis of carbon, hydrogen and nitrogen was carried using a Thermo Finnigan Flash Ea 1112 CHNS-2000 analyser. A Perkin-Elmer Elemental Analyzer was used for the determination of oxygen.

2.2.19 Bulk density

Gum powder (30.0g) was placed in a 100 mL measuring cylinder and the height was noted. After 100 taps on the table, the occupied volume was read. The diameter of the cylinder was

measured with a vernier scale. The height noted is used to measure the volume from the formula:

Volume = Π r2 h

The inverse of this volume is the bulkiness.

2.2.20 Tap density

Gum (10 g) was transferred to a graduated 100 mL measuring cylinder. The volume occupied after tapping was determined.

Data are means ± standard deviation of triplicate determinations (mg 100 g^{-1}). Means followed the same superscript in each column are not significant ($P < 0.05$).

2.2.21 Hausner's ratio

This was calculated as the ratio of tapped density to bulk density of the gum sample.

2.2.22 Compressibility index (C %)

This was calculated using the equation.

Compressibility = (Tapped – bulk density)/Tapped density x 100.

3.0 RESULTS AND DISCUSSION

3.1 Organoleptic and phytochemical properties

Table 1 presents the phytochemical test results. Treatment of the gum with ruthenium red showed a red colour confirming the obtained product as gum. Phytochemical tests carried out on *Raphia hookeri* gum confirmed the presence of high concentration of carbohydrates as a violet ring was formed at the junction of two liquids on reaction with Molisch's reagent. The carbohydrate can be several forms of polysaccharides. Protein (1.63%) and nitrogen (90.28%) content is relatively low compared to the value (3.06%) and (16.6%) reported by Eddy *et al*.[15],[11] The difference may be due to losses during processing. The low protein and lipid contents coupled with absence of fibres confirm the emulsifying properties of raffia gum.[16] Vitamins contents are also low but comparable to those reported in literatures. The presence of flavonoids and alkaloids in the exudates suggest medicinal properties. The biological roles of alkaloids include protection against allergies, inflammations, free radicals, microbes, virus and tumors.[17] This could suggest gum's possession of antimicrobial properties.

Table 1: Phytochemical Test results.

Parameter	Result
Alkaloids	0.37 ± 0.02
Phenols	0.01 ± 0.01
Carbohydrates	80.27 ± 0.01
Protein	1.63 ± 0.01
Lipids	0.8 1± 0.01
Fibre	0.00
Flavonoids	0.42 ± 0.01
Saponin (mg/100)	2.4 1± 0.01
Phytic acid	6.2 2 ± 0.02
Soluble oxalate	8.71 ± 0.02
Total oxalate	18.1 ± 0.01
Ascorbic acid	6.40 ± 0.01
Niacin	3.70 ± 0.01
Thiamine	0.11 ± 0.01
Riboflavin	0.64 ± 0.02

3.3 Physico-chemical test results

The results of the physico-chemical tests are shown in Table 1. The present study was taken to characterize *Raphia hookeri* as a pharmaceutical excipient. Organoleptic properties showed that the gum while wet was brownish yellow but changed to deep brown on drying. It changed to light brown when powdered. The gum was slightly soluble in water and a dispersion of it yielded a gummy solution. The gum was practically insoluble in ethanol, acetone and chloroform.

The bulk and tapped density gave an insight on the packing and rearrangement of the particles and the compaction profile of the material. The compressibility index of raffia gum was 22.46, implying that the raffia gum has good flow property with moderate compressibility. This is important in scale up processes involving this gum as an excipient in qa pharmaceutical formulation. Therefore minimal modification of formulation containing this gum will be needed in its process development.

The total ash and acid insoluble ash values of raffia gum were found to be 3.5% $^{w}/_{w}$ and 1.2%$^{w}/_{w}$ respectively. Ash value reflects the level of adulteration or handling of the drug. Adultration by sand or earth is immediately detected as the total ash is normally composed of inorganic mixtures of carbonates, phosphates, silicates and silica. Therefore, the low value of total ash and acid insoluble ash obtained in this study indicated low level of contamination during gathering and handling of crude *Raphia hookeri* gum.

The swelling characteristic of raffia gum was studied in two different media: 0.1 hydrochloric acid and water. The swelling was similar in water and. The result showed that raffia gum has a good swelling index suggesting that the gum may perform well as binder as well as in controlled release dosage forms.

A 1% $^{w}/_{v}$ suspension of raffia gum in water gave a PH of 6.1. The near neutral pH of raffia gum implies that when used in uncoated tablets, it may be less irritating to the gastrointestinal tract. It may be a useful application in formulation of acidic, basic and neutral drugs. Knowledge of pH of an excipient is an important parameter in determining its suitability in formulation since the stability and physiological activity of most preparation depends on pH.

Table 2: Physico-chemical analysis.

Parameter	Result	Parameter	Result /Concentration (mg/L)
Solubility in cold water	7.6	Iron (Fe) (mg/mL)	8.06 ± 0.057
Solubility in hot water	8.8	Magnesium (mg/mL)	5.18 ± 0.004
Solubility in acetone	0.0	Calcium (mg/mL)	4.40 ± 0.010
Solubility in ethanol	0.0	Zinc (mg/mL)	0.35 ± 0.021
Solubility in chloroform	0.0	Nickel (Ni)	0.37 ± 0.022
Loss on drying (%)	0.41 ± 0.01	Cadmium (Cd)	0.13 ± 0.003
Total Ash (%)	3.1	Angle of repose (^{o})	42.0 ± 0.01
Acid insoluble ash (%)	1.21	Bulk density (BD) (g/mL)	0.895 ± 0.01
pH (at 27.8^{o}C)	6.1	Tap density (TD) (g/mL)	0.984 ± 0.02
Crude protein (%)	3.2	Hausners ratio	1.1
Crude lipid (%)	1.1	Compressibility index	22.46
Swelling index	20		
Viscosity			
Manganese (Mn) (mg/L)	1.37 ± 0.023		
Chromium (Cr) (mg/mL)	O.75 ± 0.009		
Cobalt (Co) (mg/mL)	0.32 ± 0.008		

The moisture content of raffia gum was low, suggesting its suitability in formulation containing moisture sensitive drugs. Given suitable temperature moisture will lead to the activation of enzyme and the proliferation of micro organisms, thereby affecting the shelf life of most routine formulations. It is important to investigate the moisture content of material because the economic importance of an excipient for industrial application lies not only on the cheap and ready availability of the biometral but the optimization of production processes such as drying, packaging and storage.

3.4 FTIR study

Figure 1 Presents peaks and frequencies of IR absorption as well as functional groups deduced from the FTIR spectrum of raffia gum. From the results obtained from Figure 1, the gum displayed strong OH vibrations at 3415 cm-1. CH stretches at 2942. 85.[18] CH deformation consisting of stretching and bending vibrations occurred at 1415. 00. C-O vibration occurred at 1053.52. A symmetric CH stretch was observed at 2350cm -1 while the OH stretch due to the -COOH functional groups appeared at 1625 and 1515.00 cm -1.[18] A C=O stretch due to an acetyl group was observed at 1253 cm-1.[19] C-O stretch was found at 1053.52 while C-H bending vibrations due to alkynes were found at 558 and 649 cm-1.

The FT-IR spectrum of raffia gum confirms the hydroxyl (OH-) group, CH- stretching of aliphatic, C – O suggestive of polysaccharide structure.

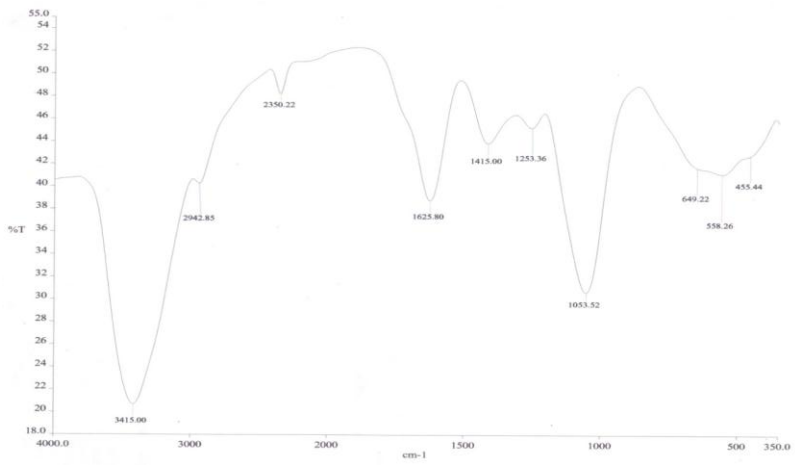

Fig. 1: FT-IR spectrum of *Raphia hookeri* gum powder.

4.0 CONCLUSION

The result obtained in this study was established for the first time regarding the fundamental characteristic of the gum obtained from the incised trunk of *Raphia hookeri* which showed excellent properties well established for pharmaceutical excipient. Though more research is still needed in this regard the major concern is that *Raphia hookeri* can be used as a pharmaceutical excipient in the binding properties and in controlled release tablet formulations. The physicochemical composition of the raffia palm gum showed that it has greater percentage of carbohydrate contents, total oxalate and calcium. Crude fibre, starch and lignin were absent. The mineral elements compositions are within the range specified by WHO. Raphia gum was also found to be pH sensitive and may therefore be useful in intestinal/colon drug delivery.

ACKNOWLEDGEMENT

We acknowledge the University of Ibadan Central Laboratory, Ibadan, Nigeria for making some of their facilities available for this study.

Source of support: Nil; Conflict of interest: None declared.

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