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Natural hydrogel obtained from leaves of C*ochorus Olitorious* plant in South-South Nigeria. 1: Preliminary characterization studies

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Abstract

Plant products serve as an alternative to synthetic products because of their local accessibility, eco-friendly nature and lower prices compared to imported synthetic products. The present study was undertaken to evaluate the natural gum obtained from the leaves of *Cochorus Olitorious* and its possible application as a pharmaceutical excipient. Phytochemical examination such as solubility, swelling index, pH, granule formulation of the extracted hydrogel and physico-technical characterization studies as Hausner's Quotient, compressibility index and angles of repose were carried out on the hydrocolloids. All values of physicochemical properties were represented as mean of standard deviations and data analyzed by Spearman"s rank correlation coefficient and t-test whose calculated two tailed T value at P(0.05) was 3.14 indicating no significant difference between the two variables (hydration capacity and swelling index) signifying a direct proportionality relationship between them. The Swelling index and Hydration capacity of the hydrocolloid in distilled water display such relationship as: Cochorus Olitorious = Sodium Carboxymethyl Cellulose(NaCMC) >Tragacanth>Benthonite. The results obtained suggested that the gum could be a good pharmaceutical excipient especially as a tablet binder, suspending agent and emulsifier.

Keywords: *Natural Hydrogel ,Cochorus Olitorious, South- South Nigeria.*

Introduction

In recent years plants gum has evoked tremendous interest in Pharmacy due to their diverse pharmaceutical applications as diluents, binders, disintegrants in tablets, thickners in oral liquid, protective colloid in suspension, gelling agents in gels and bases in suppositories (Udeala and Chukwu 1985) (Zatz and Kushla 1996). The gum are cheap and easily available thus making them attractive substitutes for expensive semi-synthetic and synthetic excipients. Most plant gum are polysaccharide macro molecules that dissolve more or less upon contact with water and form colloidal solution (Mohammed, 2012). Apart from the use of hydrogels in finished medicines newer ones have been found in the preparation of cosmetics, textiles, paint and paper industries. Hence the demand for this substance is increasing and new sources are getting tapped.

With the increase in demand for natural hydrocolloids, it has become necessary to explore the newer sources to meet the industrial demand (Antesh, Kumar 2012). The plant *Cochorus Olitorious* belong to the kingdom Plantae, order Mavales, family Malvaceae, subfamily Crewidae, genus Cochorus and species Olitorious. (Jansen, 2004). *Cochorus Olitorious* plant is an Afro-Arabian variety. The plant is an erect herb up to 2m tall usually strongly branched. The stem is reddish, fibrous and tough. The leaves are 6-10cm long, 3.5 – 5cm broad, elliptic-lanceolate, apicaily acute or acuminate, flowers pale yellow, bracts lanceolate, peduncle shorter than the petiole and pedicels 1-3cm very short. (Emuh, et al 2013) (Nkomo and Kambizi 2009). The growth of *Cochorus Olitorious* seedlings is fast. Flowering starts about a month after germination and continues for 1-2 months depending on soil type and conditions. The seeds are sown between February to June in carefully prepared soil (Chen and Saad 1988). While perhaps better known as a fibre crop *Cochorus Olitorious* is also a medicinal vegetable eaten from Tanganyika to Egypt (Emuh, et al. 2011). It is quite popular for its leaves that are used as an ingredient in an okra Slimy Arabian port herb known as molokhiya. It is a popular vegetable in West Africa among the Yorubas in Nigeria where it is commonly known as Ewedu. In the south-south part of Nigeria, this plant known as Ahihiara grows profusely. Interest in the gum is that very few people in this area knows about the plant especially in the pharmaceutical world. The plant, due to environmental degradation may go extinct if not fully explored. Interest in this work is not only in the hydrogel but also in possible biomedical value that is being investigated. The *Cochorus Olitorious* has been reported to be used in folk medicine as a demulcent, febrifuge and a tonic and the jute a folk remedy for aches, pains, dysentery, enteritis, fever, pectoral pain and tumors (Mabubal Islam, 2008). It has also been demonstrated that *Cochorus Olitorious* possessed antibacterial activity and also induces apoptosis in human hepatocellular carcinoma (Paola et al 1998) (Gbadamosi, 2012). The objective of this work is to isolate the hydrogel from the leaves of *Cochorus Olitorious* on a laboratory scale and carry out preliminary studies on its use as a pharmaceutical excipient. Generally chlorophyll or pigments are present in the plant which should be removed before isolating the hydrocolloid. The plant material must be treated with petroleum ether and chloroform to remove pigment and chlorophyll and then with distilled water (Babereh, et al. 2012). Care should be taken when drying the final isolated /extracted gum. It must be dried at a very low temperature of not more than 50^0c or in a vacuum. The dried material is stored carefully in a desiccator to prevent further moisture uptake or degradation (Sahlim, et al 2004).

A number of factors stimulated global interest in new excipient/ diluent development. Some drugs show incompatibilities with many of the currently used excipients for example, Atenolol – Polyvinyl pyrollidone (PVP) and atenolol-magnesium stearate (Bostan t, et al 2011). One of the most common drug-excipient incompatibilities is the reaction between aldehydic sugars, such as lactose and primary and secondary amines, leading to formation of Schiff bases and this complex series of reaction, lead to browning and discoloration of the dosage form. (Sonali, et al 2010). Despite being a carrier of choice for dry powder in aerosol formulations, lactose may need to be replaced with a different carrier, such as mannitol or sucrose, when formulating primary and secondary amine drugs. Also magnesium stearate is incompatible with aspirin, some vitamins and most alkaloidal salts (Allen and Huner 2003).

Materials and Method

The materials used are all of pharmaceutical grade and include: Acetone, n-hexane (Kernel China) Sodium meta bi-sulphite, ethanol (BDH, Germany) and distilled water, Grinding machine (Corona China),Table centrifuge (Pec Medical, USA), p^H meter (Hanna-H198107, USA), Soxhlet extractor. The leaves of *Cochorus Olitorious* were obtained from local market in Choba, Rivers State, Nigeria.

Extraction of the Hydrogel

The mucilage was isolated from freshly dried and coarsely powdered leaves of C*cochorus Olitorious* using a grinding machine (Corona China). The weighed materials was defatted with n-hexane using a Soxhlet extractor, then treated with ethanol for 12hours. The air dried powder was then mercerated with hot water containing 1.5g of Sodium meta bisulphite (an antioxidant) in the ratio 1:17 with intermittent stirring for 12 hours to enhance the release of mucilage. The dispersion was filtered through a muslin cloth upon application of little pressure to obtain a viscous solution. The hydrocolloid (impure) was precipitated from water using acetone in the ratio1:1.The Precipitated gum was washed severally with acetone, redispersed in water, centrifuged at 2500rpm for 20 minutes. The supernatant obtained was treated with acetone to expunge the gum. The substance obtained was air dried, pulverized using a mortar and pestle and passed through a 250(micron meter) aperture and then stored in a dessicator.

Phytochemical Examination

Preliminary test namely, Ruthenium red test, Molisch test and Iodine test were performed to confirm the nature of gum obtained (Umer Qadir, et al 2014).

Ruthenium Red Test:

This test is used to confirm the presence of gum. A small quantity of dried powder was taken and mounted on a slide with Ruthenium red solution. It was observed under a light microscope.

Molisch Test:

This test is used to confirm the presence of carbonhydrate in the gum. 0.1g of dried gum powder was placed in a clean test tube. 2 drops of molisch's reagent freshly prepared was also introduced. Concentrated sulphuric acid was gradually added on the side of the tube to form a layer below the aqueous solution. Observations were then made.

Iodine Test:

This test is to confirm the presence or absence of starch in the gum. 0.1g of dried gum powder was added to 1ml 0.2% iodine solution in a test tube and the mixture observed.

Physicochemical Properties Of The Gum

The separated gum was evaluated for its solubility, swelling index and p^H value (Vimot ,2013) .

Solubility Test:

The gum was evaluated for solubility in water, ethanol and acetone. A 1gm quantity of gum was weighed and placed in a clean test tube containing 10ml of distilled water. The mixture was shaken vigorously and observed for solubility. This was also done using 10ml of ethanol, acetone and chloroform. The same procedure was repeated for the reference gums such as Tragacanth, Sodium Carboxy Methyl (NaCMC) and Bentonite.

Swelling Index:
The method used by Ohwavworhua and Adelakum (2005) was adopted. A 1.0g quantity of each test sample was adequately measured and placed separately in each of 15ml plastic centrifuge tubes and the volume occupied was noted. 10ml of distilled water was added to each tube from a 10ml measuring cylinder and stoppered. The contents were shaken vigorously,the mixture was allowed to stand for 10 minutes after which they were immediately centrifuged at 1000rpm for 10 minutes in a bench centrifuge (Pec Medicals, USA). At the end of the centrifugation process, the supernatant was carefully decanted and the volume of sediment measured. The swelling index was calculated using the relation:

$$S = V_2/V_1$$

Where S = swelling index

V_1 = volume occupied by the gum prior to hydration

V_2 = volume occupied by the gum after hydration.

This was repeated thrice and mean value obtained.

p^H of various dispersions.
This was done by shaking various concentrations of 0.1, 0.2, 0.5 and 1% w/v dispersion of the sample in water and the p^H determined using a p^H meter (Hanna-H198107 USA). p^H determination were made in triplicate and the mean p^H value calculated.

Proximate Analysis
Ash and Acid insoluble ash contents were determined according to AOAC procedure (AOAC 1990), Nitrogen content was determined using Hach method (Oladipo and Nwakaoha 2010) and protein content calculated as: Nitrogen X 6.25. Fat and crude fibre content were obtained from hexane extraction according to Entwistle and Humlar(1994). Carbonhydrate content was estimated as 100% - (moisture + fat + protein + fibre + ash).

Determination of Moisture Content
A 3gm weight of gum powder was put into the moisture content analyser (Sartereons Germany). The machine was then set to 130^0C for 5 minutes. The value of the moisture content of the gum was read and recorded. The procedure was repeated twice and the mean value was taken as the moisture content.

Determination of Hydration Capacity
The method used by Musa, et al (2008) was adopted. 0.5g of the gum powder was placed in a tarred 5ml stoppered centrifuge tube. 10 ml of distilled water was added and shaken vigorously for 2 minutes. It was then allowed to stand for 10 minutes during which it was mixed by inverting the tube three times at the end of 5 and 10 minutes. The sample was centrifuged at 1000rpm for 10 minutes. The aqueous supernatant was then carefully removed and the tube with the sediment was reweighed. The hydration capacity was calculated as ratio of the weight of sediment to initial weight of dry powder. The procedure was done in triplicate and the mean taken (Muazu, et al 2014).

Physico-Technical Characterisation Of The Gum (Granules)
The granules of the gum as obtained was evaluated for its Flow rate, Bulk and Tapped density, Angle of repose, Hausner's Quotient and Compressibility index.

Bulk and Tapped Densities
A 15.0g quantity(W) of each granule sample was accurately weighed and placed in a 100ml measuring cylinder. The volume V_0 occupied by each of the samples without tapping was noted. After 100 taps on the bench, the occupied volume V_1 was read. The Bulk and Tapped densities were calculated as the ratio of weight to volume for V_0 and V_1 respectively.

$$D_{Bulk} = W/_{V0}$$
$$D_{Tapped} = W/_{V1}$$

Angle of Repose (θ)
The static angle of repose θ was measured according to the fixed funnel and free standing cone method (Sharn, 2008). A funnel was clamped with its tip 2cm above a plain paper placed on a flat horizontal surface. A 4gm quantity of the gum (granule) was weighed and carefully poured through the funnel until the apex of the cone thus formed just reached the tip of the funnel. The height (h) of the granule cone and the mean diameter (D) of the base of the powder cone were determined and the tangent of the angle of repose was calculated using the equation:

$$\mathbf{Tan\ \theta = 2h/_D}$$

The procedure was carried out for both the reference gums in triplicate and mean standard deviation taken.

Hausner's Quotient
This was calculated as the ratio of Tapped density (D_t) to Bulk density (D_b) of the samples
Hausner's Quotient = Dt/Db

Flow Rate
15.0g of the granules was introduced into a closed glass funnel of tube diameter 1mm and efflux tube length 2cm and clamped to a retort stand 2cm above a plain horizontal surface. With the use of a stop watch, the time taken for the 15g granule to flow (pass) through the funnel after removal of finger tips at the closed end was noted. This was also done in triplicate and mean volume was taken.

The flow rate is calculated as:

$$\frac{\text{Weight of granule (g)}}{\text{Time of flow (s)}}$$

Compressibility Index

This was calculated using the equation

Compressibility (%) =

$$\frac{\text{Tapped density} - \text{bulk density}}{\text{Tapped density}} \times 100$$

Results

Percentage Yield

Percentage yield of gum (%) =

$$\frac{\text{Weight of gum (extract) (g)}}{\text{Weight of raw material}} \times 10$$

Average weight of the natural gum = 30.75g

Weight of dried powdered leaves = 150g

Percentage yield (%) = $\frac{30.75g}{150g} \times 100 = 20.5\%$

Table 1: Preliminary Confirmatory Test for the Dried Gum Powder

Test	Observation	Inference
Ruthenium test: Small quantity of dried gum powder mounted on a slide with ruthenium red solution and observed under a microscope.	Pink color develops	Gum present
Molisch Test: 0.1g of dried mucilage powder + Molisch's reagent + Conc. H_2So_4 on the side of a test tube.	Violet color observed at the junction of the two layers.	Carbohydrate present
Iodine Test: 0.1g of dried gum powder + 1ml 0.2N Iodine solution	No color observed in solution	Starch absent

Table 2: Results of some Physico-Chemical Properties of the Gum with Standard Gums

Test	Cochorus Olitorious	Tragacanth	Sodium CMC	Betronite
SOLUBILITY TEST (i) 1g of test sample + 10ml distilled water + shaking	Soluble in water forming a mucilagenous gel	Slightly soluble in water forming a mucilagenous gel	Soluble in water forming a mucilagenous gel	Insoluble in water
(ii) 1g of test sample + 10ml ethanol + shaking	Insoluble in ethanol	insoluble	insoluble	insoluble
(iii) 1g of sample + 10ml of acetone + shaking	insoluble	insoluble	insoluble	insoluble
SWELLING INDEX IN DISTILLED WATER	5.5 ± 0.06	1.4 ± 0.06	5.5 ± 0.17	1.0 ± 0.06
HYDRATION CAPACITY	5.0 ± 0.02	2.2 ± 0.15	2.8 ± 0.16	1.8 ± 0.10

Table 3: p^H of Various Concentrations (% $^W/_V$)

Concentration (% w/v)	Mean triplicate p^H determination			
	Cochorus Olitorious	Tragacanth	Sodium CMC	Bentonite
0.1	6.7	6.3	7.5	8.3
0.2	7.0	6.9	7.6	8.4
0.5	7.3	7.0	7.8	8.6
1.0	7.4	7.2	8.0	8.8

Table 4: Descriptive Statistics of Physico-Chemical Properties of Gums With n = 4. where n = C. Olitorious, Tragacanth, NaCMC and Bentonite.

Parameters	Mean + SD	Coefficient of Variation	Standard Error
Swelling Index	3.35+2.45	0.743	1.155
Hydration Capacity	2.86+1.33	0.535	0.765
Flow Rate	20.63+1.25	0.060	0.625

Table 5: Spearman's Rank Correlation Coefficient (r)

Gums	Swelling Index (Mean)	Hydration Capacity (Mean)	Rank Swelling Index	Rank hydration Capacity	d_i	d_i^2
1	5.50	5.02	3	4	1	1
2	1.40	2.12	2	2	0	0
3	5.60	2.80	4	3	-1	1
4	1.00	1.52	1	1	0	1

Where 1 = C. Olitorious, 2 = Tragacanth, 3 = NaCMC 4 = Bentonite

Table 6: Result of Proximate Analysis of the Extracted Gum

GUM Content	Percentage (%) Value
Moisture	2.10
Carbohydrate	19.02
Lipid	9.70
Ash	13.14
Acid insoluble ash	1.45
Protein	3.75
Fibre	6.73

Table 7: Results of Physico-Technical Characterization of Formed Granules of Extracted Gum and Standard

	Cochorus Olitorious	Tragacanth	Sodium CMC	Bentonite
Bulk Density (gl ml)	0.29+0.01	0.37+0.03	0.33+0.03	0.60+0.00
Tapped Density (g / ml)	0.38+0.02	0.53+0.03	0.43+0.03	0.85+0.15
Hausner's Quotient	0.30+0.10	1.43+0.07	1.30+0.15	1.42+0.0
Angle of repose (Θ)	30.5+0.45	25.2+0.25	28.4+0.35	42.0+0.56
Flow rate (F_R) $^{g}/_{sec}$	21g/sec	20g/sec	22g/sec	19.5g/sec
Compressibility index (%)	24.2+5.57	30.1+3.52	23.3+1.65	29.4+0.35

Discussion

The average yield of dried dispersible Cochorus Olitorious gum obtained was found to be 20.5%. The gum was subjected to preliminary phytochemical tests, physicochemical and physicotechnical characterization and results summarized as in Tables 1, 2 and 7 respectively. Treatment of the aqueous dispersion with ruthenium red gave pink colour confirming the product obtained as a polysacharide gum (Bisnagit, et al 2006). The product was also positive to Molisch's test signifying the presence of carbonhydrate while a negative result was obtained in the Iodine test showing that starch is absent (Ophrad, et al 2014).

Results in Table 2 shows some of the physicochemical parameters of both test and reference gums. While C. Olitorious was very highly soluble in water yielding a brown viscous solution signifying it as a hydrophilic polymer, it was practically insoluble in ethanol, acetone and other organic solvents. The high solubility profile of the hydrocolloid relates it to being a good pharmaceutical excipient that could impart improvement in mouth feel, pourability of liquid formulations, ability to extend shelf life, encapsulate flavours, emulsify beverages, broad viscosity property, ability to retain moisture, provide elasticity and freeze thaw ability (Christopher and Dupon, 2011). The swelling and hydration characteristic of C. Olitorious gum was studied and compared with other standard gums. The result show that the gum has high swelling index and hydration capacity relative to NaCMC but greater than that of Tragacanth and Bentonite as in Table 2. The swelling ability of any polysaccharide depends upon its water retention or absorption capacity and the high water absorption (hydration) capacity was found to be 5.0$\pm$0.02 and this could indicate the extracted gum to have a good suspending property (Ohwuavwunhua, 2010). Also the good hydration capacity as a formulation variable may enhance drug release from matrix systems (Munday and Cook, 2000), hence the gum from C. Olitorious could be useful as a binder and excipient for sustained release drug formulation. Statistically upon descriptive analysis of the physicochemical properties of the hydrocolloid, result obtained as in Table 4, show only slight difference and low values of coefficient of variance, standard deviation and error about the mean. This confirms that the method used was precise and accurate. Also using the Spearman's rank correlation coefficient (r) to find the T value as in Table 5 by process of ranking, the calculated T value on a two tailed test at P(0.05) was 3.14 indicating a positive association between hydration capacity and swelling index since there is no significant difference about their mean .

The results of the physico-technical characterization shows the compressibility index value of 20.5%. Since the compressibility index has been proposed as an indirect measure of bulk density, particle size, surface area and cohesiveness of powders (granules), the value obtained may indicate possible acceptability of the gum as a Pharmaceutical adjuvant. Hausner's Quotient value of 1.30 is also acceptable (Carr, 1965). The tangent of angle of repose is equal to the coefficient of friction and the lower the friction the greater the flow of granules. From the results obtained the angle of repose is 30.5^0 which from scale of flowability (Carr, 1965) is classified as good hence the dried *Cochorus Olitorious* gum has shown to have good flow property and could be utilized as a good tableting excipient at ambient room temperature and humidity in the tropics (Dertmar, 2008). Knowledge of the p^H of an excipient is an important parameter in determining its suitability in a formulation since stability and physiological activities of most preparations depends on p^H (Koops, 2005). From the results obtained in Table 3, the p^H of C. Olitorious gum increased slightly with increase in concentration of the gum and was between 6.7 and 7.4 indicating the gum to have almost a neutral p^H hence its possible application as a universal excipient for both basic and acidic drugs. Results of the proximate analysis reveals the presence of proteins, carbohydrate and fibres though purity of the gum was not fully ascertained further work is to be carried out using the differential scanning colorimetry (DSC) for determining the level of purity though the plant has been in use as an edible source and food thickner in some parts of Nigeria especially the Igbos and Yorubas. From these results therefore, it can be inferred that the extracted hydrogel from the dried leaves of C. Olitorious has the potential of being a viable pharmaceutical excipient. Further purification and evaluation are ongoing and will constitute separate reports. This will include possible test as binder for tableting and as gelling agents for suspension and emulsion formulations.

Conclusion

Natural gums are promising biodegradable polymeric materials. Many studies have been carried out in many fields including food technology and pharmaceuticals using gum. It is clear that natural gum have many advantages over their synthetic counterparts. However there is a need to develop other natural sources as well as modifying existing ones for the formulation of novel drug delivery system, biotechnological application and other delivery systems. From the present preliminary studies, it could be concluded that *Cochorus Olitorious* gum can be a good excipient in both liquid and solid pharmaceutical formulation even at lower concentrations ($0.5\%^{w}/_{v}$) compared to other standard gums because of its non-toxic, water-soluble nature, high hydration capacity and swelling index. Its vast growth in the south-south part of Nigeria should be well utilized to avoid wastage and the plant going extinct in the nearest future. Its wide cultivation can give employment to the youths using mechanized farming.

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