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Isolation and Characterization of *Cola acuminata* Gum as a Potential Pharmaceutical Excipient

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Authors' contributions

This work was carried out in collaboration between all authors. Author EAB designed the study, wrote the protocol and the first draft of the manuscript, managed the literature searches and experimental procedures. Author MAM managed the analyses of the study performed and the spectroscopy analysis and author OI managed the toxicological experimental process. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Gums are polysaccharides composed of varying chemical compositions and a wide range of molecular weights. Appropriate evaluation of their physicochemical properties assists in determining their suitability as pharmaceutical excipients in the preparation of various dosage forms and drug delivery systems. The present study focused on characterization of a novel natural polymeric gum extracted from the pods of *Cola acuminata* plant for its possible application as pharmaceutical excipients.
Methodology: The novel *Cola acuminata* gum (CAG) was extracted by soaking the sliced *Cola acuminata* pods in water and precipitated using acetone. Phytochemical screening and characterization of physicochemical properties such as particle size distribution and densification, organoleptic and flow properties, solubility in various solvents, pH, moisture sorption, swelling

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index and apparent viscosity were carried out on the extracted gum using standard and official methods. Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC) and Wide angle X Ray Diffractometry (WAXD) were also employed to further characterize the gum. Acute toxicity and in vivo skin irritation of the gum on rats were determined using standard methods.

Results: The results revealed that CAG is tasteless, odorless and brown in colour. The dry CAG powder is a hydrophillic polysaccharide gum containing mixture of monosaccharides and possesses good compressibility and flow properties. It is slightly acidic with pH of 5.37 and exhibited pH dependent swelling in water to produce viscous mucilage with pseudoplastic flow. LD_{50} of the gum in rat is greater than 5000 mg/Kg and Primary Irritation Index (PII) is zero. DSC revealed a glass transition temperature of 224.5°C ; WAXD showed predominantly diffused diffractogram with only two sharp peaks at 2θ = 20.9° and 2θ = 26.6° while FTIR spectrum showed characteristic bands at 3427 cm^{-1}, 2935 cm^{-1}, 1625 cm^{-1}, 1230 cm^{-1}, 1045 cm^{-1}, 870 cm^{-1} and 770 cm^{-1} which indicate the amorphous nature of the gum.

Conclusion: CAG obtained from a waste product exhibited good physicochemical properties, it can be considered as a novel pharmaceutical excipient with a great potential for wide range of applications in varieties of pharmaceutical formulations from simple dosage forms
to advance drug delivery systems. Its safety for both oral and topical applications was also established.

Keywords: Cola acuminata gum; polysaccharides; compressibility; pharmaceutical excipients; toxicity; pseudoplastic.

1. INTRODUCTION

Both natural and synthetic polymers have wide applications as pharmaceutical excipients ranging from the preparation of simple dosage formulations to the design of complex drug delivery systems [1]. Pharmaceutical excipients control the physicochemical characteristics, release profiles and bioavailability of drugs from their formulated products. The capability of excipients to provide their intended functions on the formulated products depends on the inherent physicochemical properties of the excipients itself which greatly influence the choice, concentration, mode of incorporation and other considerations that may influence the final product [2].

Natural polymers have gained attention and are often preferred as pharmaceutical excipients over synthetic polymers because they are less expensive, non-toxic, readily available, biocompatible, biodegradable and capable of chemical modification to provide tailor-made materials for drug delivery system [3]. Gums are examples of natural polymers, they are polysaccharides composed of large forms of units with varying chemical compositions and a wide range of molecular weights. These properties impart the needed flexibility which promotes their use as excipients. An appropriate evaluation of the physical and chemical properties such as solubility, water sorption, swelling capacity, pH, viscosity, etc, will determine their suitability in the formulation of various dosage forms and drug delivery systems. Toxicological evaluations are also necessary to ensure their safety. Examples of gums that have been used as excipients in pharmaceutical formulations include Albizia gum, Cashew gum, Guar gum, Karaya gum and Khaya gum [2,4,5]. The search for a new, cheap and useful natural gum from apparently a waste product which can be optimized as an alternative pharmaceutical excipient stimulated the interest in this present study.

Cola nut (cola spp) is a genius of about 125 species (family Sterculiaceae).It is native to the tropical rain forest of Africa, *Cola accuminata* is one of the species very common in Nigeria; it is usually cultivated in commercial quantity in the western part, widely chewed in the northern part and used for traditional ceremonies in the eastern part of the country. *Cola acuminata* seeds contain xanthine derivatives such as caffeine, theophylline and theobromine. The pharmacological effects of cola nut seeds include stimulation of central nervous system and gastric acid secretion. It is also a weak diuretic and possesses positive chronotropic, analeptic and lipolytic properties. Cola pods are the chambers that contain several cola nuts seeds, each of the pods can weight over five pounds [6,7]. While *Cola acuminata* seeds are of great economic value, the pods are obviously waste products usually thrown away after opening to remove the seeds. Although the chemical

constituents and uses of cola nut seeds have been documented [8,9,10], information about the constituents and characterization of the gum from the *Cola acuminata* pods have not been reported yet. Therefore, the objective of this study is to conduct phytochemical, physicochemical and toxicological characterization of the novel *Cola acuminata* gum (CAG) for its optimization as a potential pharmaceutical excipient.

2. METHODOLOGY

2.1 Materials

CAG was extracted from the empty cola pods collected from traders in Elele town, Rivers State, Nigeria, after the removal of the cola nuts. Female albino rats weighing between 250 - 300 g were used for toxicity studies. All other materials used were of analytical grades.

2.2 Methods

2.2.1 Extraction of *Cola accuminata* gum

Freshly harvested cola nut pods were washed thoroughly with distilled water, and then sliced into smaller pieces. A 2 kg quantity of the sliced pods was weighed and soaked in 5 liters of distilled water containing 0.1% w/v sodium metabisulphite. The container was covered with a lid and left undisturbed for 24 hours after which the viscous mucilage produced were separated from the pods by passing it through a muslin cloth. Acetone was used to precipitate the cola gum from the viscous mucilage. The ratio of the quantity of acetone to gum mucilage is 3:1. The precipitated gum was washed repeatedly with more acetone to remove the remaining water until the gum became brittle. It was later dried at 60°C for 1 hours, the dried mass was pulverized to fine powder and stored in an air tight ambered coloured bottle. The percentage yield of the gum was calculated as follows:

$$\text{Percent yield} = \frac{\text{Weight of the CAG extracted}}{\text{Weight of the cola pod}} \times 100 \quad (1)$$

2.2.2 Phytochemical screening of *Cola acuminata* gum

2.2.2.1 Test for secondary metabolites

Various phytochemical tests were conducted on the extracted CAG to determine its secondary metabolites constituents such as carbohydrates, reducing sugars, tannins, alkaloids, anthraquinones, saponins, alkaloids, cardiac glycosides and flavonoids. These tests were carried out and recorded according to standard procedures [11].

2.2.2.2 Acid hydrolysis and chromatographic analysis

Acid hydrolysis was carried out on 5% w/v of CAG as follows; 0.25 g of the gum was dispersed in 5 ml of distilled water, 5 ml of 1% dilute sulphuric acid was added and warmed on water bath for 10 minutes, and then filtered. The filtrate was used for chromatographic analysis. 5% w/v of aqueous solutions of different monosaccharides such as glucose, fructose, xylose, galactose and ribose sugars were also prepared. Samples of these monosaccharides along with the sample from the hydrolyzed filtrate of CAG were spotted on No1 chromatographic paper using fine capillary tube. Solvent system n-butanol: Acetic acid: water in ratio 4: 1: 5 were used to develop the chromatogram for 6 hours in a chromatographic tank using ascending technique. The chromatogram was then air dried activated in an oven at 40°C and then sprayed with aniline phthalate solution and respective positions and colours of the sugars spots were marked. The hydrolysable sugars detected from *Cola acuminata* gum are recorded.

2.3 Evaluation of Some Physicochemical Properties of *Cola acuminata* Gum

2.3.1 Determination of organoleptic properties

Organoleptic properties such as colour, odour and taste of the sample of CAG were assessed by three panels consisting of six assessors and their observations were recorded.

2.3.2 Analysis of particle size and particle size distribution

A 30 g quantity of the gum powder was used for the analysis. Five sieves of aperture sizes 1000 µm, 500 µm, 250 µm, 150 µm, 100 µm, were used. They were arranged in a stack with the largest pore size sieve at the top and the smallest pore size sieve at the bottom. The stack was mounted on an Endecott's test sieve shaker. The weighed amount of the powder was placed on the top sieve and covered with the lid. The stack of sieves was mechanically shaken for 25 min. The weight of powder retained on each

sieve and the fines collected were taken and the values were used to calculate the average diameter of the particles (Dav) using the formula [12]:

$$Dav = \Sigma \frac{(\% \text{ retained} \times \text{mean aperture size})}{100} \quad (2)$$

2.3.3 Assessment of flow rate and angle of repose

A 30 g quantity of the gum powder was allowed to flow through an orifice of a funnel to determine flow rate. The funnel was fixed with the tip of the funnel 10 cm above the base. Time taken for the weighed powder to flow out completely from the orifice was recorded. This was performed in triplicate. Flow rate was obtained by the equation below:

$$\text{Flow rate} = \frac{\text{Weight of powder (g)}}{\text{Time (sec)}} \quad (3)$$

Furthermore, the angle of repose was determined by calculating tan θ from the height and radius of the cone formed by the powder as it flowed out of the orifice and subsequently obtaining the inverse of tan θ.

2.3.4 Determination of bulk and tapped densities

A 30 g quantity of the gum powder was gently poured through a short stemmed glass funnel into a 100 ml graduated glass cylinder. The volume occupied by the powder was noted. The cylinder was then tapped 50 times on a bench to obtain a constant volume. The volumes before and after tapping were used to calculate bulk density and tapped densities in (g/ml) respectively. Furthermore, Hausner's quotient and Carr's compressibility index used to determine the flow and compressibility properties of powder were obtained from the equations:

$$\text{Hausner's quotient} = \frac{\text{Tapped density}}{\text{Bulk density}} \quad (4)$$

$$\textbf{Carr's compressibility} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100 \quad (5)$$

2.3.5 Moisture sorption studies

The method described by Mahmud et al. [13] was adopted. A dried evaporating dish was weighed and 1 g of CAG was weighed into it. The new weight of the dish was noted and then placed over saturated sodium chloride solution in a glass desiccator to maintain a relative humidity (RH) condition of 75% for a period of 5 days. The sample was removed, the weight was determined after the fifth day and thereafter transferred into another desiccator containing activated silica gel (desiccant) for another 5 days after which the sample was removed again and new weight was determined. The moisture contents of the sample under different RH conditions were later calculated.

2.3.6 Determination of pH of *Cola acuminata* gum

The pH of the gum mucilage (5% w/v) was measured with an Oaklon pH meter (Model 1100). The pH meter was set to neutral (7.4) at a room temperature of 28 ^{o}C and the electrode was immersed into the mucilage. The reading on the meter recorded. Triplicate measurements were made.

2.3.7 Determination of solubility of *Cola acuminata* gum in various solvents

The solubility of the gum was determined in distilled water, acetone, chloroform and ethanol. 100 mg of the gum was accurately weighed and added into screw-capped test tubes containing 10 ml of respective solvents. The contents were mixed continuously by placing the tubes in a mechanical shaker for 3 hours at 50 rpm at 25°C and afterwards left overnight. After 24 hours, 5ml of the supernatant solution was withdrawn, into small pre-weighed evaporating dish and heated to dryness over a digital thermostat water bath (Model, HHS, Mc Donald Scientific International). The weights of the dried residues with reference to the volume of the solutions were determined using a digital electronic balance (Model, XP-300, Denver instrument, USA) and expressed as the percentage solubility of the gums in the respective solvents [14].

2.3.8 Evaluation of swelling properties of *Cola acuminata* gum

A 1 g quantity of the powder was placed in a 100 ml measuring cylinder and tapped 200 times. The initial volume V1 of the gum in the cylinder was recorded. Phosphate buffer solution (pH 1.2) was added to the mass to reach the 100 ml mark, in the cylinder and left to stand for 24 hours. The final volume of the gum in the cylinder was then recorded as V2. The same procedure was repeated using phosphate buffer solutions of pH 7.2 and 9.2. The swelling index (S.I.) in various pH media was calculated as follows:

$$S.I. = \frac{V2 - V1}{V1} \times 100 \quad (6)$$

$V1$ is the initial volume before adding buffer and $V2$ is the final volume after adding buffer. The experiment was repeated in triplicate [15].

2.4 Determination of Apparent Viscosity of *Cola acuminata* gum

The viscosity of three samples of CAG aqueous dispersion consisting of 1% w/v, 2% w/v and 3% w/v (CAG1, CAG2, CAG3 respectively) was determined at 25°C, with the aid of Digital Synchroelectric viscometer (NDJ- 5S, China). Using spindle 2 at various rotational speed (6, 12, 30 and 60 rpm), the effect of shear rate on the viscosity of the samples was determined. All determinations were made in triplicate and the results obtained were expressed as the mean values. The rheogram for the three samples were obtained by plotting rate of shear against shear stress.

2.5 Thermal Analysis

Thermal analysis of CAG was carried out using differential scanning calorimeter (DSC Netcsch 204F1, Germany). The sample weight of 2 mg was sealed in a perforated aluminum pan and scanning was performed at temperature ranging from zero to 300°C at a heating rate of 10°C per min under an atmosphere of nitrogen.

2.6 FTIR Spectroscopy

Infrared spectrum of CAG was obtained. About 2 % w/w of the gum sample with respect to the potassium bromide (KBr) disc was mixed with dry KBr (FTIR grade, Aldrich, Germany). The mixture was ground into a fine powder using an agate mortar before compressing into a disc. Each disc was scanned at a resolution of 4 cm^{-1} over a wave number region of 400–4000 cm^{-1} using FTIR spectrophotometer (Model 500, Buck Scientific, USA) coupled to a computer with Omnic analysis software. The characteristic peaks of IR transmission spectrum were recorded.

2.7 Wide Angle X-ray Diffraction (WAXD) Analysis

To study the crystal characteristics of CAG, WAXD study was carried out using an X - ray generator PW3040/60 X'Pert PRO (Fabr: DY2171 PANanlytical, Netherlands) connected to the tube (PW3373/00 DK 147726 Cu LFF) copper anode which delivered X-ray of wavelength, λ = 0.1542 nm at a high voltage of 40 kv and an anode current of 25 mA. WAXD measurement was taken with goniometer (PW3050/60 MPD-System, PANanlytical, Netherlands) from 3.0° to 45.0° in 0.015° steps (1s per step and the WAXD diffractogram was obtained for the sample.

2.8 Toxicological Evaluations of *Cola acuminata* Gum

Toxicological evaluations carried out included acute toxicity (LD_{50}) test and skin irritation test. Swiss albino rats weighing between 250 - 300 g obtained from Animal House, Department of Pharmacology and Toxicology, Madonna University were used for these studies. The animals were apparently healthy and were preconditioned for two weeks before the commencement of the work. The care and handling of all the animals used were conducted in compliance with National Regulations for Animal Research. University Ethical Committee reviewed the protocols, which were consistent with International Animal Welfare Guidelines.

2.8.1 Experimental design of acute toxicity test (LD_{50})

Twenty one animals were used for this study. The method of Lorke [16] that described a procedure for the investigation of acute toxicity of an unknown chemical substance with an estimation of the LD_{50} was adopted for the study. It was carried out in two stages. A stock solution of the CAG in water was made and appropriate calculated oral doses of the substance relative to the body weight were administered. The first stage involved the use of different preliminary trials doses of the solution. In this stage the animals were divided into three groups of three animals each. Group A1 received 10 mg/kg of the CAG aqueous dispersion; Group B1 received 100 mg/kg, while Group C1 received 1000 mg/kg. The animals were constantly monitored for the first 2 h, intermittently for 4 hours and then over night. The number of dead animals per group was recorded at the end of 24 h. From the result, the second stage was performed using the doses of 1500 mg/kg, 2500 mg/kg, 3500 mg/kg and 5000 mg/kg for four groups each containing three animals. Then they were monitored as stated above and the dead noted.

2.9 Skin Irritation Test

Draize et al. [17] method was adopted for this test. Nine animals were used for the experiment.

The hairs on the backside area (about 3cm^2) of the rats were removed a day prior to the day of the experiment. The rats were divided into 3 groups. For the animals in Group 1, nothing was applied to the shaved bare skin. A 5% w/v aqueous dispersion of CAG was applied on group 2, while 1.0% v/v aqueous solution of formalin was applied as a standard irritant on Group 3. The application sites were observed for irritant responses (erythema and edema) at specific time interval and graded by the same investigator according to standard visual scoring scale shown in Table 1.

3. RESULTS AND DISCUSSION

3.1 Percent Yield of Extracted *Cola acuminata* Gum

The percent yield of the CAG obtained by extraction from *Cola acuminata* pods was 9.8±1.3 %. The method of extraction of CAG was simple and cost effective involving precipitation of the gum from the mucilage using limited quantity of organic solvent. Acetone used is cheap and has lower boiling temperature which allows easier solvent recovery. Extraction of other natural gums involved complex, cumbersome and capital intensive processes [18,19,20]. The simplicity of the extraction process of CAG makes it easy to translate the small scale laboratory experimental procedure to large scale commercial process, whereas for some other natural gums, there may be need for extensive modification between laboratory experimental extraction process and large scale commercial production. The extraction process equally restricted exposure of CAG to toxic chemicals that can compromise its biocompatibility. This is a good advantage of natural polymers over synthetic polymers.

CAG has a peculiar advantage over other natural gums in terms of ready availability for commercial production because the source from which it is obtained. Cola trees are cultivated in large quantities for commercial purpose across many developing countries which ensure its ready availability [6,7]. Moreover, CAG is obtained from the cola pod, a waste product usually thrown away after harvesting cola seed. Other gums are obtained from different parts of various plants such as seeds, fruits, leaves or barks which have other valuable commercial uses as food and medicinal purposes, using these parts of the plants for large scale production of gums may lead to shortage of that parts for other uses and can lead to price increase for such parts. Extraction of CAG from the pods of the cola trees is a demonstration of conversion of waste to wealth, leading to production of a novel gum that can be used as an alternative local source of pharmaceutical raw material/excipient in the formulation of various dosage forms and drug delivery systems as demonstrated in other developing countries that have harnessed their local sources of raw materials [19,20,21].

3.2 Phytochemical Screening and Chromatographic Analysis of *Cola acuminata* Gum

Results of preliminary phytochemical screening as shown in Table 2 indicated the presence of carbohydrates and absence of other secondary metabolites, while the results of chromatographic analysis after acid hydrolysis shown in Table 3 indicated the presence of hydrolysable polysaccharides that yielded mixture of reducing sugars; pentose (ribose and xylose) and hexose (glucose). This is a confirmation that *Cola acuminata* gum is composed of polysaccharides just like other natural gums. Gums are polysaccharides made up of a monosaccharide or mixed monosaccharides, many of them may be combined with uronic acids and on hydrolysis yield a mixture of sugars and uronic acids. They contain hydrophilic molecules, which can combine with water and swell to form viscous solutions or gels [5,19].

3.3 Evaluation of Some Physicochemical Properties of *Cola acuminata* Gum

3.3.1 Particle size analysis and particle size distribution

The results of physicochemical properties of CAG are shown in Table 4. CAG is brown in colour, odourless and tasteless, the particle size distribution for CAG powder is as follows; 500 µm (21%), 250 µm (40%), 150 µm (24%) and 100 µm (15%). The calculated average particle size of CAG is 256 µm. Influence of inter-particulate forces such as van der Waals and electrostatic bonds in fine particles with high surface to mass ratio makes them to be more cohesive than coarse particles which are influenced more by gravitational force. Flow problems are likely to occur as the size falls to 100 µm and below, because of increase of the cohesive forces among the particles [22]. Conversely, the flow properties of powders are improved when the particles are large and the

Table 1. Standard scoring scale for skin irritation study

Skin responses score	
Erythema and eschar formation	**Scale**
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet-redness) to slight eschar formation (deep injuries)	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1.0 mm)	3
Severe oedema (raised more than 1.0 mm and extending beyond exposure area)	4
Total possible score for irritation	8

particle size distribution is narrow, this is due to the reduction in inter - particulate cohesive forces that prevent the free flow of smaller particles. Particles within the range of 250 µm are usually relatively free flowing; however, larger particles may lead to less strong tablets due to the fact that they have lesser surface areas for bond formation as compared to smaller particles [23]. Therefore, an optimal particle size and size distribution is required to obtain good flow properties, compaction and hardness. The result of particle size distribution and calculated average particle size indicated that CAG powder particles fell within the optimal size and size distribution necessary for good flow properties which in turn can enhance good compressibility, uniformity of weight and drug content, consequently consistent dissolution and drug release when use as a binder in tablet formulation [24,25].

Table 2. Phytochemical Constituents of *Cola acuminata* Gum

Secondary metabolites	Result
Alkaloids	Absent
Tannins	Absent
Starch	Absent
Saponins	Absent
Glycosides	Absent
Flavonoids	Absent
Carbohydrates	Present
Anthraquinones	Absent

3.3.2 Assessment of flow rate and angle of repose

As shown in Table 4 the angle of repose and flow rate of CAG powder were 26.25 ^{o} and 4.75 g/sec respectively. The rougher and more irregular the particle surface and the smaller the particle size, the higher the frictional force in powder particles, the higher the angle of repose and the lesser the flowability. Granules with angle of repose below 30°C exhibit good flow [26], while granules with angle of repose above 40° would flow with difficulty [27]. The results of the angle of repose and flow rate indicated that CAG powder exhibited good flow properties. Powder materials with good flow properties are necessary for accurate die fill which is required to produce less variation in uniformity of weight of tablets.

Table 3. Identified Monosaccharides from Hydrolysed CAG sample

Reference sugars	Result
Fructose	Absent
Galactose	Absent
Glucose	Present
Ribose	Present
Xylose	Present

3.3.3 Bulk and tapped densities

Other parameters for assessing properties of powders such as bulk and tapped densities, Hausner's quotient and Carr's compressibility are also shown in Table 4. Bulk and tapped densities are used to assess the flow properties, densification and porosity of powder particles; they also provide information on tablet hardness and disintegration time and uniformity of weight. Hausner's quotient is indicative of inter-particle friction and cohesiveness of powder particles and can be used to predict the flow behaviour of the powder [28,29], while Carr's Compressibility is a measure of the compressibility of powder, it is an assessment of powder's ability to compact and decrease in volume when pressure is applied; it is a direct measure of the potential bridge strength and stability of granules. Carr's index is predictive of powder flow properties and its suitability in production of strong tablets which

can withstand pressure. A powder material with compressibility index of less than 10% and Hausner's ratio of 1.00 - 1.11 is said to have excellent flow, compressibility index of 11 - 15% and Hausner's ratio of 1.12 -1.18 describes a material that has good flow characteristics, while compressibility index of 16 – 20% and Hausner's ratio of 1.19 – 1.25 describes a particle with fair flow properties. Compressibility index of 21% and above with Hausner's ratio of 1.26 – 1.34 is said to have poor flow properties [30,31]. The results of bulk and tapped densities of CAG powder confirmed its good flow properties. Hausner's ratio and Carr's compressibility of CAG are 1.1 and 9.52% respectively; therefore, exhibited excellent flow properties and satisfactory compressibility. These values confirmed the results of particle size / size distribution and flow rate / angle of repose to prove that CAG is a potential good binder with requisite properties necessary in the formulation of tablets with desired uniformity of weight, drug content, hardness, dissolution and drug release profiles.

Table 4. Physico-chemical properties of *Cola acuminata gum*

Parameter	Results
Colour	Brown
Odour	Odourless
Taste	Tasteless
pH of mucilage	5.37 ± 0.15
Moisture Absorbed	67.60 ± 3.01
Average particle diameter (Dav)	256 µm
Flow Rate (g/sec)	4.75
Angle of Repose (°)	26.25
Bulk density (g/ml)	0.57
Tapped density (g/ml)	0.63
Hausner Quotient	1.11
Carr's compressibility (%)	9.52
Swelling Index (%)	**Values**
Buffered pH 1.2	263 ± 1.6
Buffered pH 7.2	375 ± 3.2
Buffered pH 9.2	517 ± 2.1
Solubility (mg/ml)	**Values**
Water	8.0 in 100
Acetone	insoluble
Ethanol	insoluble
Chloroform	insoluble

3.3.4 Moisture sorption studies

The results of moisture sorption as presented in Table 4 showed that CAG absorbed considerably significant amount of moisture when exposed to an environment of high relative humidity (75%). In the presence of desiccant, the absorbed moisture was lost indicating the hydrophilic nature of the gum. In contrast, hydrophobic polymers will not show any significant moisture absorption when exposed to an environment of high relative humidity [32]. The observation from moisture sorption studies confirmed the susceptibility of CAG just like other natural gums to possible chemical and microbial degradations resulting from water molecules absorbed from the environment when they are not properly stored. The quality and integrity of the gum can therefore be preserved by storing it in air tight container in the presence of desiccant.

3.3.5 Solubility and Swelling properties of *Cola acuminata* gum

As shown in Table 4, the gum was insoluble in the three organic solvents (acetone, chloroform and ethanol), but slightly soluble in water. More so, it exhibited high swelling capacities in water. Hydrophobic polymers are usually insoluble in water but soluble in most organic solvents where as hydrophillic polysaccharides such as gum with numerous sugar molecules are either soluble or partially dissolve in water but are insoluble in organic solvents [5,32]. The observed partial solubility and extensive swelling capacities of *Cola acuminata* gum in water may be due to the fact that the polymer molecules have a linear molecular arrangement. Linear polymers are less soluble in water and consequently, exhibit appreciable swelling profiles than those with branched components [33]. The swelling of a linear polymer in water without dissolution to form viscous mucilage is an indication that it is cross-linked. The cross linking tie the macromolecular chains together by primary covalent bonds thereby transforming each compound into a single giant molecule [34,35]. The partial solubility and the swelling ability of *Cola acuminata* gum to form a viscous mucilage in water makes it a suitable viscosity enhancer that can retard rapid sedimentation of suspended particles according to Stoke's law, thereby making it a potential suspending agent.

3.3.6 pH of *Cola acuminata* gum

The pH of CAG was 5.37 which indicated that it was slightly acidic, most plant gums are slightly acidic because they are essentially polyuronides consisting of sugar and uronic acid. Slightly acidic polymers such as xanthan, pectin, alginic acid, polyacrylic acid are anionic polymers that ionize in alkaline pH to become negatively charged [36]. Unlike nonionic polymers,

physicochemical properties of ionic polymers such as viscosity, swelling and bioadhesion are greatly and significantly affected by the ionic strength of the surrounding in which such polymers are subjected. Ionic hydrogels have limited swelling when exposed to the surrounding where its own ionization is hindered, but in a favourable environment where it can ionize, such hydrogel will have appreciable increase in swelling profile. The swelling and viscosity of anionic hydrogels increases when the pH of the medium changes from acidic to alkaline [37,38]. This makes some ionic hydrogels to be suitable for pH dependent drug delivery systems. Table 4 revealed the swelling property of polymeric CAG in different pH media, the swelling index was in the order of pH 1.2 < pH 7.2 < pH 9.2. In this case, ionization of Cola accuminata gum was hindered in acidic medium while alkaline media favoured its ionization thereby enhancing its swelling in higher pH media, the higher the degree of ionization the higher the swelling profile, this may be the reason why CAG had the highest swelling profile in pH 9.2. Since the swelling profiles of CAG has been shown to be pH dependent, the possible mechanism of drug release from solid dosage forms prepared with CAG may be by swelling. Therefore, the use of CAG as tablet matrix or coating materials for pH dependent, enteric coating and colon targeted drug delivery system may be a possibility.

Furthermore, substances with strong acidic or alkaline pH are potentially corrosive and may cause irritation to the skin. It is assumed that products with slightly acidic pH (similar to that of healthy skin; pH of 5.5) will be safe and most comfortable to the skin and qualify for *in vivo* skin irritation animal test [39,40]. The pH of CAG aqueous dispersion was found to be 5.37 (close to that of healthy skin) and it is a preliminary indication that it is not corrosive and qualified to undergo confirmatory in vivo animal test for skin irritation [41].

3.3.7 Viscosity of CAG dispersion in water

The results of viscosity test of CAG are shown in Fig. 1. It was observed that the viscosity increased with increase in the concentrations of CAG from 1% w/v to 3% w/v in the polymer dispersions. This behaviour is attributable to the intermolecular interaction or entanglement of polymer chains, thereby increasing the effective macromolecule dimension and molecular weight [42]. When hydrogel polymers are used in the formulation of solid dosage forms such as matrix tablets, the viscosity behaviour of gel formed by such polymers is very important. Increase in polymer concentration in a matrix tablet, increases the viscosity of the gel layer formed around the tablet when it come in contact with suitable liquid medium and thus leads to the formation of gel layer with a longer diffusional path, making the gel more resistant to dilution and erosion. This could cause a decrease in the effective diffusion coefficient of the drug and therefore reduction in drug release, thereby controlling the drug dissolution. The mechanism of drug release from a matrix also changes from erosion to diffusion as the polymer concentration increases [43]. The formation of a thick gel by CAG aqueous dispersion, which increased with increase in concentration indicated that it can be useful in controlled drug delivery systems. The viscosity of a polymer dispersion is affected by the nature and concentration of the polymer and the rate of shear applied on the fluid. The viscosity increased as the concentration of the gum increased while the viscosity decreased with increase in shear rate. At all concentrations CAG exhibited shear - thinning non-Newtonian pseudoplastic flow as shown in Fig. 1. This behaviour is found to be associated with most hydrogel polymers. The possible explanation is that, at low shear rates, the macromolecules of the polymer solutions are coiled and entangled causing immobilization of water entrapped within the molecules, leading to the high viscosity of the polymer solution [44]. Upon application of higher rate of shear, the randomly coiled entangled polymer chain tends to disentangle and align themselves in the direction of flow rates of shear. This orientation reduces the internal resistance of the molecules to flow and allows a greater rate of shear at each successive shearing stress, this together with the release of some of the entrapped water within the macromolecules results in an effective lowering of viscosity. A good suspending agent will increase the viscosity of a suspension when its concentration is increased to retard the sedimentation rate of dispersed particles in the formulation, but at higher shear rate (by shaking or agitation of the container), it will exhibit shear thinning effect that makes the suspension less viscous and allow the removal of the suspension by pouring from the container [45]. This quality of a good suspending agent was demonstrated by CAG.

3.3.8 DSC analysis

According to Fig. 2, DSC thermogram of CAG showed no sharp peak melting point of crystalline substance but rather exhibited broad endothermic peaks of amorphous nature. The

first relatively broad endothermic peak at 64.1°C corresponds to the desolvation temperature; the second broader endothermic point at 224.5°C is the glass transition temperature, while the third exothermic peak at 279.6ºC is the decomposition temperature of CAG. The thermogram indicated the thermostability of the gum to high temperature before degradation [46].

3.3.9 FTIR Spectroscopy

Fig. 3. presents the FTIR spectrum of CAG. The spectrum displayed features typical of polysaccharides with the characteristic functional groups [47]. The presence of a broad absorption band at 3427 cm^{-1} is representing hydrogen bonded OH stretching vibration, while the sharp absorption band located at 2935 cm^{-1} is due to C-H stretching while the bands at 870 cm^{-1} and 770 cm^{-1} indicated the C-H bending. The absorption band that appeared at 1625 cm^{-1} is due to the asymmetric stretching vibration of carboxylate group. The C=O stretches bond due to acetyl groups is present at 1230 cm^{-1} while bending ether bond (CH_2–O–CH_2) is manifested as a characteristic band at 1045 cm^{-1}.

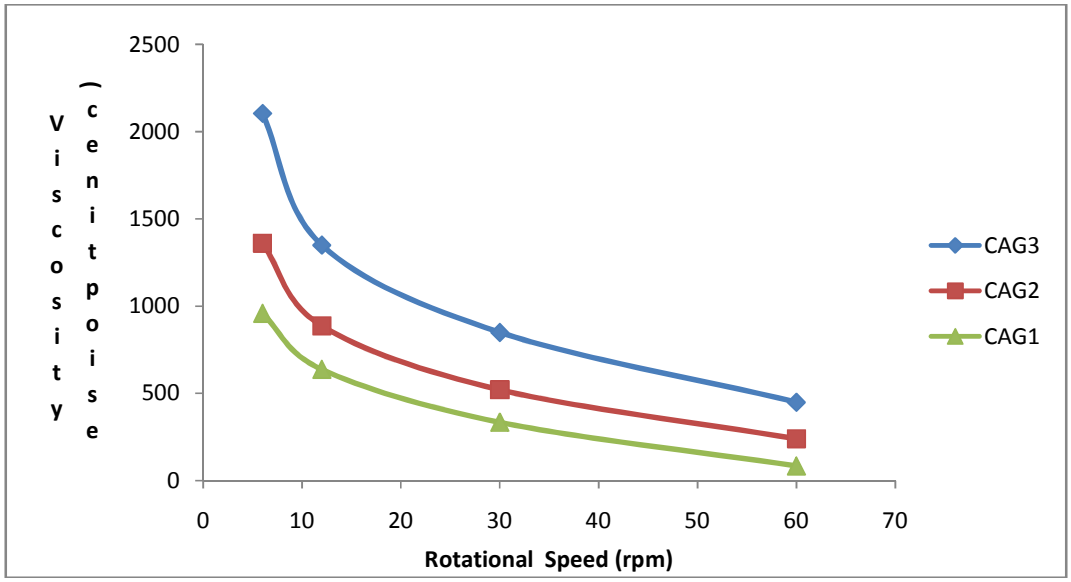

Fig. 1. Effects of concentration and shear rate on viscosity of CAG dispersion in water

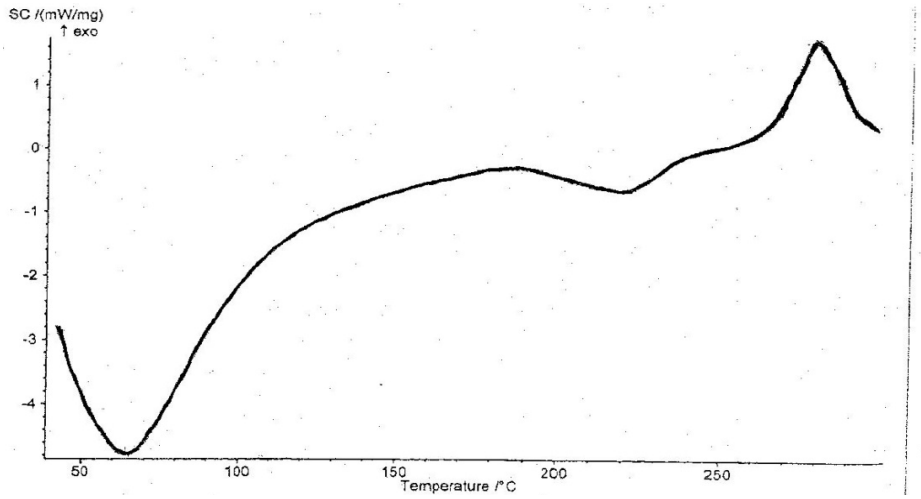

Fig. 2. DSC thermogram of CAG

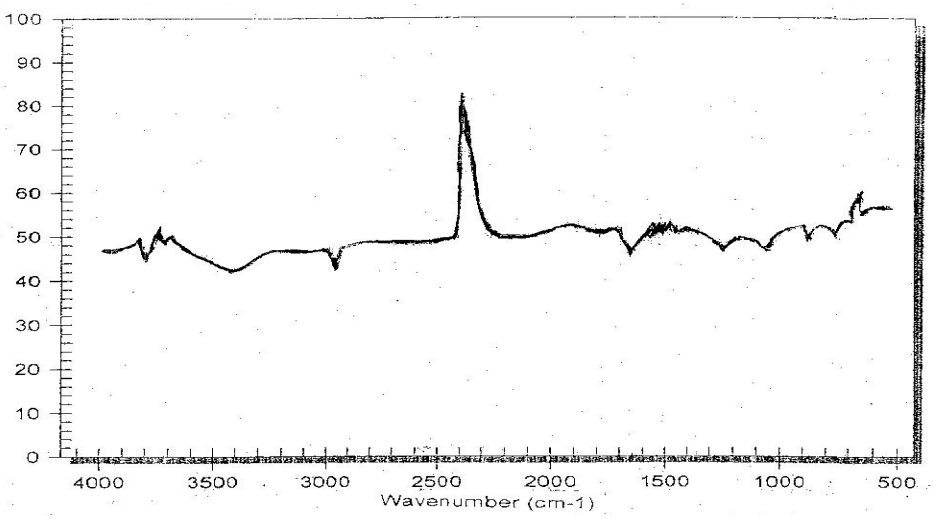

Fig. 3. FTIR spectrum of CAG

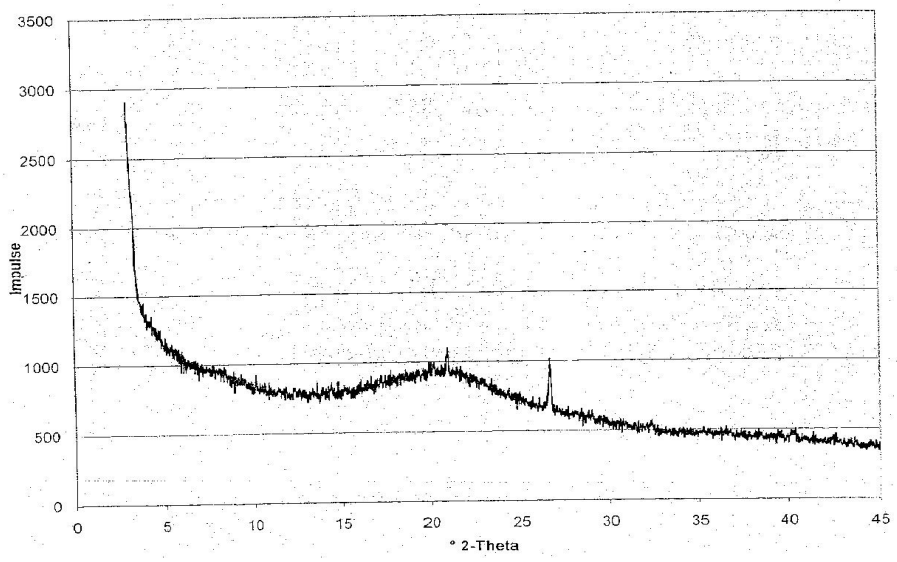

Fig. 4. X-ray diffraction patterns of CAG

3.3.10 X-ray diffraction analysis

WAXD analysis of polymer matrices gives information on the crystalline state of the matrices, sharp band corresponding to crystalline region and diffused band corresponding to amorphous region (60). Fig. 4, shows the WAXD diffractogram of CAG which was mostly diffused with only two sharp intensities at $2\theta = 20.9°$ and $2\theta = 26.6°$ because of its amorphous nature. This observation confirmed the result of DSC and FTIR analyses. Many natural gums have also been reported to exhibit diffraction patterns indicating their amorphous nature [48,49]. Crystallinity of a polymer is of a great importance for its use as retardant for drug delivery; highly amorphous polymer tends to prevent rapid release of embedded drug particles compared to its crystalline form, [44,50]. Amorphous nature of CAG might make it useful in controlled drug delivery system.

3.3.11 Acute toxicity studies

From the result of acute toxicity studies, the oral Lethal Dose (LD_{50}) of CAG in mice was estimated to be greater than 5000 mg/kg body weight since no adverse sign of toxicity or death of animal was observed up to the maximum dose of 5000 mg/kg body weight used for this study.

Table 5. Results of skin irritation study for CAG

Skin responses	Time (Hrs)	Score		
		Rat 1	Rat 2	Rat 3
Erythema and scar formation	1	0	0	0
	24	0	0	0
	48	0	0	0
Oedema formation	1	0	0	0
	24	0	0	0
	48	0	0	0
Primary Irritation Index (PII)		PII = 0/6 = 0.00	PII = 0/6 = 0.00	PII = 0/6 = 0.00

Average Primary Irritation Index = $\frac{0.00 + 0.00 + 0.00}{3}$ = *0.00*

This indicated that CAG is practically non-toxic in mice because, LD_{50} value greater than 5000 mg/kg body weight are of no practical interest [46]. The non-toxicity of CAG therefore, is an indication of the possibility of its use in the preparation of foods and orally administered pharmaceutical products such as suspensions, emulsions, granules, tablets, etc. The safety margin of CAG can be said to be very high in the sense that the concentration that is required in most foods and orally administered pharmaceutical products is quite insignificant when compared with the ceiling dose adopted in this studies. This is a confirmation that CAG, like other natural polysaccharides have advantages over synthetic polymers which include non toxicity, biocompatibility and biodegradability [3,5].

3.3.12 Evaluation of skin irritation

According to Table 5, the results of skin irritation studies on rats in response to application of CAG aqueous dispersion compared to the standard formalin solution gave 0 scale levels for both erythema and oedema. This shows that CAG was very compatible with no visible irritation to the animals' skin. This is an indication that CAG can be safely used in the formulation of dermatological preparations such as creams, gels, emulsions and lotions, etc.

4. CONCLUSION

From results of various investigations conducted, it can be concluded that CAG obtained from a waste material possesses good physicochemical properties desirable in formulation of varieties of pharmaceutical products including simple dosage forms and advance drug delivery systems. It also demonstrated potential safety necessary for use in both oral and topical applications. It can therefore be considered as a potential novel pharmaceutical excipient that may serve as an alternative raw material in pharmaceutical industries.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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