JOPAT Vol. 16(1) 96- 113, January – December, 2017.
ISSN 1118 - 1028

EVALUATION OF THE SUSPENDING PROPERTIES OF *Cola Acuminata* GUM ON CALAMINE SUSPENSION

EMMANUEL ADELAJA BAMIGBOLA[1*], OLUWAYEMISI AGNES OLORODE[2] AND DORCAS ALY UZIM[3].

[1]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Niger Delta University, Bayelsa State, Nigeria.

[2] Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, Niger Delta University, Bayelsa State, Nigeria.

[3] Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmacy, Madonna University, Elele, Rivers State, Nigeria.

ABSTRACT

Many natural gums are employed as suspending agents in the formulation of pharmaceutical suspensions. The search to develop locally available natural gum from apparently a waste product as an alternative suspending agent stimulated the interest in this present study. *Cola acuminata* gum (CAG) extracted from *Cola acuminata* pods was subjected to some preliminary physicochemical and phytochemical evaluations. Calamine suspensions were formulated with CAG between the concentration range of 1 – 4 % w/v and compared with suspensions formulated with two standard suspending agents (tragacanth and acacia gums). Sedimentation volume, flow rate, rheology and redispersibility were used as evaluating parameters. Skin irritation test was carried out on albino rats using 5 % w/v aqueous dispersion of CAG and calamine suspension containing 4 % w/v of CAG to ascertain the gum's suitability in the formulation of dermatological products. The results revealed that CAG is a hydrophillic polysaccharide gum containing mixture of monosaccharides and swells in water to produce viscous mucilage. At all concentrations employed, CAG possessed superior suspending properties relative to other gums. The suspending abilities of the gums are in the order of CAG > tragacanth > acacia. Comparatively, the performance of 1% w/v of CAG was similar to that of 3 % w/v of tragacanth gum. CAG is a potential alternative suspending agent in the preparation of pharmaceutical suspensions. The skin irritation test showed that CAG can be safely used in the formulation of dermatological products to be applied to the skin.

Key words: *Cola acuminata gum*, suspending agent, dermatological suspension, skin irritation.

*Author for correspondence; e-mail: bamigbolae@yahoo.com; Tel. +2348035859878

INTRODUCTION

A pharmaceutical suspension is a heterogeneous coarse dispersed system containing insoluble solid particles (internal phase) dispersed through a liquid (external phase). Unlike colloidal systems, the solid particles in suspensions will eventually settle down, because of their sizes (usually greater than 1µm) which are sufficiently large enough for sedimentation. The settling of the dispersed phase on standing necessitates the inclusion of suspending agents to reduce the rate of settling and permits easy re-dispersion of any settled particulate matters [1, 2, 3]. Both synthetic and natural polymers can be used as suspending agents, but the use of natural polymers for pharmaceutical applications is attractive because they are economical, readily available, non-toxic, amenable to chemical modifications, and potentially biocompatible. Of increasing importance is the fact that plant resources are renewable and if cultivated or harvested in a sustainable manner, they can provide a constant supply of raw material [4, 5].

Natural polysaccharide gums obtained from plants have been employed as structured vehicles to formulate deflocculated suspensions to impart viscosity to the system in order to retard the rate of sedimentation of suspended particles. Such examples include tracaganth gum, khaya gum, cashew gum, *Albizia zygia* gum and *Moringa oleifera* gum [5, 6, 7]. The suspending properties of CAG extracted from the pods of *Cola acuminata* was therefore considered for evaluation in this study. No work had been carried out on the suitability of this gum for this purpose.

Cola nut (cola spp) is a genus of about 125 species (family Sterculiaceae), it is native to the tropical rain forest of Africa. *Cola acuminata* 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 [8, 9]. *Cola acuminata* seeds are of great economic value, but the pods are waste products usually thrown away after the removal of the seeds from it. Although the chemical constituents of cola nut seeds have been documented, the constituents of the gum from the pods have not been reported yet [10, 11, 12]. Interestingly, no investigation has been done on its suitability as a suspending agent, therefore, the objective of this study it to explore the potential of the highly viscous mucilage of CAG as a suspending agent.

Calamine powder was chosen for this investigation because it belongs to the group of non - diffusible, insoluble (hydrophobic) solutes in aqueous medium and requires a suspending agent when prepared in liquid dosage forms [13]. Calamine suspension is an anti-pruritic dermatological preparation usually applied on the skin to treat mild pruritic conditions such as poison ivy, poison oak, sunburn, rashes, chickenpox, insect bites and stings [14]. Dermatological products are usually applied to the skin for therapeutic or cosmetic purpose. Human skin is quite sensitive to many chemical substances therefore; the potential for dermatological products or ingredients used in their formulation/preparation to cause skin irritation/corrosion needs to be carefully evaluated as part of the overall safety assessment process. Dermal irritation is defined as the production of reversible damage of the skin following the application of a test substance for up to 4 hours. Assessment is generally based on the degree of erythema/eschar and/or oedema caused after a single topical application of a test substance on the test animal skin using Draize score. On the other hand, dermal corrosion is defined as production of irreversible damage to skin; namely, visible necrosis through the epidermis and into the dermis, following a single topical application of a test substance for up to 4 hours. The general method usually employed in the testing guideline for assessment of skin irritation/corrosion involves the use of in vivo animal studies [15]. The updated version of the testing guideline recommends that prior to undertaking the described *in vivo* animal test for irritation/corrosion of any substance, a sequential testing strategy based on the stepwise measurement of pH and use of acceptable validated *in vitro* tests of the substance is employed as a guide for selecting substances to be considered for *in vivo* animal experiment [16, 17]. The considerations from the sequential testing strategy are as follows:

- A substance with a pH below 2.0 or above 11.5 should not be considered for *in vivo* animal test, such a substance is suspected to be corrosive.
- A substance found to be corrosive based one of the alternative *in vitro* corrosive tests should not be considered for *in vivo* animal test.

MATERIALS AND METHODS

Materials

CAG was extracted from the empty cola pods collected after the removal of the cola nuts. Other materials used were calamine fine powder (William Ransom Plc, England), Acacia gum powder (S.D. Fine, Mumbai), Tragacanth gum powder (Searle co, England) and female albino rats. All other materials used were of analytical grades.

Extraction of CAG

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 was 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 in a hot air oven at 60 ^{o}C for 1 hours, the dried mass was pulverized to fine powder which was passed through sieve number 100 and stored in an air tight amber coloured bottle. The percentage yield of the gum was calculated as follows:

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

Evaluation of some physicochemical properties of CAG

Determination of pH of CAG

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.

Determination of solubility of CAG in various solvents

The method of Ramani *et al.* (1996) was adapted to determine the solubility of the gum 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 revolutions per minutes (rpm) at 25 ^{o}C and afterwards left overnight. After 24 hours, 5 mL of the supernatant solution was withdrawn, into small pre-weighed evaporating dish and heated to dryness over a digital thermostat water bath (Model, HHS, McDonald 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 [18].

Evaluation of swelling properties of CAG

The swelling properties of CAG were evaluated using the method of Rafiee-Tehrani *et al.* (2007). A 1g 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. Water 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 swelling index (S.I.) was calculated as follows:

$$S.I. = \frac{V2 - V1}{V1} \times 100 \ldots\ldots\ldots\ldots\ldots (2)$$

V1 is the initial volume before adding water and V2 is the final volume after adding water. The experiment was repeated in triplicate [19].

Phytochemical screening of CAG

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 by Harbone (1989) [20].

Acid hydrolysis and chromatographic analysis

Acid hydrolysis was carried out on 5 % w/v of CAG as follows; 0.5 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 ^{o}C and then sprayed with aniline phthalate solution and respective positions and colours of the sugars spots were marked. The hydrolysable sugars detected from CAG are recorded.

Preparation of calamine suspensions

10 % w/v calamine suspension containing 1 % w/v of CAG was prepared as follows: 10g of calamine powder and 1g of CAG powder were triturated together in a mortar with 20 mL of distilled water to form a smooth paste; 0.1g of benzoic acid was added as a preservative, distilled water was added gradually with constant stirring. The mixture was transferred into a 100 mL measuring cylinder, water was used to rinse the pestle and mortar and the volume was made up. The mouth of the cylinder was covered and then shaken vigorously for 1 minute and kept at room temperature of about 29 ^{o}C on a vibration free surface. The procedure was repeated using 2 %, 3 % and 4 % w/v CAG. The above procedure was repeated using tragacanth gum and acacia gum.

Sedimentation volume

The sedimentation volumes of the various suspensions prepared were monitored to observe any decrease in volume. The observations were recorded hourly for the first 4 hours on day 1, then monitored and recorded daily for the first 7 days and thereafter weekly for 7 weeks [1]. The sedimentation volume was calculated using the following equation:

$F = 100\ V_u / V_o$ ………………………….. (3)

where V_u is the ultimate volume of the sediment at a particular time and V_o is the original volume of the suspension when it was prepared. Comparative evaluation of suspensions containing 1% and 3% w/v of CAG and Tragacanth gum was carried out by plotting the sedimentation volumes of the suspensions against the storage time.

Flow rate

The time required for 10 mL of each suspension sample to flow through a 10 mL pipette from a fixed height was determined. The flow rate (in mls^{-1}) was calculated using the formula:

$$\text{Flow rate } (\eta_\alpha) = \frac{\text{Volume of pipette (ml)}}{\text{Flow time (s)}} \ldots\ldots (4)$$

Rheological study

Effect of type and concentration of suspending agent

The effects of type and concentration of the suspending agents on the rheological properties of the suspensions were evaluated by determining the viscosity (poise) of various suspensions containing different suspending agents at different concentrations were determined at 25 ^oC using the Brookfield Synchroelectric viscometer, model LVF (Brookfield Laboratories, Massachusetts) at the speed of 30 rpm using spindle 4. All determinations were made in triplicate and the results obtained were expressed as mean values.

Effect of shear rate

The effects of different shear rates on the rheology of the suspensions were evaluated by determining the viscosity (poise) of three suspensions each containing different suspending agents at 2 % w/v concentration at 25 ^oC, using the Brookfield Synchroelectric viscometer; model LVF (Brookfield Laboratories, Massachusetts). This was done using spindle 4 at varied speed (30, 60 and 90 rpm) since drag force is known to alter with change of rotational speed [21]. All determinations were made in triplicates and the results obtained were expressed as mean values. The rheogram for the three suspensions were obtained by plotting rate of shear against shear stress.

Redispersibility test

Fixed volumes of all the suspensions (50 mL) were kept in bottles and stored at room temperature on a vibration free surface. After 7 days, the bottles containing the suspensions were shaken manually to redisperse the sediments. The number of time required to redisperse the suspensions were noted and recorded as redispersibility number. The presence of deposit if any after re-dispersion was also noted [7].

Skin irritation test

Skin irritation test was conducted using Draize (1994) method. The study was carried out with the permission of the Institution's Animal Ethic Committee following the principles of Good Laboratory Practices and Animal handling according to NIH guide for the care and use of laboratory animals was followed [22]. Twelve female albino rats weighing about 250 - 300 g 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 4 groups. For the animals in Group I, nothing was applied to the shaved bare skin. A 5 % w/v aqueous dispersion of CAG was applied on group II, while calamine suspension containing 4 % w/v of CAG was applied on group III. A 1.0 % v/v aqueous solution of formalin was applied as a standard irritant on Group IV. 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 below [23]:

Table 1: Standard scoring scale for skin irritation study

SKIN RESPONSES	
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 (injuries in depth)	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**

RESULTS

Percent yield of extracted CAG

The percent yield of CAG obtained by extraction from *Cola acuminata* pods was 9.8 $\pm$ 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 has lower boiling temperature which allows easier solvent recovery. Extraction of other natural gums involved complex, cumbersome and capital intensive processes [24, 25, 26]. 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 [8, 9]. 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 [25, 26, 27].

Evaluation of some physicochemical properties of CAG

As shown in Table 2, CAG is light brown to reddish brown in colour. 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. [28, 29] The observed partial solubility and extensive swelling capacities of CAG in water may be due to the fact that the polymer molecules have a linear molecular arrangement. It has been reported that linear polymers are less soluble in water and consequently, exhibit appreciable swelling profiles than those with branched components [30]. 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 [31, 32]. The partial solubility and the swelling ability of CAG 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 accounting for its use as a suspending agent.

The pH of CAG indicates that it slightly acidic, most plant gums are slightly acidic because they are essentially polyuronides consisting of sugar and uronic acid. Slightly acidic polysaccharides such as tragacanth, xanthan and alginic acid are anionic polymers that ionize in suitable pH media to become negatively charged [33, 34]. Physicochemical properties such as viscosity, swelling and bioadhesion of ionic polymers are significantly affected by the ionic strength of the surrounding medium in which the polymers are subjected. In a favourable environment where they can ionize, such hydrogels will have appreciable swelling profile. This may be another contributory factor to the appreciable swelling profile of CAG in aqueous medium [33, 35]

Table 2: Some physico-chemical properties of CAG

Parameters	Description (values)
Colour	Light brown to reddish brown
pH of mucilage	5.37 $\pm$ 0.15
Swelling Index (%)	375 $\pm$ 3.2
Solubility	**Values (mg/ml)**
Water	8.0 in 100
Acetone	insoluble
Ethanol	insoluble
Chloroform	insoluble

Phytochemical screening and chromatographic analysis of CAG

Results of preliminary phytochemical screening as shown in Table 3 indicated the presence of carbohydrates and absence of other secondary metabolites. The results of chromatographic analysis after acid hydrolysis shown in Table 4 indicated the presence of hydrolysable polysaccharides that yielded mixture of reducing sugars; pentose (ribose and xylose) and hexose (glucose).

Table 3: Phytochemical constituents of CAG

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

Table 4: Identified monosaccharides from hydrolyzed CAG sample

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

This is a confirmation that CAG 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 [25, 29].

Sedimentation volume

The effects of type and concentration of suspending agents on the sedimentation volume are shown in Table 5 while the effects on flow rate, viscosity and redispersibility are shown in Table 6. The results indicated that the concentration of the suspending agents was directly proportional to the sedimentation volume and viscosity but inversely proportional to the flow rate and redispersibility number. Generally, sedimentation volume decreases with time of storage for all the suspensions indicating increase in the settling down of the dispersed particles. With reference to Table 5, the suspensions prepared with acacia gum (1 - 4 % w/v) behaved characteristically like a flocculated suspensions because the dispersed particles settled down very rapidly with a clear supernatant region. The possible reason for this observation is that, unlike most gums which produce viscous aqueous solution at low concentrations, acacia gum is extremely soluble in water, not being able to form solutions of appreciable viscosity until the concentration is up to 37 % w/v at 25 ^{o}C [33]. This concentration is much higher than the maximum concentration (4 % w/v) employed in these experiments. Because of the poor viscosity of acacia, it is not capable of providing appreciable suspending action, it is therefore usually mixed with tragacanth, starch and sucrose to form what is commonly known as Compound tragacanth powder BP. [36]. The result obtained in Table 6 buttressed this fact, showing that the viscosities of the calamine suspensions prepared with acacia gum is much lower compared with the ones prepared with CAG and tragacanth gum.

Unlike acacia gum, CAG and tracaganth gum acted like structured vehicles with appreciable viscosities in all the concentrations employed to produce deflocculated suspensions with dispersed particles settling down very slowly with a cloudy supernatant. This is a peculiar characteristic of natural polysaccharide gums; the mechanism of action is by formation of multi-molecular layer around individual hydrophobic solid particles to produce dual effects of wetting and suspending of the particles, leading to the deflocculating of the system [37, 38]. The superior suspending ability of CAG over tragacanth gum manifested in higher sedimentation volumes and viscosities of its suspensions with lower flow rate and redispersibility numbers at similar concentrations employed. There were no significant reductions in the sedimentation volumes of suspensions containing 3% w/v and 4 % w/v of CAG throughout the period of the study, the suspensions were too viscous, difficult to flow and redispersed. As a matter of fact 1 % w/v of CAG demonstrated a suspending ability comparable to 3 % w/v of tragacanth gum with reference to sedimentation volume and viscosity and as shown in Figure 1 and Table 6 respectively. This is an indication of the superiority of CAG over tragacanth gum. The suspending ability of the suspending agents employed in this study was in the order of CAG > Tragacanth gum > Acacia gum.

Table 5: Sedimentation volume (%) of calamine suspensions formulated with different concentrations of suspending agents

Suspending Agent Conc.(%w/v)	Sedimentation Volume (%) Time (hour)					Time (days)			Time (weeks)						
	0	1	2	3	4	1	3	5	1	2	3	4	5	6	7
CAG															
1%	100	95	94	93	91	83	78	73	69	60	56	52	49	48	47
2%	100	100	99	98	97	87	78	76	74	70	69	67	66	64	63
3%	100	100	100	100	100	100	100	100	100	100	95	93	92	90	88
4%	100	100	100	100	100	100	100	100	100	100	100	98	96	95	94
Tragacanth															
1%	100	42	32	31	30	28	27	26	26	26	26	26	26	26	26
2%	100	98	97	96	94	88	79	73	70	47	39	32	30	29	28
3%	100	99	98	97	95	89	85	78	74	63	57	53	50	48	46
4%	100	100	99	98	97	96	93	91	86	70	60	56	54	52	50
Acacia															
1%	100	28	27	26	26	26	26	26	26	26	26	26	26	26	26
2%	100	28	28	28	27	27	27	27	27	27	27	27	27	27	27
3%	100	35	33	31	30	30	30	30	30	30	30	30	30	30	30
4%	100	37	35	33	32	32	32	32	32	32	32	32	32	32	32

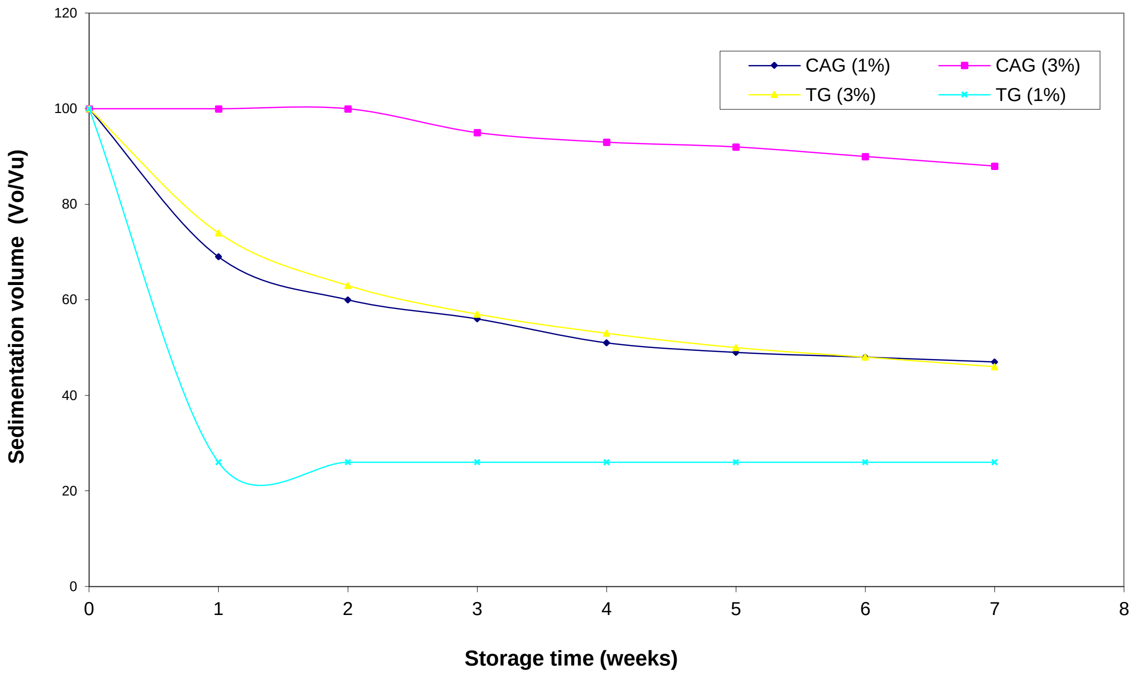

Fig1: Sedimentation volume (Vu/Vo) vs. storage time (weeks) of calamine suspensions formulated with 1% w/v and 3 % w/v of CAG and tragacanth gum

Note: CAG (1%) - *Cola acuminata* gum (1% w/v), CAG (3%) - *Cola acuminata* gum (3% w/v)
TG (1%) - Tragacanth gum (1% w/v), TG (3%) - Tragacanth gum (3% w/v)

Flow rate

The results of flow rate are presented in table 6. Generally, the flow rate is inversely proportional to the concentration and viscosity for all the suspending agents. Increase in concentration of the suspending agent led to increase in viscosity and subsequently, decrease in the flow rate. The flow rates of the suspensions were in the order of acacia > tragacanth > CAG. However, Suspensions containing 3 % w/v and 4 % w/v of CAG were too viscous and could not flow through the pipette, this shows that pourabilty of the suspensions from the containers will likely be a problem. On the other hand, this is an indication that CAG may be a useful thickener or gelling agent in other pharmaceutical formulations, drug delivery systems, cosmetics and food products which requires highly viscosity agents.

Rheological study

Results of rheological evaluations as shown in table 6 indicated that increase in concentration of suspending agent led to increase in the viscosity of the suspensions. At any particular concentration, the viscosity of the 3 suspending agents is in the order of CAG > Tragacanth > Acacia, indicating the superior suspending properties of CAG over the 2 other suspending agents. However, it was observed that suspensions containing 3 - 4 % w/v of CAG formed stiff viscous gels. The viscosity of the gels was difficult to obtain. Figure 2 indicated that all the suspensions exhibited pseudo plastic flow, because increase in shear rate (as a function of spindle rpm) led to decrease in viscosity. This observation is similar to that of other natural gums which exhibited pseudoplastic behaviour. Materials with pseudoplastic flow can be referred to as shear thinning systems that can exhibit thixotropic behaviour which is an essential characteristic needed for a good suspension. [7, 27, 38].

Table 6: Effect of the type and concentration of suspending agents on the flow rate, viscosity and redispersibility of calamine suspensions

Suspending agents	Concentration (% w/v)	Flow rate (mls^{-1})	Viscosity (poise)	Redispersibility number
CAG	1.0	0.89	2.71	18
	2.0	0.64	3.21	8
	3.0	**	**	**
	4.0	**	**	**
Tragacanth gum	1.0	1.02	1.31	24
	2.0	0.97	2.10	12
	3.0	0.85	2.86	5
	4.0	0.66	3.05	2
Acacia gum	1.0	1.99	0.69	6
	2.0	1.87	0.81	4
	3.0	1.66	0.96	2
	4.0	1.54	0.12	2

**Too viscous to be determined

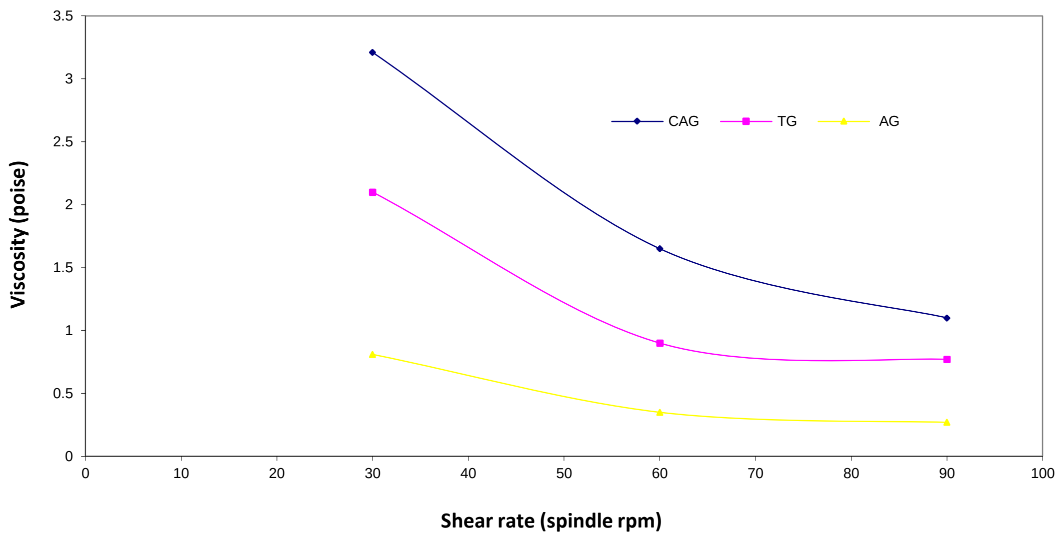

Fig 2: Effect of shear rate on the viscosity of calamine suspension formulated with 2 % w/v of various suspending agents

Note: CAG - *Cola acuminata* gum (2 % w/v), TG - Tragacanth gum (2 % w/v), AG - Acacia gum (2 % w/v)

Suspension that undergo thixotopy will remain sufficiently viscous at rest to prevent sedimentation of the dispersed particles but upon agitation (application of stress) the viscosity is reduced to produce good flow characteristic necessary for pouring to remove and administer the required dose [38, 39, 40]. The rheological property of the suspensions indicated the good suspending property of all the materials.

Redispersibility test

Redispersibility number provides a relative means for evaluating the redispersion of particles, even after storage, and gives an idea of electrokinetic (zeta potential) of the suspension. A greater redispersibility number show that the particles are clumped together as tightly packed aggregates, possessing affinity at their surface levels that could eventually form a cake. This only proves that greater energy is required to decrease inter surface potential and energy barrier. A lower redispersibility number shows that particles are in loose aggregates and can easily be dispersed by small agitation. This is generally affected by its settling velocity which is affected by viscosity. Therefore, as a general rule, the higher the viscosity, the slower the settling rate, and consequently, the lower the redispersibility number [39].

From the results obtained as indicated in table 6, the ease of re-dispersion of all the suspensions improved with increase in viscosity. However, suspensions containing 3 - 4 % w/v of CAG were too viscous and difficulty to flow and redisperse. Incorporation of CAG at concentration of 3 % w/v and above changed the structure of the suspensions to gel, thereby reducing the flow property of the suspensions.

In other words, as the viscosity increased, the redispersibility number decreased. This observation was similar to results obtained by other researchers. Ogaji *et al* [40] reported that the number of time required for redispersion of paracetamol suspensions formulated with

Adansonia digitata gum and Sodium Caboxymethylcellulose decreased with increase in the concentrations of these suspending agents with resultant increase in viscosities of the suspensions. Similarly, Moghimipour *et al*. [41] reported that increase in concentration of tragacanth as a suspending agent from 0.5 to 1.0 % w/v significantly increased the viscosity and sedimentation volume with improved ease of redispersion of magnesium hydroxide suspension.

The possible explanation for this is that, increase in concentration led to increase in viscosity and increase in viscosity retards the settling velocity of the suspended particles and at the same time probably reduced the inter particle attraction making the particles loosely packed together making redispersion easier. More so, the ease of re-dispersion followed the order of acacia > CAG > tragacanth. In all concentration employed, suspensions prepared with acacia have very low viscosities and behaved relatively like flocculated suspensions with the suspended particles settling down at once within a short period of time. Expectedly, acacia suspensions were all easily re-dispersed. Suspensions prepared with CAG were much easier to redispersed that of tragacanth because, the settling rate of particles in suspensions prepared with CAG were much slower than those prepared with equivalent amounts of tragacanth, because of the higher viscosity of CAG compared to tragacanth. Therefore, it's expected that the particle aggregates that settled down in CAG suspensions are less tightly packed and can be easily re-dispersed with less effort compared with tragacanth suspensions. Easy redispersibility is an important property of a good suspension to ensure dosage uniformity and prevent caking which may remain even after vigorous shaking for a specified time [7]. This encourages patient compliance to medication regimen.

Suspensions containing 3 - 4 % w/v of CAG were too viscous and difficulty to flow and redisperse indicating that small quantity of CAG may be needed in the formulation of suspensions with appropriate characteristics. Notwithstanding, the high viscosity of CAG is an indication for another use as stabilizer, thickener or gelling agent in certain formulations requiring high viscosity such as in pharmaceutical, food and cosmetic industries. It is also of economic value in the sense that a little quantity of CAG will be required in suspensions.

Evaluation of skin irritation

The pH of CAG was determined as a pre-requisite to *in vivo* animal test in order to evaluate the potential of causing any irritation when used in the formulation of a dermatological preparation. A substance with acidic or alkaline pH may cause irritation to the skin. It is assumed that product with a slightly acidic pH (similar to that of healthy skin; pH of 5.5) will be safe and most comfortable to the skin [42, 43]. The pH of CAG aqueous dispersion was found to be 5.37 $\pm$ 0.15 and according to OECD TG 404 [15], it could be assumed that it is not corrosive and qualified to undergo confirmatory in vivo animal test for skin irritation.

The results of skin irritation studies represented in Tables 7 and 8 showed that CAG aqueous dispersion and calamine suspension compared to the standard formalin solution giving 0 scale levels for both erythema and oedema. This shows that CAG alone was very compatible with no visible irritation to the animals' skin and also when used as a suspending agent in the formulation of calamine suspension. Thus CAG proved to be safe in the formulation of dermatological calamine suspension for application on skin. This is an indication that it could also possibly be safe in the formulation of other dermatological preparations such as cream, gel, emulsion and lotion.

Table 7: 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

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

Table 8: Results of skin irritation study for calamine suspension

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

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

CONCLUSION

CAG has been found to have superior suspending properties compared to standard suspending agents such as tragacanth and acacia. It has the potential as an alternative suspending agent and also as a thickener and gelling agent in pharmaceutical, cosmetic and food industries. It is equally suitable for preparation of dermatological products due to its compatibility with skin. It is a potential locally available economical pharmaceutical raw material/excipient that can be obtained from a waste product. Other pharmaceutical applications of CAG as a dermatological patch, film forming agent, gelling agent, binder and emulsifier are being studied.

REFERENCES

1. Banker, G.S., Rhodes, C.T. Disperse systems. In: Banker, S.G., Rhodes, C.T., eds. Modern

Pharmaceutics. 3rd ed. New York, NY: Marcel Dekker, Inc; 1988. p. 305 - 318.

2. Kibbe, H.A. Hand book of pharmaceutical excipient. 3rd ed. London: Pharmaceutical Press; 2000. p. 41.

3. Kulshreshtha, A.K., Singh, O.N., Wall, G.M. Pharmaceutical suspensions: From formulation development to manufacturing. 1st ed. New York (USA): Springer Science Business Media; 2010. p. 1- 4.

4. Malafaya, P.B, Silva, G.A., Reis, R.L. (2007). Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. *Adv. Drug Deliv. Rev.* **59**: 207-233.

5. Beneke, C.E., Viljoen, A.M., Hamman, J.H. (2009). Polymeric plant-derived excipients in drug delivery. *Molecule*. **14**: 2602-2620.

6. Femi-Oyewo, M.N., Adedokun, M.N., Olusoga, T.O. (2004). Evaluation of the suspending properties of *Albizia zygia* gum on sulphadimidine suspension. *Trop. J. Pharm. Res.* **3** (1): 279 – 284.

7. Panda, D.S., Choudhury, N.S.K., Yedukondalu, M., Si, S., Gupta, R. (2007). Studies on a natural gum for its application as a suspending agent; http://priory.com/pharmacy/suspensionGum suspendingAgent.html, sourced on the 15th of April 2009.

8. Ibu, J.O., Iyama, A.C., Ijije, C.T., Ishmael, D., Ibeshim, M., Nwokediuko, S. (1986). The effect of *Cola acuminata* and *Cola nitida* on gastric acid secretion. *Scand. J. Gastroenterol.* **21**:39-45.

9. Erbe, L. Encyclopedia Americana, Int edn, USA: Groiller Inc; 1993. p. 216.

10. Ogutuga, D.B.A. (1975). Chemical composition and potential commercial uses of kola nut, *Cola nitida*. *Ghana. J. Agric. Sci.* **8**:121-125.

11. Attawodi, S.E., Mende, P., Pfundstein, B., Preussemann, R., Spiegelhalder, B. (1995). Nitrosatable animes and nitrosamide formation in natural stimulants: *Cola acuminata*, *Cola nitida* and *Garcinia cola*. *Food. Chem. Toxicol.* **33** (8): 625-630.

12. Jayeola, C.O. (2001). Preliminary studies on the use of kolanuts (*Cola nitida*) for soft drink production. *J. Food. Tech. Afr.* **6** (1): 25-26.

13. Martins, A, Swarbrick, J, Cammarata, A. Physical pharmacy, 4th ed. Baltimore, USA: Lippinccott Williams and Wilkins 2001. p. 479-481.

14. Cydulka, R.K., Garber, B. Dermatologic presentations. In: Marx, J.A. ed. Rosen's emergency medicine: Concepts and clinical practice. 7th ed. Philadelphia, Pa: Mosby Elsevier; 2009: p. 118.

15. Anonymous: OECD guideline for the testing of chemicals, No. 404: Acute dermal irritation/corrosion. Paris, France 2002. p.13.

16. Worth, A.P., Cronin, M.T.D. (2003). The use of pH measurements to predict the potential of chemicals to cause acute dermal and ocular toxicity. *Toxicol.* **169**: 119-131.

17. Anonymous: OECD guideline for the testing of chemicals, No. 413: *In vitro* skin irritation/ corrosion - Human skin model test. Paris, France 2004. p.8.

18. Ramani, C.C., Puranik, P.K., Dorle, A.K. (1996). Study of diabietic acid as matrix forming material. *Int. J. Pharm.* **137**:11-19.

19. Rafiee-Tehrani, M., Ghaffari, A., Navaee, K., Oskoui, M., Bayati, K. (2007). Preparation and

characterization of free mixed-film of pectin/chitosan/Eudragit® RS intended for sigmoidal drug delivery. *Eur..J. Pharm. Biopharm.* **67**: 175 - 186.

20. Harbone, J.B. Phytochemical methods. 2nd ed. London: Chapman and Hall; 1989. p 10, 121.

21. Mahmud, H.S., Oyi, A.R., Allagh, T.S. (2008). Studies on some physicochemical properties of *Khaya senegalensis* gum. *Nig. J. Pharm. Sci.* **7** (1): 146-152.

22. Anonymous: NIH Publication No. 85-23. Revised edn. USA: U.S. Department of Health, Education and Welfare; 1985.

23. Draize, J.H., Woodard, G., Calvery, H.O.(1994). Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp.Ther.* **82**:377-390.

24. Abdulsamad, A, Bhatia, P.G., Ojile, J.E. (2006). Emulsifying and suspending properties of cashew gum. *Nig. J. Pharm. Res.* **5** (1):40-47.

25. Gupta, V., Puri, R, Gupta, S., Jain, S., Rao, G.K. (2010). Tamarind kernel gum: An upcoming natural polysaccharide. *Syst. Rev. Pharm.* **1**:50-54.

26. Uzondu, A.L., Nwannunu, N., Bamigbola, E.A. (2011). Some physicochemical properties of the gum obtained from the seeds of *Brachystegia eurycoma*. *Nig. J. Pharm. Res.* **9** (2):1-12.

27. Kumal, S.V., Sasma,l D., Pa,l S.C. (2008). Rheological characterization and drug release studies of gum exudates of *Terminalia catappa* Linn. AAPSPharmSciTech. doi:10.1208/s/12249 – 008 9101-5.

28. Umeka, M.J., Yeole, P.G. (2008). Characterization and evaluation of natural copal gum - resin as film forming material. *Int. J. Green Pharm.* **2** (1): 37- 42.

29. Jani, G.K., Shah, D.P., Prajapati, V.D., Jain, V.C. (2009). Gums and mucilages: versatile excipients for pharmaceutical formulations. *Asian. J. Pharm. Sci.* **4** (5): 308-312.

30. Lima, R.D.N., Lima, J.R., de Salis, C.R., Moreira, R.D. (2002). Cashew tree (*Anacadium occidentale* L.) exudate gum: A novel biolingand tool. *Biotechnol Appl. Biochem.* **35**: 45-53.

31. Gennero, A.R. Remington. The science and practice of pharmacy. 20th edn. US: Lippincott Williams and Wilkins; 2000. p. 307.

32. Florence, A.T., Attwood, D. Physicochemical principles of pharmacy. 4th edn. London (UK): Pharmaceutical Press; 2006. p. 273, 296 - 302.

33. Reza, M., Quadir, M.A., Haide,r S.S. (2003). Comparative evaluation of plastic, hydrophobic and hydrophilic polymers as matrices for controlled - release drug delivery. *J. Pharm. Sci.* **692**: 274-291.

34. Andrew, G.P., Laverty, T.P., Jones, D.S. (2009). Mucoadhesive polymeric for controlled drug delivery. *Eur. J. Pharm. Biopharm.* **71**(3):505-510.

35. Omidian, H., Park, K. (2008). Swelling agents and devices in oral drug delivery. *J. Drug. Del. Sci. Tech.* **18** (2): 83-93.

36. Martins, A., Swarbrick, J, Cammarata, A. In: Physical pharmacy, 3rd edn. Philadelphia, USA: Lea and Febiger; 1991. p. 465, 533-534.

37. Abdulsamad, A., Bhatia, P.G., Ojile, J.E. (2006). Phytochemical, toxicological and viscosity studies of cashew gum and its dispersion. *Nig. J. Pharm. Res*. **5**(1): 85-90.
38. Patrick, J.S. Martin's Physical pharmacy and pharmaceutical sciences, 5th edn. Baltimore, USA: Lippincott Williams and Wilkins; 2006. p. 503, 565.
39. Aulton, M.E. Pharmaceutics: The science of dosage form design. 2nd edn. Philadelphia, USA: Churchill Livingstone; 2002. p. 57-58, 84-86, 273.
40. Ansel, H.C., Allen Jr., L.V., Popovich, N.G. Ansel's Pharmaceutical dosage forms and drug delivery systems. 8th edn. USA: Lippincott Williams and Wilkins; 2005. p. 395.
41. Ogaji, J.I., Omachi, J.A., Iranloye, T.A.(2012). Effect of *Adansonia digitata* gum on some physicochemical properties of paracetamol pediatric suspension formulations. *Int. J Res. Pharm. Sci.* **2** (2): 79 - 80.
42. Moghimipour, E., Handali, S., Salimi, A., Mansoum, M. (2013). The effect of electrolyte concentration and polymer content on the rheological behaviour of magnesiun hydroxide suspension. *British J. Med. Med. Res.* **3** (4): 1982.
43. Parsons, D., Bowler, P.G., Myles, V., Jones, S. (2005). Silver antimicrobial dressings in wound management: A comparison of antibacterial, physical and chemical characteristics. *WOUNDS.***17** (8):231
.