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CHARACTERIZATION OF GUM FROM *SESAMUM INDICUM* LEAVES AS A SUSPENDING AGENT IN A PEDIATRIC PHARMACEUTICAL SUSPENSION

Ndidi C. Ngwuluka[1*], Munirat Akanbi[1], Inalegwu Agboyo[1] and Onyinye J. Uwaezuoke[2]

[1]Department of Pharmaceutics and Industrial Pharmacy, Faulty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria.

[2]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Olabisi Onabanjo University, Ago-Iwoye, Nigeria.

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***Correspondence for Author:**

*** Dr Ndidi C. Ngwuluka**, Department of Pharmaceutics and Industrial pharmacy, Faulty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria.

Ndidi.Ngwuluka@biodrudel.com

ABSTRACT

Studies are undertaken to develop excipients from plant resources due to their availability, low toxicity and biodegradability. In this study, a novel excipient, was extracted from the leaves of *Sesamum indicum;* rheology, structural elucidation by FTIR and phytochemical analysis were undertaken. Its suspending properties were determined by employing techniques such as sedimentation volume, viscosity, ease of redispersibility, and buffering in comparison with acacia and mainly tragacanth. The percentage yield of the gum was 1.96% with residual moisture of 5.6%. A quantity (0.25g) with relative viscosity of 5.2 of sesamum gum can be used to achieve a sedimentation volume, 10mL on the 14th day with ease of redispersibility of 12-14secs. In summary, sesamum gum exhibited the potential to produce acceptable suspensions with quick restoration of homogeneity and no formation of aggregates. Furthermore, buffering further stabilized the viscosities of the suspensions. Hence, sesamum gum is a potential pharmaceutical excipient for pharmaceutical suspensions that is accessible, cost effective and environmental friendly.

Keywords: Sesamum, suspending agent, excipients, natural gums, pharmaceutical, suspensions.

INTRODUCTION

Natural gums and mucilages are biopolymers extracted mainly from plants and have wide applications in the food and pharmaceutical industries as emulsifiers, emulsion stabilizers, suspending agents, binding agents, thickeners, humidifiers, drug release modifiers and coating agents [1-3]. They are often preferred to synthetic polymers because they are biodegradable, non-toxic, inexpensive, freely available; pliable to both chemical and biochemical modification and thus can compete with synthetic biodegradable polymers [4-6]. Natural gums and mucilages hydrate and swell when in contact with water and so have been employed in preparation of single unit dosage forms [7].

Sesamum indicum L. as a plant dates back to 3050-3500 B. C evidenced by the archeological finding at Harappa [8]. Sesame is an annual self pollinating plant with an erect, pubescent, branching stem, and 0.60 to 1.20 m tall. The leaves are ovate to lanceolate or oblong while the lower leaves are trilobed and sometimes ternate and the upper leaves are undivided, irregularly serrate and pointed [9]. The older cultivars have smooth and flat leaves while the nonshattering cultivars have cupped leaves with leaf-like outgrowths on their lower side. Some cultivars have many branches, while others are relatively unbranched [9]. The gummy matter in the leaves when mixed with water forms mucilage which is used in the treatment of infant cholera, dysentery and diarrhea [10]. The leaves due to the mucilaginous exudates are used for soap or shampoo [8]. However, very little is known about its leaves' potential as a pharmaceutical excipient.

Oral delivery remains the most convenient route of administration and pharmaceutical suspensions are employed in a proportion of the patient population who has difficulty in swallowing such as pediatrics and geriatrics. Furthermore, oral suspensions have high patient compliance, reduced side effects and improved bioavailability [11]. Though there are a number of oral excipients available, the quest is still on to find more excipients especially from natural origin due to their low cost, low or no toxicity, availability and biodegradability. Therefore, this study was undertaken to assess the suspending ability of the gum from *sesamum indicum* leaves for utilization in pharmaceutical suspensions. Suspensions were prepared with sesamum gum in comparison to tragacanth (mainly) and acacia. Suspending properties were assessed by employing techniques such as sedimentation volume, viscosity, and ease of redispersibility.

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MATERIALS AND METHODS

Materials

Sesamum indicum leaves, acacia (BDH Chemical Ltd, Poole, England), tragacanth (Steculia Gum, Halewood Chemicals Ltd, Stanwell Moor, Staines, Middlesex, England), amaranth solution, sulphadimidine (Merck Group, Darmstadt, Germany) and raspberry syrup. Other chemicals were of analytical grade and utilized as received.

Extraction of gum from *sesamum indicum* leaves

Sesamum indicum leaves were purchased from farin gada market, Jos at the onset of rainy season. The leaves were identified at the Federal College of Forestry, Jos, Nigeria by Joseph Azila of the Department of Forestry Technology. The voucher specimen with voucher number FHJ 93 was kept at the department's herbarium for future reference. The extraction of the gum was undertaken by adding gradually 2.23kg of fresh leaves of *sesamum indicum* into 8.4L of hot water (100^{o}C). This was allowed to macerate for 6 hours and afterwards, the brown slimy solution was expressed and separated from the leaves with a muslin bag. Mucilage was precipitated from the solution by washing several times with absolute alcohol, air-dried for two days at ambient temperature and milled. The gum obtained was weighed and percentage yield calculated thus:

$$\%\ \text{Yield} = \frac{\text{Weight of gum}}{\text{Weight of leaves}} \times 100$$

Equation 1

Phytochemical Analysis

Sesamum gum was tested for proteins, starch, alkaloids and tannins. To test for protein, sufficient quantity of 0.1N HCl was added to the 100mg of the gum and boiled for 20 minutes with periodical addition of 0.1N HCl to prevent drying. After 20 minutes, the sample was allowed to cool and to 1mL of clear solution, 2mL of sodium hydroxide was added and then 2mL of copper sulphate. The presence of a violet colour is indicative of protein. Other tests were undertaken as described in Treas and Evans' Pharmacognosy [12].

Moisture content determination

A quantity of 5g of sesamum gum was weighed and placed in an oven at 40^{o}C. It was weighed at intervals until a constant weight was obtained. The percentage moisture content was calculated from the equation below:

$$\%\ \text{Moisture Content} = \frac{\text{Weight loss}}{\text{Initial Weight}} \times 100$$

Equation 2

Fourier Transform Infra-Red (FTIR) spectroscopy of sesamum gum

Fourier Transform Infra-Red spectrum of sesamum gum was obtained with a PerkinElmer spectrometer (PerkinElmer Spectrum 100, Beaconsfield, United Kingdom) over a range of 4000-650cm^{-1}.

Rheological characterization of sesamum gum

Sesamum gum (500mg) was uniformly dispersed in 50mL of deionized water. Rheological studies were undertaken with Haake MARS (Modular Advanced Rheometer System) rheometer (Thermo Scientific Inc., USA) with RheoWin PC software. The rheometer was fitted with a cone and plate geometry; 35mm in diameter and cone angle of 1^{o} – sensor C35/1^{o}Ti and was set in oscillatory mode. Basic viscosity was obtained by subjecting the gum to a shear rate range from 0.00 - 200.00 1/s over 180 seconds; yield stress obtained from a shear rate range of 0.200 – 200 pa over 200 seconds and oscillatory frequency sweep from 50.00 – 0.05 Hz. These studies were done the first day and the dispersed gum was stored under ambient temperature for three days and the studies repeated.

Preparation of sulphadimidine suspensions

The standard formula (Table 1) for the preparation of sulphadimidine oral pediatric suspension B.P.C. 1993 was employed. With the B.P.C formula as standard, 100mL was prepared for each suspension. However, the suspending agents and their concentrations were varied for each suspension. Briefly, the appropriate weight of the required gum for each suspension was weighed and triturated in a mortar with 10g of sulphadimidine powder added in geometric progression. Afterwards, 20ml of raspberry syrup was added to form a smooth paste while 2ml of benzoic acid solution and 1ml amaranth solution were diluted with 50ml chloroform water double strength. The chloroform mixture was gradually added to the content in the mortar with constant trituration. The mixture was placed in a labeled pre-calibrated bottle and water was added to make up to 100ml and shaken before it was left to stand.

Table 1: Compositions of the suspensions prepared

Composition	Formulation							
	F1	F2	F3	F4	F5	F6	F7	F8
Sulphadimidine powder (g)	10	10	10	10	10	10	10	10
Compd. Tragacanth (g)	4	3	2	1	-	-	-	-
Acacia (g)	-	-	-	-	1	-	-	-
Sesamum gum (g)	-	-	-	-	0.25	0.5	0.75	1.0
Amaranth solution (mL)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Benzoic acid (mL)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Raspberry syrup (mL)	20	20	20	20	20	20	20	20
Double strength Chloroform (mL)	50	50	50	50	50	50	50	50
Water q.s to mL	100	100	100	100	100	100	100	100

Characterization of sulphadimidine suspensions

Determination of viscosities of sulphadimidine suspensions

The viscosities of the suspensions were determined using a U-tube viscometer in comparison with distilled water. Briefly, through the tube (L), the U-tube viscometer was filled with the suspension to be analyzed slightly above the mark G with a 1mL pipette. The volume was adjusted such that the bottom meniscus is at the G mark and with a siphon attached to the end of the tube (N). The suspension in the viscometer was sucked up above point E. The suction pressure was released and the time taken for the bottom of meniscus to fall from mark E to mark F was recorded. This was done daily for two weeks and the relative viscosity was calculated from equation below:

$$\text{Relative Viscosity} = \frac{T - T_0}{T_0} \qquad \text{Equation 3}$$

Where T is the time of flow of suspension in seconds and T_0 is time of flow of distilled water in seconds.

Determination of sedimentation volume

Sedimentation volumes were determined by placing 100mL of the suspensions in measuring cylinders and stored on a vibration-less work bench and undisturbed. Initial volumes were recorded and the volumes of sediments were observed and recorded daily for two weeks.

Ease of redispersibility of suspensions

Ease of redispersibility of suspensions was determined by placing the suspensions in capped bottles and the bottles were shaken in the same direction for the same number of times. The time taken for uniform dispersion was observed and noted. This was done daily for two weeks and weekly for another two weeks.

Determination of pH of suspensions

Determination of pH was undertaken with homogenous suspensions twice a week for two weeks. Hence the suspensions were re-dispersed each time pH is determined. The pH of the mucilage of sesamum gum was also determined.

Determination of densities of suspensions

A pycnometer was employed to determine the weight of 100mL of suspension in relation to 100mL of water. Having obtained the weights, the densities were calculated.

The influence of thaw-freeze method on the characteristics of the suspensions

The suspensions were subjected to low temperatures (0^oC and 4^oC) and high temperatures (40^oC and 60^oC). At each temperature, the characters of the suspensions such as color, viscosity and redispersibility were assessed after 24 hours.

Influence of buffer on the stability of suspensions

Phosphate buffers of pH 6.5, 7.5 and 8.0 were prepared; sulphadimidine suspensions were prepared and different suspensions had the different pH buffers. The buffers were used instead of water to make up the suspension to 100mL. The suspensions were characterized based on viscosity, sedimentation volume and ease of redispersibility.

Statistical analysis

The influence of pH on relative viscosities of suspensions was subjected to analysis of variance (ANOVA) and statistical significant test analysis was used for sedimentation volume.

RESULTS AND DISCUSSION

Physical characters, yield, photochemical analysis moisture content

Sesamum gum extracted was brown in color and was milled to fine powders which appeared fluffy. The phytochemical analysis of sesamum gum indicated that it contained protein and carbohydrate (glucose units). The percentage yield was 1.96% which can be expected from a plant extract. The residual moisture content after drying was 5.6%.

Fourier Transform Infra-Red (FTIR) spectroscopy of sesamum gum

Spectrum obtained of sesamum gum as shown in Figure 1 indicates the possible presence of alkyl, hydroxyl and carbonyl groups as well as amino groups. The broad peak between 3540 and 3000 cm^{-1} is characteristic of the presence of hydroxyl groups. The peaks at 2918.75 cm^{-1} and 1599.95 cm^{-1} indicate the possible presence of alkyl groups and amide groups respectively. The peak at 1414.93 cm^{-1} is possibly due to C-O-H bending while 1024.43 cm^{-1} may be due to O-C stretching. Hence the FTIR spectrum corroborates the outcome of phytochemical analysis indicating the possible presence of protein and carbohydrate.

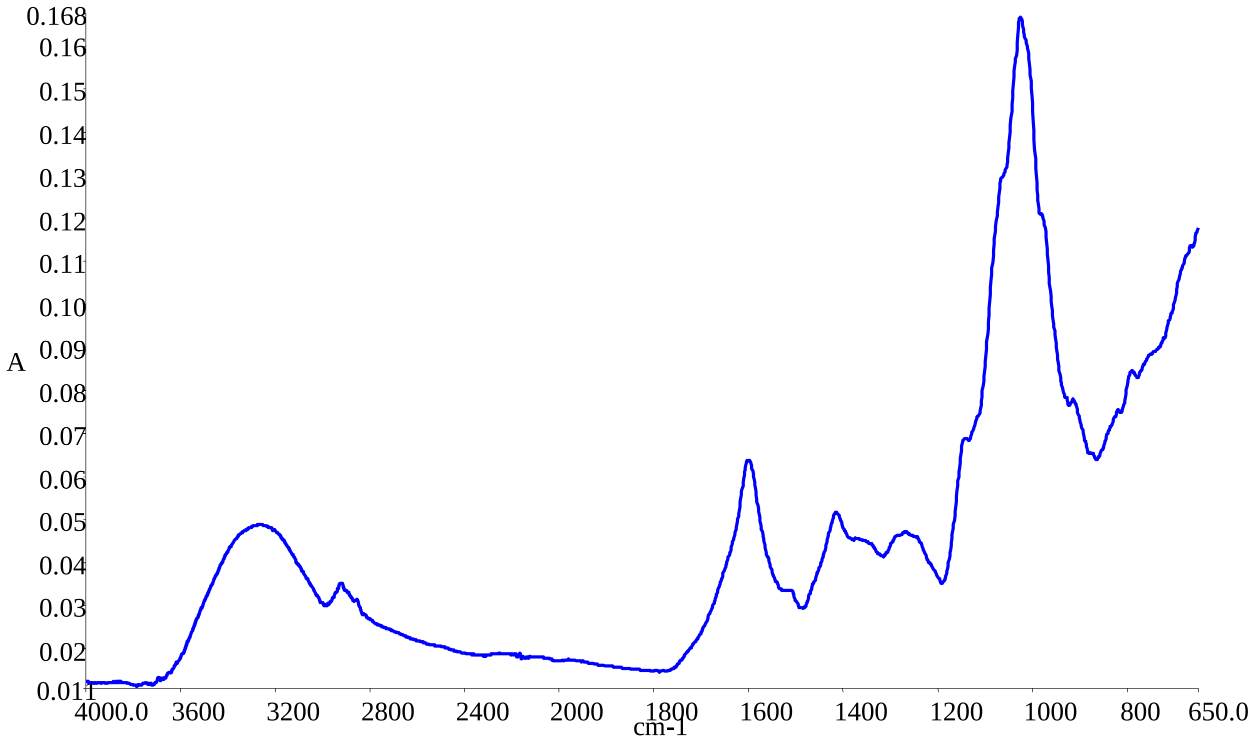

Figure 1: FTIR spectrum of sesamum gum powder.

Rheological characterization of sesamum gum

The viscosity rheogram of sesamum gum indicates the gum exhibited non-newtonian pattern of flow. Its shear thinning behavior is shown in Figure 2a. However, after three days of storage, the viscosity decreased and the shear required for initiation of flow decreased. Frequency sweep is used to characterize the structure of a material - to indicate if the material behaves as a liquid, gel, entangled solution or a three-dimensional network. Figure 2b shows the visco-elastic behavior of sesamum gum. The elastic and loss moduli increased in a parallel pattern as frequency increased until about 60 rad/s with elastic modulus higher than loss modulus indicating the ability of sesamum gum to behave as a three-dimensional network. As the frequency increased above 100 rad/s, there was a slight alternate decrease and increase of the loss modulus but elastic modulus remained higher. Furthermore, the elastic and loss moduli of sesamum gum after three days were parallel up to about 60 rad/s and elastic modulus was higher. However, after 100 rad/s, the loss modulus was higher indicating that the gum began to behave as a liquid from that point. After the storage of the dispersed gum, its resistance to deformation decreased due to modification that occurred during storage.

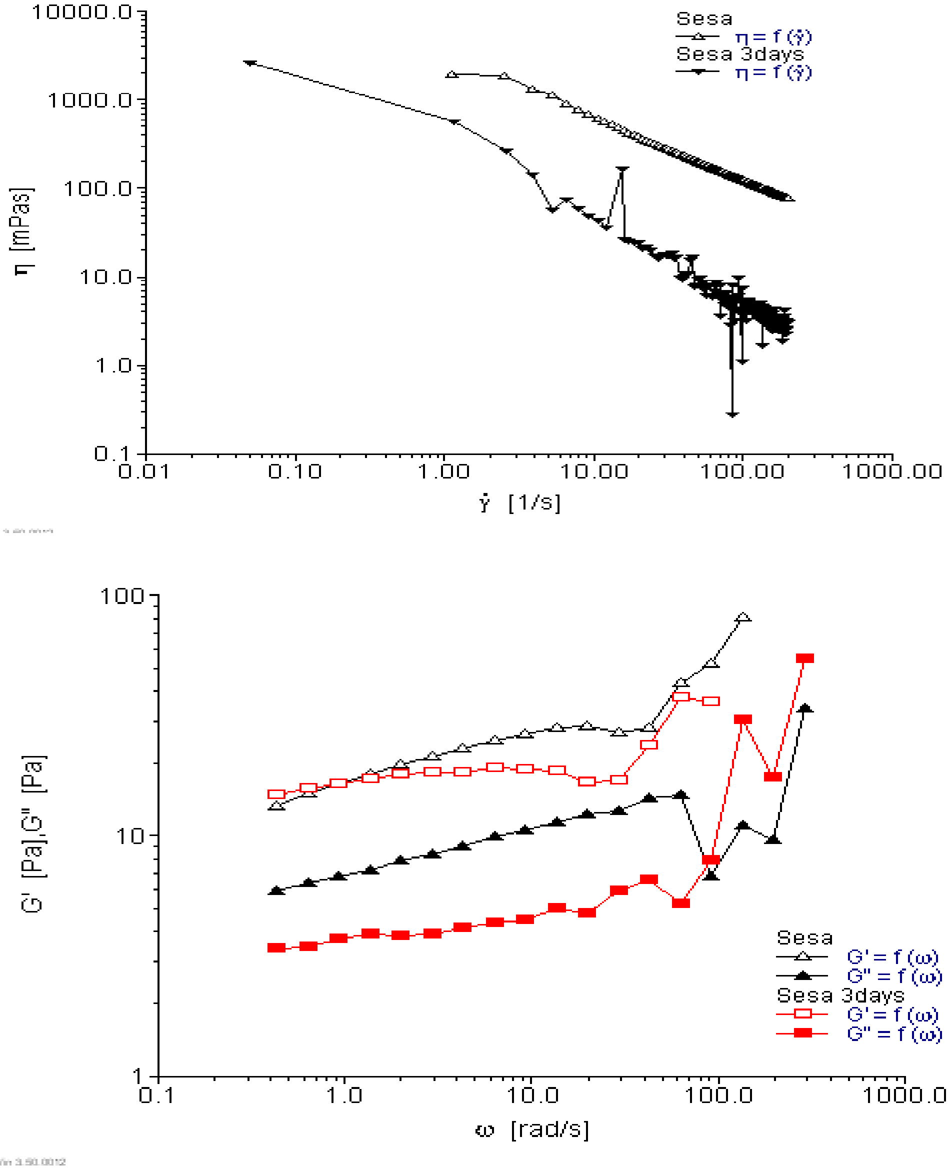

Figure 2: a) Viscosity as a function of shear rate for sesamum gum and; b) viscous and elastic modulus as a function of angular frequency for sesamum gum.

Viscosities of the non-buffered and buffered suspensions

Relative to viscosity of water which is 1.0 millipascal seconds, sesamum indicum was very viscous at low concentration and so more than 1g was not used to ensure pouring of the suspensions. Furthermore, 0.25g of sesamum gum was more viscous than 4g tragacanth as shown in Table 2. However, on storage and as the days progressed, the viscosity decreased significantly while that of tragacanth was not significant. Even when buffers were added to sesamum indicum mucilage, there was still decrease in viscosity. Natural gums though safe and are applied as thickeners, emulsifiers, viscosity enhancing agents and suspending agents have one of the setbacks as decrease in viscosity over time. The decrease in viscosity exhibited by natural gums is associated with autocatalytic hydrolysis due to the natural pH (pH 5 – 6) [13], bacterial growth [14] and enzymatic degradation [15]. Other setbacks include uncontrolled rate of hydration, thickening, and microbial contamination; and requires functionalization or modification to overcome these problems [4, 6]. Despite these, more studies are undertaken develop excipients from plant resources because of their low toxicity, availability and degradability. It was observed that drop in viscosity of sesamum mucilage was rapid and may be due to autocatalytic hydrolysis at pH 5 -6. This may be so as microbial culturing of sesamum gum did not yield any significant outcome to suggest that the decrease in viscosity could be due microbial contamination. However, when sesamum gum was incorporated into sulphadimidine suspension, the drop in viscosity of the suspension was not statistically significant. This may be due to the possibility of some interactive mechanisms between other components of the suspension and sesamum gum. Suphadimidine is known to bind to some excipients such as sodium starch glycolate, Veegum and Amberlite [16]. Sulphadimidine is also known to interact and form complexes with aromatic carboxylic acids by hydrogen bonding [17, 18]. It is envisaged that physical interactions such as hydrogen bonding may have occurred between sulphadiminde and sesamum gum (containing a polysaccharide) preventing autocatalytic hydolysis of the gum thereby preventing significant decrease in viscosity. The addition of buffers further stabilized the viscosity especially in suspensions that buffer pH 6.5 was added. Statistical evaluation (ANOVA) corroborated the experimental observation stating that effect of pH had no significant effect on the viscosities of the mucilages of the gums but did have a significant effect on the viscosities of the suspensions.

Determination of sedimentation volume

The sedimentation volumes of suspensions prepared with sesamum gum (0.25g and 1.0g) as suspending agent decreased over time; from 25mL to 10mL and 46 to 26 mL respectively within two weeks (Figure 3). Higher concentration of sesamum gum yielded more suspension of particles over time. Furthermore, addition of buffer decreased the rate of sedimentation of particles and hence increased the sedimentation volume of the suspension. With pH 6.5, the decrease in sedimentation volume over time for 0.25g sesamum gum was from 27 to 24Ml; 30 to 23mL with pH 7.5 and 30 to 28mL with pH 8.0. That of 1.0g sesamum gum was from 47 to 41mL with buffer pH 6.5, 49 to 38mL with buffer pH 7.5 and 48 to 35mL with buffer pH 8.0. This is indicative that the presence of buffers prevented rapid sedimentation of particles. In sum, the decrease in sedimentation volumes was not statistically significant except in the case of suspension prepared with 0.25g sesamum gum without buffer. The use of 4g tragacanth as suspending agent yielded lower sedimentation volumes in comparison to sesamum gum. Buffer pH 6.5 improved the sedimentation volume of tragacanth in comparison to non-buffered suspensions and suspensions with buffers pH 7.5 and 8.0. The sedimentation volume with pH 6.5 was 26 to 24 over time but was 11 to 10mL in non-buffered suspension and 15 to 13mL and 16 to 14mL with pH 7.5 and 8.0 respectively over time. It was observed that lower concentration (0.25g) of sesamum gum can be employed to achieve the same sedimentation volume as 4g tragacanth (Figure 3). And higher concentration such as 1.0g can be used to further increase the sedimentation volume of suspended particles. There is due to a positive correlation between sedimentation volume and viscosity. As concentration of sesamum increases, the viscosity increases with subsequent increase in sedimentation volume.

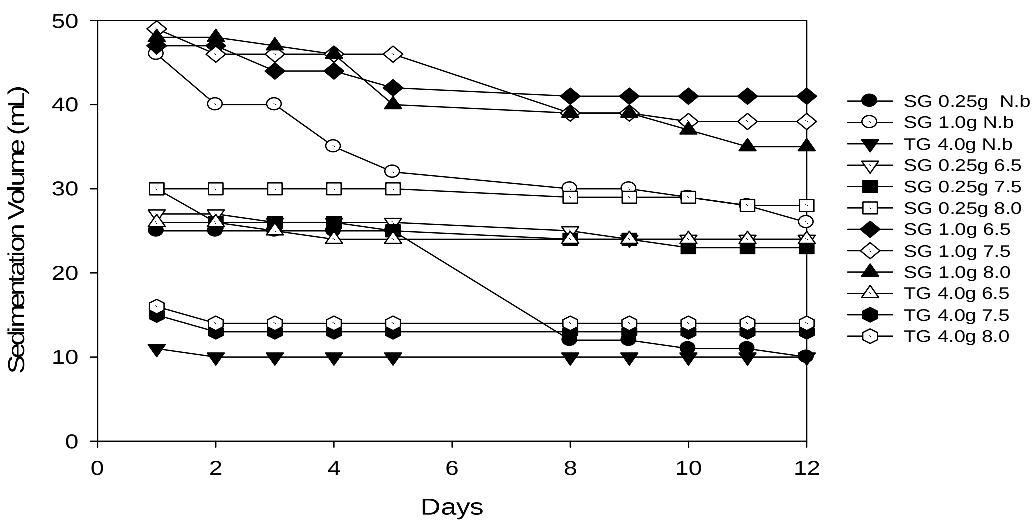

Figure 3: Comparative influence of different pH on sedimentation volume of suspensions with different concentrations of suspending agents.

Table 2: Relative viscosities of non-buffered and buffered suspensions

Day	0.25% SG mucilage				1.0% SG mucilage				4% Tragacanth mucilage				0.25% SG in suspension				1.0% SG in suspension				4% Tragacanth in suspension			
	N.b	**6.5**	**7.5**	**8.0**	**N.b**	**6.5**	**7.5**	**8.0**	**N.b**	**6.5**	**7.5**	**8.0**	**N.b**	**6.5**	**7.5**	**8.0**	**N.b**	**6.5**	**7.5**	**8.0**	**N.b**	**6.5**	**7.5**	**8.0**
1	5.2	6.2	6.3	6.0	21	24	22	24	2.5	2.7	2.4	2.4	3.6	3.4	3.5	3.8	10	9.3	9.6	11	3.2	3.4	3.2	3.2
2.	3.4	3.3	3.9	5.1	18	20	20	19	2.6	2.6	2.6	2.5	3.5	3.4	3.5	3.8	9.5	9.3	9.6	10	3.1	3.3	3.2	3.2
3.	2.1	3.1	2.4	3.5	8.0	20	17	15	2.6	2.4	2.4	2.6	3.3	3.5	3.4	3.5	9.3	9.3	9.6	9.6	3.1	3.3	3.2	3.2
4.	1.6	2.4	2.0	3.3	7.1	20	9.1	8.5	2.5	2.5	2.5	2.5	3.1	3.4	3.3	3.4	9.2	9.3	9.6	9.4	3.1	3.3	3.1	3.2
5.	1.6	2.4	1.6	2.2	2.1	16	4.2	6.4	2.6	2.4	2.3	2.5	3.0	3.3	3.3	3.4	9.1	9.3	8.6	9.4	3.1	3.3	3.1	3.2
8.	1.5	2.0	1.6	1.7	1.6	8.9	1.7	2.1	2.5	2.3	2.3	2.4	3.1	3.4	3.2	3.4	8.5	9.2	8.5	9.4	3.1	3.3	3.1	3.1
9.	1.6	1.6	1.6	1.7	1.6	3.8	1.6	1.7	2.4	2.3	2.3	2.4	3.1	3.4	3.1	3.1	8.7	9.2	8.6	8.7	3.2	3.3	3.1	3.1
10	1.5	1.5	1.3	1.7	1.6	2.8	1.5	1.5	2.3	2.2	2.3	2.4	3.1	3.4	3.2	3.2	7.8	9.2	8.5	9.1	3.1	3.3	3.1	3.1
11	1.5	1.5	1.3	1.2	1.5	1.6	1.6	1.4	2.3	2.2	2.2	2.3	3.1	3.4	3.1	3.2	8.1	9.2	8.5	8.7	3.1	3.3	3.1	3.1
12.	1.4	1.5	1.3	1.2	1.5	1.6	1.6	1.5	2.3	2.2	2.2	2.3	3.1	3.4	3.1	3.1	7.9	9.0	8.4	8.5	3.1	3.3	3.1	3.1

N.b means non-buffered while 6.5, 7.5 and 8.0 refer to the different pH buffers. SG – sesamum gum

Ease of redispersibility of suspensions

Suspensions prepared with sesamum gum (0.25 and 1.0g) as suspending agent were redispersible within 20 seconds over time in comparison with acacia gum with ease of redispersibility decreasing on storage to 40 seconds (Table 3). Incorporation of buffers had no significant influence on ease of redispersibility of the suspensions. Ease of redispersibility determines the acceptability of a suspension which should be free-flowing on agitation and pouring [19]. Furthermore, ease of redispersibility ensures uniformity of dosage and so redispersibility in less than 20 seconds of the suspensions prepared with sesamum gum may afford uniformity of dosage with no formation of aggregates.

Table 3: Ease of Redispersability (in Seconds) of Suspensions at Different pH

Day	0.25% w/v Sesamum gum				1% w/v Sesamum gum				4% w/v Tragacanth				1% AG
	N.b	pH 6.5	pH 7.5	pH 8.0	N.b	pH 6.5	pH 7.5	pH 8.0	N.b	pH 6.5	pH 7.5	pH 8.0	N.b
1	12	10	10	12	11	12	10	11	8	10	9	10	20
2	12	10	13	10	11	8	10	13	11	12	11	16	25
3	8	12	11	10	12	10	11	10	10	17	14	13	23
4	9	10	11	13	10	10	9	10	13	18	14	15	26
5	10	11	13	10	13	11	10	10	13	18	15	15	22
8	12	14	14	13	15	13	15	15	16	18	14	18	22
9	12	13	13	15	12	12	11	14	14	16	16	16	22
10	13	14	12	16	14	14	13	14	18	15	15	17	25
11	14	16	12	14	12	11	13	13	18	18	16	18	27
12	13	14	13	15	13	13	15	15	18	18	16	15	26

AG stands for acacia gum

Determination of pH of suspensions

The pH of sesamum mucilage decreased significantly over time from 7.5 for 1.0g sesamum to 4.81 within two weeks. It is envisaged that the hydrolysis of the gum may have yielded some acidic component(s) leading to the decrease in pH. However, the suspensions prepared with sesamum gum were significantly stable with slight decrease in pH. Tragacanth mucilage also decreased in pH from 6.77 to 5.52 while the suspensions were stable as well. In fact, the pH profile of suspensions with 1.0g sesamum gum was comparable to those of tragacanth (Figure

4). In sum, it is reported that polysaccharides increase in viscosity with pH due to increase of the ionization degree of carboxylate groups in polysaccharide molecules [20].

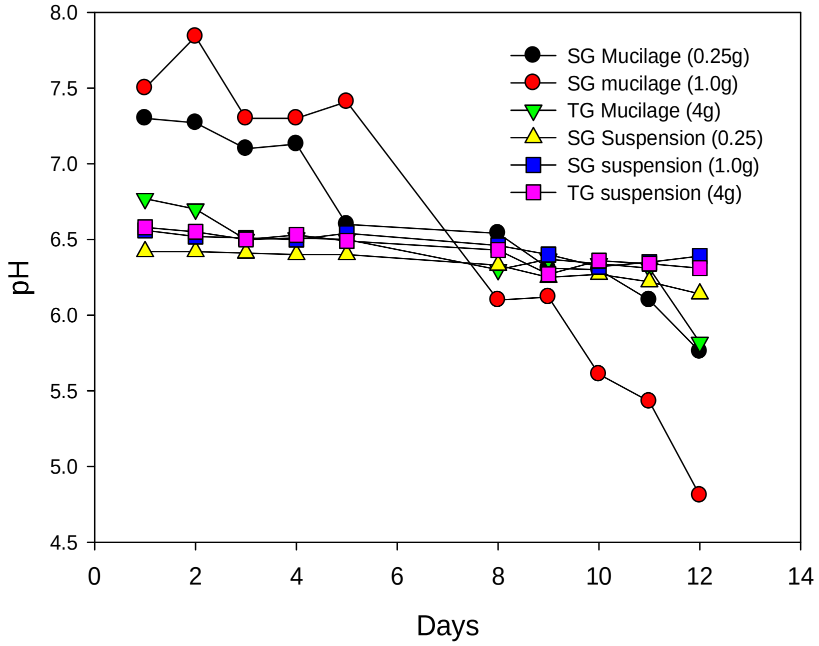

Figure 4: **Change in pH of mucilages and suspensions on storage**

Determination of densities of suspensions

As expected, as the concentration of suspending agent increased the densities of the suspensions increased (Figure 5). The density of particles influences their rates of sedimentation and the ease of redispersibility. It is well known that denser and larger particles sediment faster than smaller and less dense particles. Furthermore, upon agitation, the forces acting on the particles to induce mobility depend on the mass of the particles and the difference in density between the particles and the liquid [21]. The densities observed with suspensions prepared with sesamum gum are indicative that sedimentation would not be rapid after shaking thereby enabling the patient time to withdraw the required dose for administration.

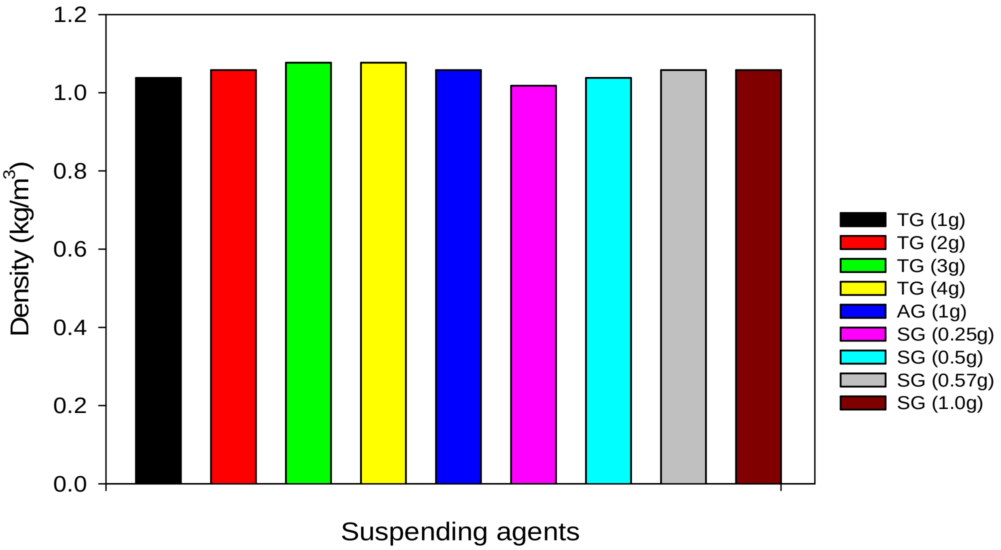

Figure 5: Densities of the suspensions with different suspending agents and their concentrations.

Effect of temperatures on suspensions

At low temperatures (0 and 4^{o}C), there was no significant change in integrity or viscosity of the suspensions. This is appreciable as suspensions are usually stored at low temperature during administration. At ambient temperatures, the suspensions were also visibly stable. There was no color change, no appreciable decrease in viscosity and the suspensions were easily redispersible. However, when the temperature was increased; there was decrease in viscosity indicating that heat exerts a viscosity-thinning effect on the suspensions.

CONCLUSION

Sesamum indicum was compared with acacia gum and mainly tragacanth, a commonly used suspending agent in pharmaceutical suspensions. It produced acceptable suspensions which would yield uniform dose due to its ease of redispersibility and no formation of aggregates. Sesamum gum holds promise as a pharmaceutical excipient which is accessible, cost-effective and environmentally friendly in comparison to synthetic gums and polymers. It can be employed as a thickener and suspending agent for formulation of pharmaceutical suspensions.

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