ORIGINAL PAPER

Nig. J. Pharm. Res. 2016, 12 (1) pp 37-47
ISSN 0189-8434 **Available online at http://www.nigjpharmres.com**

Emulsifying and Suspending Properties of *Enterolobium cyclocarpum* Gum

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: The thermodynamic instability of emulsions and suspensions necessitate the incorporation of emulsifiers and suspending agents respectively, in order to stabilize the formulations and ensure administration of accurate doses.
Objective:*Enterolobium cyclocarpum* gum was characterized and evaluated for its emulsifying and suspending properties in comparison with official acacia gum.
Method: Physicochemical and phytochemical characterization of *Enterolobium cyclocarpum* gum (ENCG) were carried out. Emulsifying properties were assessed using creaming rate, globule-size distribution and viscosity measurements. Metronidazole suspensions containing 1.0 -5.0%w/v of ENCG (or acacia) were assessed by sedimentation volume, pH determinations, viscosity and flow rate. Primary emulsions containing ENCG (or acacia) were formulated. Polysorbate-80 was also incorporated in the formulations to enhance the surface activity of the system.
Results:ENCG contains anthraquinones, cardiac glycosides and flavonoids. It is a highly viscous, weakly acidic gum (pH 5.96 at 25^0C), with a high hydration power and swelling index of 15.14. Acacia emulsions were more stable than ENCG emulsions, however, polysorbate-80 at 8.95 %v/v, reduced the creaming rate of the emulsions. Globule size and viscosity (25^0C) ranked ENCG emulsions > acacia emulsions. Sedimentation volume decreased over time with ENCG suspensions demonstrating faster sedimentation rate. The pH of the suspensions remained relatively unchangedSuspensions containing ENGG were more viscous and the flow rates ranked ENCG suspensions < acacia emulsions.
Conclusion: The results suggest that *Enterolobium cyclocarpum* gum is a highly viscous, poorly emulsifying gum but it hassuspending properties comparable with official acacia gum.
Keywords: *Enterolobium cyclocarpum*, Emulsions, Suspensions, Pharmaceutical excipients.

INTRODUCTION

Gums are natural heteropolysaccharides with high molecular weight. They are secondary plant metabolites, often viscous in nature, that are formed as a result of microbial infection or as a result of mechanical injury on a plant (Adeyanju *et al*., 2014). Natural gums, though hydrophilic and are generally biodegradable and non-toxic, have the tendency to undergo uncontrolled hydration (Jani *et al*, 2009). Uzeala (1988) classified gums on the basis of their source of origin as plant seeds (examples include locust bean, guar gum and macuna gum), seaweed extracts (examples include alginates, agar and carrageenan), tree exudates (examples include tragacanth gum, khaya gum, acacia gum and raphia gum), citrus fruits (pectin), animal skin and bones (gelatin), and gums obtained by fermentation processes (examples include Xanthan and lichen gum). Documented reports of the use of Natural gums as emulsifying and suspending agents in pharmaceutical formulations abound in literature (Ngwuluka *et a*l, 2012; Asantewaa *et a*l, 2011; Femi-Oyewo *et al*, 2004). Natural gums also have extensive use as binders, mucoadhesives and release modifiers (Adetunji *et al*, 2015; Bamiro *et al*, 2010; Emeje *et al*, 2009; Kalu *et al*, 2007; Adetunji *et al*, 2006). However, the expensive production of some of these commercially available gums has culminated in the attraction for cheaper and easily available natural gums that have desirable and comparable properties (Adetunji and Odole, 2015).

Mexican walnut gum, obtained from the incised trunk of *Enterolobium cyclocarpum* tree (Family: Mimosaceae), is structurally a beta – (1→3) -galactan, while some of the galactoses are 6-0 linked and others occur as terminal residue. There are evidences that support the presence of α-L-arabinofuranose, β-L-arabinopyranose and β-D-glucuronic acid as terminal and internal residues (Leon-de-Pirto G, *et al*., 1994). This study was aimed at investigating the emulsifying and suspending properties of the gum obtained from Mexican Walnut (*Enterolobium*

cyclocarpum; Family: *Mimosaceae*). Characterization of the gum carried out and its efficacy as an emulsifying and suspending agent (in metronidazole suspensions) was studied in comparison with Acacia gum BP. Metronidazole was chosen as the model drug for the suspensions because of its low solubility, and thus requires a suspending agent to ensure administration of accurate doses.

MATERIALS AND METHODS

Materials

The materials used include castor oil BP (Drugfield Pharmaceuticals Ltd., Nigeria), Acacia gum BP (Titan Biotech Ltd., India.), Analytical grade chloroform water (D/S) and Diethylether (BDH Chemicals Ltd., England.), Ethanol (96%) (M.R.S. Scientific Ltd.) and sodium lauryl sulphate (Sigma-Aldrich Co, St Louis, MO, USA). Ultra-pure de-ionized water (UPDW) was used. All other chemicals used were of reagent grade and their use was modified as described.

Gum Collection and Extraction

The brown coloured gum was obtained as exudates from the incision of the trunk of *Enterolobium cyclocarpum* tree (family: Mimosaceae) authenticated at the Forest Herbarium, Ibadan (FHI No: 110388), Nigeria. The gum was weighed, allowed to dry and then thoroughly washed in chloroform water (D/S) to remove associated earth particles. The washed exudates were spread on sterile drainers at a room temperature of 27±1 ^{0}C for a period of 3 hours, and dried in the oven at a temperature of 50 ^{0}C for 48 hours. The dried gum was blended using a laboratory blender (Waring® capacity blender Z272205, Sigma Aldrich Co, USA) , and thereafter soaked in acetone sufficient to cover the entire quantity of the dried gum for 24 hours. The resulting solution was filtered through muslin cloth, freeze dried and screened through a 120-µm mesh sieve (Bamiro *et al*, 2010; Adetunji *et al.* 2015). The percentage yield of the gum was calculated.

Characterization of the gum

Determination of particle size and photomicrograph

The particle size and distribution of the gum samples were determined using a light microscope. The photomicrographs of the gum samples were also observed showing the shapes of the different gum samples.

Determination of Phytochemical Characteristics

(i) Test for alkaloids: Two milliliters of a 10% HCL was added to 2 gm of *Enterolobium cyclocarpum* gum (ENCG) powder and boiled on a water bath. The resulting solution was filtered and the filtrate was analysed for the presence of alkaloids using Wagner's and Dragendorff's tests.

(ii) Determination of the presence of Saponin: Exactly 1 gm of ENCG was boiled with 10 mL of distilled water in a test tube. This was filtered while hot and 2.5 mL of the resulting filtrate diluted to 10 mL and shaken vigorously for two minutes (Sofowora, 1993)

(iii) Determination of the presence of Anthraquinones: An amount equal to 1 gm of ENCG was extracted with 10 mL ether, filtered while hot and 10 mL of 10 % Ammonia solution was added to the filtrate in the test tube (Sofowora, 1993).

(iv) Determination of the presence of Tannins: An amount equal to 1 gm of ENCG was boiled with 10 mL of distilled water for 5 minutes filtered while hot and cooled. A few drops of ferric chloride reagent were added to the filtrate (Sofowora, 1993).

(v) Keller-Killiani test for the presence of Glycosides: An amount equal to 2 gm of ENCG was dissolved in 5 mL of acetic acid containing a trace of ferric chloride and transferred to the surface of concentrated sulphuric acid (Evans, 1992)

(vi) Shinoda test for Flavonoids: An amount equal to 2 gm of ENCG was dissolved in 5mL of aqueous potassium hydroxide solution. 1.5mL of glacial acetic acid was added to the resulting solution (Evans, 1992)

Determination of Physicochemical Characteristics

(i) Determination of elemental constituents: The elemental constituents of ENCG were determined by an atomic absorption spectrophotometer Z-2000 (Hitachi HiTech, Tokyo, Japan).

(ii) Determination of the viscosity of gum: Aqueous dispersion containing 1 %w/v of ENCG was made and left for 1 hour to hydrate. The viscosities were then determined at -4 ^{o}C, 25 ^{o}C, 37 ^{o}C, 40 ^{o}C and 55 ^{o}C using a Brookfield viscometer model RVVDV-II+P (Brookfield Eng Labs Inc Middle Boro, MA, USA) at 100rpm using a spindle with code 02.

(iii) Determination of pH: A 2 %w/v aqueous solution of the air-dried ENCG was made. A glass-rod stirrer was used to gently stir the solution for about 5 minutes prior to determination of the pH using a 10 Accumet pH meter (Denver, CO). The process was carried out in quadruplicates. (Emeje *et al*, 2007; Sinko, 2011).

(iv) Determination of Drying Rate and Moisture content: The moisture content was determined using a NDMX-50 moisture analyser pi023602 (Japan). The drying rate determination was performed with 2 gm of the gum powder. The powder was placed in the oven (40 ^{o}C) and heated for 60 mins, the weight of the powder was determined at different time for 60mins and the moisture loss with the drying rate was determined.

(v) Fourier Transform Infrared (FTIR) Determinations: Spectra were obtained from ENCG using a Magna-IR, 560 spectrometer . About 5 mg of the completely dried powdered gum was weighed and then

dispersed in 200 mg KBr (pellet procedure). Signal averages were obtained at a resolution of $4cm^{-1}$.

Formulation of Emulsions

Primary emulsions were preformulated using the dry and wet gum methods of emulsion preparation to determine the method that will be used in preparing the different formulations. The ratio of castor oil: water: gum used in formulating 50 mL emulsions was 4:2:1. The mucilage of the gum was prepared by dispersing the calculated amount of the gum in the water to form an emulsion. Polysorbate 80 (a surface active agent) was incorporated in the emulsions at different volumes. Similar emulsions containing acacia were also prepared. Emulsions that contained no surfactant were also formulated to determine the effect of polysorbate 80 on the stability of the emulsions. The emulsions were subjected to uniform treatment during the entire study.

Preparation of Metronidazole Suspension

The suspensions were prepared using the method of Adedokun and Oyewo (2008) with slight modifications. A 5 gm quantity of metronidazole was triturated in a mortar with 0.5 gm of ENCG powder. Small portion of water was added to the powder mix with constant stirring until a smooth paste was obtained. The suspension was transferred to a 50 mL glass measuring cylinder and the volume made up to 50 mL with water, thus forming metronidazole suspension containing 1 %w/v ENCG as suspending agent. The procedure was repeated to formulate metronidazole suspensions containing 2, 3, 4 and 5 %w/v of ENCG. Similar metronidazole formulations containing acacia as suspending agents were prepared).

Evaluation of the Emulsions

The emulsions formulated were evaluated for their creaming rate and globule size distribution and viscosity. The rates of creaming of the emulsion were determined by placing 50 mL of each emulsion formulation in graduated cylinders and storing at room temperature. The height of the cream layer was measured weekly for 5 weeks. The creaming rates of the emulsions were calculated using:

$$\text{Percentage creaming} = H_c/H_o \times 100 \quad \text{…….. (3)}$$

Where, H_c is the height of the cream layer and H_o is the height of the original emulsion. Microscopy was used to determine the globule structure of the emulsion, using a magnification of × 50. Photomicrographs were taken and all the globules appearing in each micrograph were counted and sized with the aid of photomicrograph of calibrated stage slide. Mean globule size (X) was determined using the equation:

$$X = \sum fx/\sum f \quad \text{……..…… (4)}$$

Where, f is the frequency of each size.

The count was conducted at different interval over the period of 5 weeks after the preparation of the emulsions. The average rate of increase in globule size was taken as a measure of the globule coalescence rate and hence a measure of instability of the emulsions. The viscosity of the emulsion was determined using Brookfield Viscometer. The emulsions were poured in a beaker and the base of the spindle was immersed in the beaker containing the emulsion and the viscosity determined.

Evaluation of Suspensions

The suspensions were evaluated using sedimentation volume, flow rate, pH determinations and viscosity were used as the assessment criteria. Each suspension (50 mL) was stored in 50 mL cylinder for 14 days at 25 ^oC. Observations were made every hour for 7 hours and then every 24 hours for 14 days. The sedimentation volume, F (%) was then calculated using the following equation: F(%) =100Vu/Vo where Vu is the ultimate volume of sediment and Vo is the original volume of the suspension. The time required for each suspension sample to flow through a 10 mL pipette was determined and the rate of flow in mLs^{-1} was calculated using the equation:

$$\frac{\text{Volume of Pippette (mL)}}{\text{Flow Time (secs)}} \quad \text{……..(5)}$$

Ultrapure deionized water was used as the control.

The viscosity (poise) of the samples was determined at 0 ^oC, 25 ^oC and 40 ^oC using the Brookfield Viscometer, Dr-2x-Pro. At the end of the 28 day interval, a pH meter was used to measure the pH of the sample after the observation of the ease of dispersion has been made. All the determinations were carried out in quadruplicates and the results were recorded and analysed (Adedokun and Oyewo, 2008).

RESULTS AND DISCUSSION

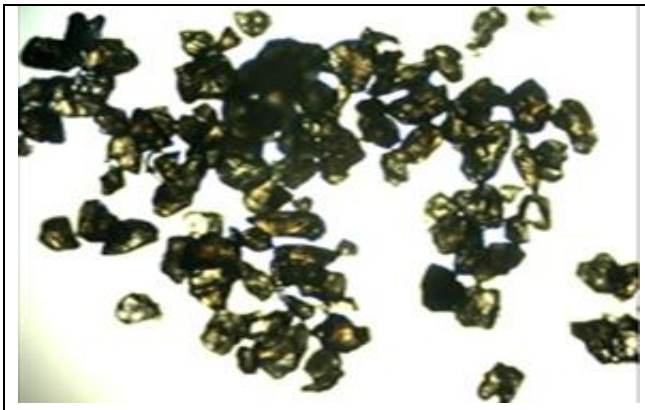
Characterization of *Enterolobium cyclocarpum* gum

The percentage yield of the dry gum obtained from the incised trunks of *Enterolobium cyclocarpum* tree was 47.24%w/w. Table 1 shows the phytochemical characteristics of the gum while the photomicrographs of *Enterolobium cyclocarpum* gum (ENCG) and acacia gum are shown in Fig. 1. The result showed that the gum contained a low concentration of heavy metals like chromium, lead, nickel, zinc, manganese and cadmium. It was observed that ENCG is devoid of alkaloids, saponins, tannins and terpenoids, but contains antraquinones, glycosides and flavonoids. The results of the physicochemical characterization of ENCG and acacia gum are shown in Table 2.. The gum under investigation (ENCG) was shown to have a moisture content of 1.80. The ENCG was observed to have a pH of 5.96, thus indicating that the gum is slightly acidic at room temperature. The degree of basicity or alkalinity of a formulation, at a particular temperature sheds light on how stable the formulation will be in terms of associated or dissociated ions. As the formulation becomes more alkaline, more undissociated base is formed (Sinko, 2011). The Functional group region of the FTIR spectra

of *Enterolobium cyclocarpum* (Fig. 2) indicated the presence of characteristic peaks at 3441 cm^{-1} (due to alkene C-H Vibrations), 2352.94 cm^{-1} and 2931.19 cm^{-1} (due to C-H stretching vibration in CH_3, CH_2) . In addition, the peak at 2123.24 cm^{-1} is assigned to C-H stretching in $–CH_3$. The fingerprint region showed peaks occurring at 1431 cm^{-1} and 1297.90 cm^{-1}, which are characteristic of the presence of unsaturated methyl groups in the compound. Moreover, the aforementioned peaks absorbed at 1431 cm^{-1} and 1297.90 cm^{-1} are characteristic of asymmetric CH_3 deformation due to $–C(CH_3)_3$ groups and cyclopropanes. Rocking vibration due to methylated benzene rings are responsible for the peak occurring at 1032.00 cm^{-1}. Peaks in the range of 545.45 cm^{-1} and 601.39 cm^{-1} have been attributed to skeletal vibration due to t-butyl groups and debranched alkenes.

Table 1: Phytochemical Analysis of *Enterolobium cyclocarpum* gum powder (+ positive, - negative)

Test	Observation
Alkaloids	-
Saponin	-
Anthraquinones	+
Tannins	-
Cardiac glycosides	+
Flavonoids	+
Terpenoids	-



A

B

Figure 1: Photomicrographs (x 50) of *Enterolobium cyclocarpum* gum (A) and Acacia gum (B)

Table 2: Physicochemical characterization

Parameters	***Enterolobium cyclocarpum* gum**	**Acacia gum**
Swelling index (%)	15.14	0
Particle density (g/cm^3)	1.39	1.12
Moisture content (%)	1.80	9.00
Total ash (%w/w)	7.67	2.68
Protein content (%)	2.69	2.56
pH (2.0%w/v)	5.96	5.73
Nitrogen content (%)	0.43	0.41

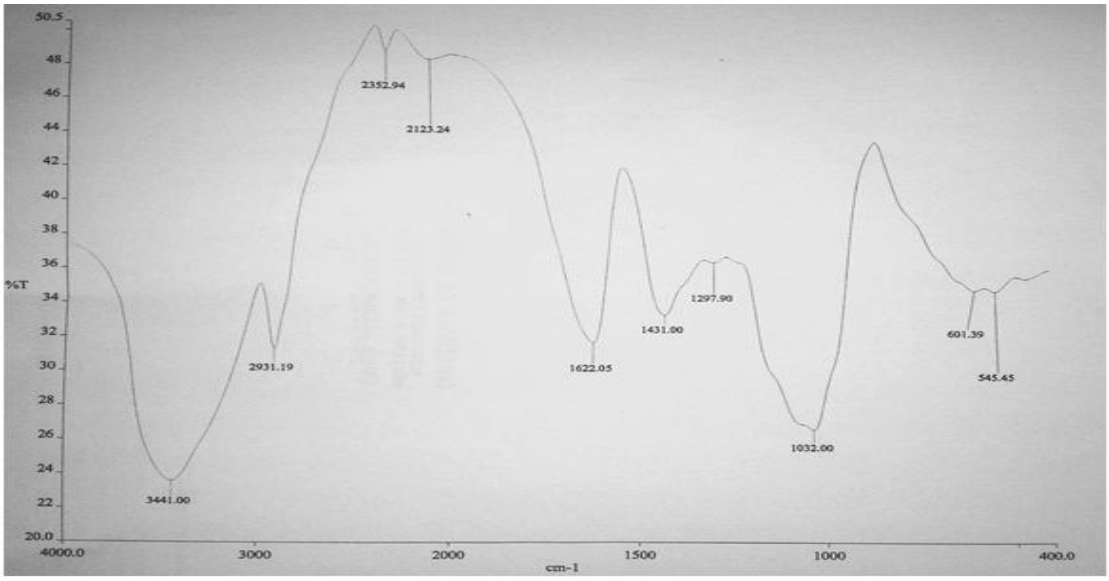


Figure 2: FTIR Plot of *Enterolobium cyclocarpum* gum

Emulsifying Properties of *Enterolobium cyclocarpum* gum

The wet gum method, which produced better emulsions in the preformulation experiments, was used in formulating the emulsions. The representative plot for the percentage rate of creaming against time for emulsions containing ENCG is shown in Fig. 3. When creaming occurs, it produces an inelegant appearance of the emulsion which is usually unacceptable to the patient. Several techniques such as addition of a thickening agent or reduction of the interfacial tension with a surfactant are commonly used to reduce creaming in emulsions. Thus, polysorbate 80, a non-ionic surfactant with a specific gravity of 1.07, was incorporated in the emulsions. From the results, it was observed that all the emulsions containing ENCG exhibited high rate of creaming creaming at all concentrations with the rate of creaming having an inverse relationship with the concentration of the gum. The emulsions containing acacia did not show any significant creaming as the emulsions formed readily on shaking the container. This was not observed for the emulsions containing ENCG which were observed to have a high volume of sediments, thus making redispersion difficult. Thus, it implies that the presence of the surfactant reduced the rate of creaming up to a certain point (known as the critical micelle concentration) observed to correlate to a predetermined concentration of 8.95 %v/v, after which further addition did not add to the aesthetic value of the formulation, but rather caused aggregation of the molecules into micelles.

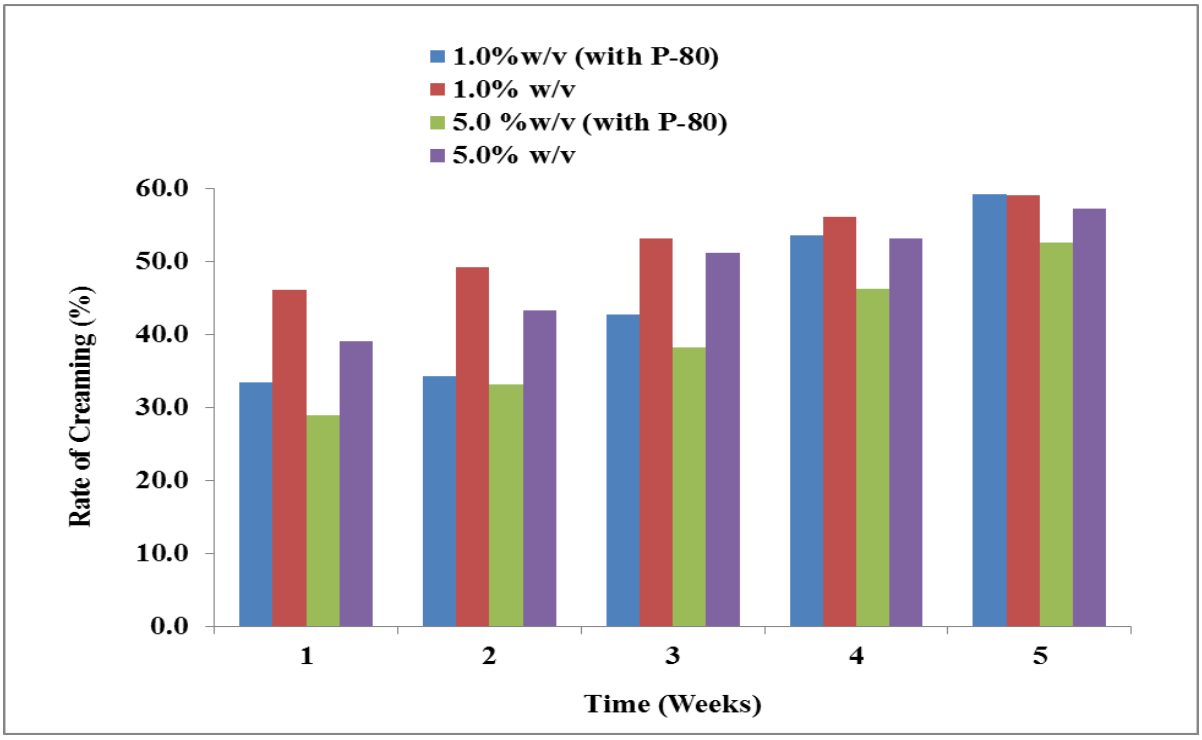


Figure 3: Plot of Rate of Creaming (%) against Time (weeks) for emulsions containing 1.0 %w/w and 5.0 % w/w *Enterolobium cyclocarpum* gum with and without surfactants

The results of the changes in the globule size (Table 4) can be attributed to the thermodynamic instability of the emulsions. The results show that there was a progressive increase in the globule size diameter as the concentration of the gum increased. However, emulsions containing ENCG had bigger sized globules than emulsions containing acacia. While reduction of globule size does not necessarily lead to increased stability, a coarse dispersion of uniform sized globules should have a better dispersion (Sinko, 2011). The globule sizes of emulsions containing acacia were relatively uniform within the concentration and thus had better stability. The viscosities of the emulsions, which are represented with the plot in Fig.4 indicates that emulsions containing ENCG were more viscous than those containing acacia at all concentrations. The emulsion containing 5.0 % w/v ENCG were too viscous to be determined. However, though viscous emulsions are expected to be more stable than mobile ones by virtue of retardation of flocculation (Asantewaa *et al*, 2011), the overall results show that the emulsions prepared with acacia gum were more stable when compared with emulsions containing ENCG. This is probably due to the effect of the uniformity of globules of acacia-containing emulsions.

Table 4: Values of globule size measurements for the emulsions

Suspending agent	Concentration (%w/v)	Globule diameter (µm)				
		Time (Days)				
		1	7	14	28	35
Enterolobium cyclocarpum	1.0	2.207	2.259	2.326	2.747	3.146
	2.0	2.262	2.267	2.268	2.268	2.268
	3.0	2.276	2.348	2.349	3.739	3.435
	4.0	2.387	2.399	3.247	3.276	3.378
	5.0	2.285	2.293	3.214	3.296	3.447
Acacia	1.0	1.193	1.193	1.193	1.183	1.181
	2.0	1.292	1.294	1.297	1.298	1.298
	3.0	1.195	1.195	1.196	1.197	1.197
	4.0	1.296	1.286	1.275	1.266	0.871
	5.0	1.375	1.374	1.367	1.365	0.893

Suspending Potentials of *Enterolobium cyclocarpum* gum

The suspending potentials of *Enterolobium cyclocarpum* gum (ENCG) was assessed in comparison with official acacia gum in metronidazole suspensions at concentrations of 1.0 – 5.0 %w/v. Sedimentation volume, pH determinations, viscosity and flow rate were the assessment parameters. Table 5 shows the percentage sedimentation volume of the suspensions on day 1 and days 2 to 14 respectively. Generally, there was a decrease in the sedimentation volume with time. The settling rate was more pronounced at a lower concentration of the gum. One aspect of physical stability in pharmaceutical suspensions is concerned with keeping the particles uniformly distributed throughout the dispersion, and although it is seldom possible to prevent settling completely over a prolonged period of time, it is necessary to consider factors that influence the velocity of sedimentation (Adetunji and Odole, 2015). The results indicated that formulations containing 5% w/v ENCG kept the particles well suspended for 14 days when compared with suspensions containing ENCG at lower concentrations (1, 2 and 3 %w/v). The results also show that acacia had lower suspending properties than ENCG at the same concentration.

Table 5: Values of sedimentation volume (%) of metronidazole suspensions containing different concentrations of suspending agents

Suspending Agent (Gum)	Concentration (%w/v)	Sedimentation Volume (%v/v) Day 1 Time (Hrs)							
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Enterolobium cyclocarpum	0	100	20	20	20	20	20	20	20
	1	100	44	44	43	43	43	43	43
	2	100	67	66	66	66	66	66	66
	3	100	86	86	86	86	86	84	84
	4	100	94	94	94	94	92	92	92
	5	100	100	100	100	100	100	100	100
Acacia	1	100	12	11	11	11	11	11	11
	2	100	14	14	14	13	13	13	13
	3	100	14	13	13	12	12	12	12
	4	100	12	12	12	12	12	12	12
	5	100	14	13	13	13	12	12	12

		Sedimentation Volume (%v/v) Day 2 -14 Time (Days)							
Enterolobium cyclocarpum	0	**2**	**3**	**4**	**6**	**8**	**10**	**12**	**14**
	1	42	42	42	41	41	41	41	41
	2	65	65	64	64	64	64	64	64
	3	84	84	84	84	84	84	84	84
	4	91	91	91	90	90	90	90	90
	5	100	100	100	100	100	100	100	100
Acacia	1	11	11	11	11	11	11	11	11
	2	12	12	11	11	11	11	11	11
	3	11	11	11	10	10	10	10	10
	4	12	12	12	12	12	12	12	12
	5	12	12	12	12	12	12	12	12

The pH determination was carried out for metronidazole suspensions containing different concentrations of the gums ranging from 1 – 5 %w/v at a temperature of 25 ^{0}C. The result (Table 6) shows that all the suspensions are weakly acidic with no significant changes in pH of the suspensions at all the concentrations, however, acacia was slightly more acidic than ENCG. According to Stoke's law, viscosity is directly proportional to the concentration of the dispersed phase. Thus, an increase in the concentration of the dispersed phase will lead to an increase in the viscosity of the medium and vice versa. The results obtained indicated an inverse relationship between temperature and viscosity (Fig. 4) with the viscosity of ENCG, which is non-newtonian in behavior (Rincon, 2011), higher than the viscosity of acacia at all temperatures.

Table 6: Values of pH for suspensions containing different concentrations of the gum

Gum Concentration (%w/v)	pH values at 25^{0}C	
	Enterolobium cyclocarpum	Acacia
1	5.97±0.04	5.77±0.11
2	5.94±0.03	5.79±0.04
3	5.94±0.05	5.73±0.03
4	5.97±0.01	5.74±0.01
5	5.95±0.03	5.76±0.12

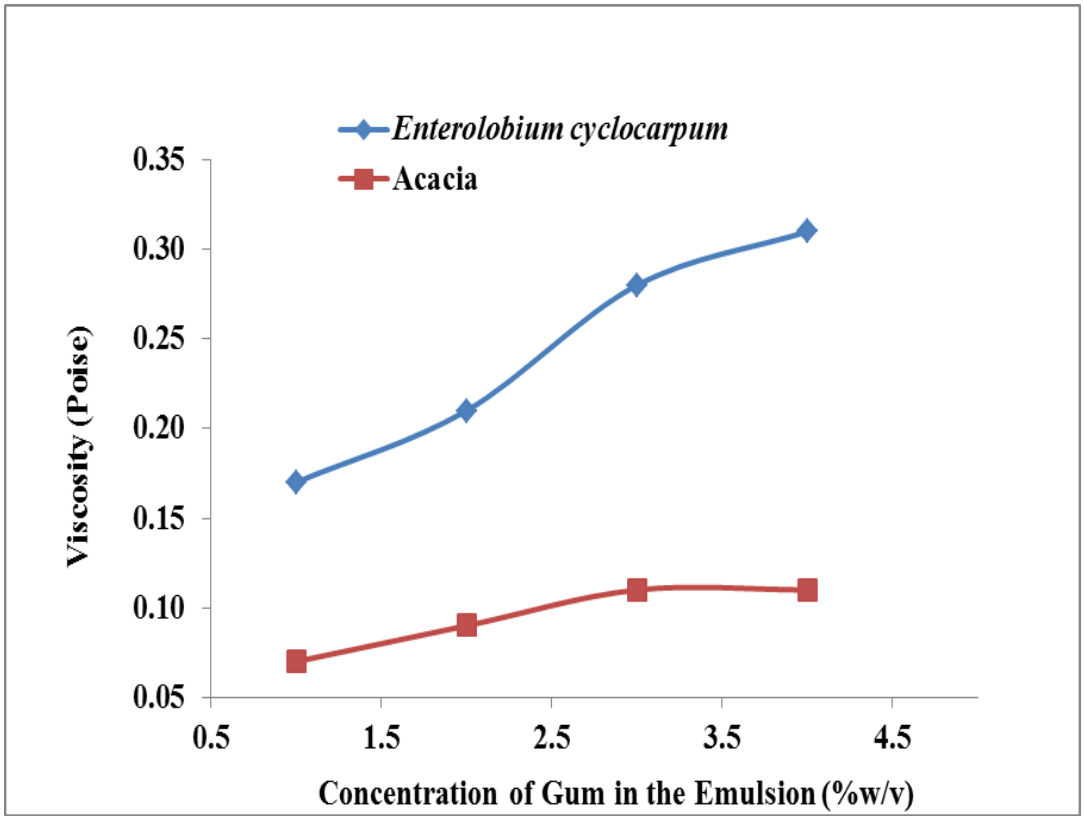


Figure 4: Plot of Viscosity (Poise) against concentration of gums used in formulating emulsions

Ideally, an increase in viscosity is expected to hinder the rate of flow (Sinko, 2011), as was observed in the flow rate of ENCG (Table 8) which was lower than acacia at the same concentration.

Table 8: Flow Rate Measurement of the Gum Samples

Gum	Concentration (%w/v)	Flow rate (mL/sec)
Enterolobium cyclocarpum	1.0	0.27
	2.0	0.26
	3.0	0.24
	4.0	0.22
	5.0	Too viscous
Acacia	1.0	0.53
	2.0	0.51
	3.0	0.45
	4.0	0.44
	5.0	0.42

CONCLUSION

The results suggest that *Enterolobium cyclocarpum* gum is a highly viscous gum with suspending properties comparable with Acacia gum BP, but with poor emulsifying properties. The presence of polysorbate 80 reduced the rate of creaming.

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Conflict of Interest: None declared

Received: 29 May, 2016

Accepted: 29 July, 2016