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Characterization and Evaluation of the Suspending Property of *Cordia obliqua* gum in Paracetamol Suspension

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Abstract

The The study aims at extracting and characterizing the gum from Cordia obliqua fruit for possible use as a pharmaceutical suspending agent. The fruits of Cordia obliqua were collected, washed thoroughly and prepared for extraction of the gum. It was precipitated with acetone and allowed to dry. The dried gum was then size reduced to a powder which was then characterized and used to formulate a suspension of paracetamol. Scanning electron microscopy revealed that Cordia obliqua gum has irregularly shaped particles mostly below 100 µm in size, the physicochemical properties properties of the extracted gum were within Pharmacopoeial specifications for good flow. The bulk, tapped and true densities of Cordia obliqua gum were 0.63, 0.75 and 1.94 respectively. The angle of repose, carr's index and hausner's ratio were 35.3 °, 16.7 %, 1.10 respectively, indicative of good flow property. Powder porosity was 19.07 %, moisture content was 11.5 %, swelling capacity and hydration capacity were found to be 87.5, and 8.8 respectively. The ash value was 2 % while the pH was 9.6. The Cordia obliqua, acacia and tragacanth gums were used as suspending agents in paracetamol suspension at concentrations of 0.1, 0.2, 0.4, 0.8 and 1.6 %w/v and were evaluated based on their viscosity, flow rate, sedimentation volume and pH. Cordia obliqua gum was found to have suspending properties that are comparable to acacia and tragacanth gums and can be used as alternative suspending agent

Introduction

Cordia obliqua, also called as clammy cherry is a flowering plant species in the genus Cordia belonging to the family Boraginaceae, it contains about 250 species of trees and shrubs that are native to the Americas but also found in Asia and Africa (Aimey *et al*., 2020). *Cordia obliqua* is found scattered throughout the mid-Himalyas up to elavations of 1,470 m (Gupta and Gupta, 2015).It is a medium sized deciduous that shows vigorous growth and is distributed in tropical, sub-tropical and warmer regions around the world (Tharun *et al*., 2020). The trees grow wild, rarely cultivated and are found growing singly. The stem bark is grayish brown, smooth or longitudinally wrinkled. Flowers are white, fragrant in large lax terminal and axillary peduncle cymes which open only at night (Ahuja *et al*., 2020). The fruit is drupe, and the seeds are mild sweet in taste (Sivakrishnan and Veeramani, 2019). Epicarp is thick while mesocarp is mucilaginous, the endocarp is hard and the seeds are mildly sweet in taste (Sivakrishnan, 2017). The gum extracted from *Cordia obliqua* has been reported to be an excellent emulsifier and tablet binder (Omar *et al*., 2017).

Medicinal uses

In folk medicine *Cordia obliqua* is used in treatment of inflammation, bronchitis, fever, wound healing, chest infection, lung disease, cholera, cold, colic, constipation, cough, headache, ulcers, urine disease and urticaria (Ahuja *et al*., 2020; Reenu *et al*., 2015). The plant is also used for cooling effects, anthelmintic, expectorant, and as a diuretic. It lessens thirst and scalding of urine, removes pains in the joints, and it is used as treatment of diseases of spleen and leprosy (Sivagnaman and Devarasu, 2019).

Suspensions are biphasic heterogeneous coarse dispersions containing essentially the insoluble particulate matter or drug suspended with the help of suspending agent(s) in a liquid medium (Murthy *et al*., 2015). The particle diameter in a suspension is usually greater than 0.5 µm (Kumar and Yagnesh, 2016). A number of patients, especially pediatric and geriatric patients, have difficulty in swallowing solid dosage forms hence formulation of a suspension will be most suitable for such patients (Jangde *et al*., 2011). Formulation of drugs as suspensions for oral administration is also useful to mask taste of unpleasant drugs and to control the rate of drug absorption (Nep and Cornwat, 2011).

Materials and Method

Machines and Equipment

Centrifuge (80-2, China), Electronic thermostatic drying box (G2X-DH.400-II, China), Metlar electronic balance (MT-2000, china), FTIR spectrophotometer (Shimadzu, Japan), Oakton pH meter (PH1100 series, Singapore), Viscometer (NDJ, China), Furnace, crucibles, measuring cylinders, beakers, volumetric flasks, Kjedahl flask, soxhlet extractor, Dessicator.

Reagents and Chemical

Acetone (BDH, England), xylene (May and Baker, England), potassium bromide (Merck, Germany), hydrochloric acid (Merck, Germany), boric acid (BDH, England), acacia (CDH, India), tragacanth (CDH, India), sulphuric acid (Merck, Germany), sodium hydroxide (Merck, Germany), petroleum ether (Merk Germany), De-ionized water.

Collection, Extraction and Preparation

The Ripened *Cordia obliqua* fruits were collected from Maiduguri metropolis Borno state, Nigeria. The method of Doharey *et al*. (2010) was adopted with modification for the extraction of the gum. A 500 g quantity of fruits was washed thoroughly with de-ionized water several times, the peel was removed, and the pulp along with the seed was refluxed with 300 mL water for 10 min at 35 °C. The aqueous extract was cooled, filtered with whatmann 40 filter paper. The filterate was treated with 10 L of acetone in a glass container then the gum was precipitated after 2 min, the precipitated gum was collected and placed on a tray and was allowed for shade dry for a week, the dried extract was then size reduced using a pestle and mortar.

Proximate analysis

Determination of moisture content (Loss on drying)

Proximate analysis

Determination of moisture content (Loss on drying)

A 1g quantity of the gum was weighed accurately into a pre-weighed petri-dish and dried in a hot air oven at 105°C. The weight of the gum was checked at intervals of 10

min, until a constant weight was obtained (Alalor and Obunezie, 2020).

Ash value

A 2 g weight of the sample was poured into a nickel crucible which was initially heated at 105 °C to a constant weight and allowed to cool. The crucible with its content was then gently heated until it was moisture free and completely charred. Subsequently, the heat was increased gradually until most of the carbon vaporised. The sample was finally heated strongly at 600 °C until the residue is free from carbon (i.e. almost white). The crucible with its content was allowed to cool and weighed. The heating and cooling step was then repeated until the residue (ash) was constant (Achor *et al.*, 2014).

The weight of the ash was then determined and the percentage ash value calculated using the equation; $Ash\ value = \frac{WA}{WSP} X100$

Where WA and WSP are weight of ash formed and initial weight of the powder respectively.

Crude lipid content determination

Two grams of the pulverized sample was used for determining the crude lipid by extracting the lipid from it for 5 h with (at 60 to 80 °C) petroleum ether in a soxhlet extractor (AOAC, 2006). Triplicate samples were extracted to obtain triplicate values that were later averaged

Protein determination

Total protein was determined by the Kjedahl method. 0.5 g of the sample was weighed in triplicate into a filter paper and put into a Kjedahl flask, 8 to 10 mL of concentrated H_2SO_4 were added and then digested in a fume cupboard until the solution became colourless. Distillation was carried out with about 10 ml of 40 % sodium hydroxide (NaOH) solution. The condenser tip was dipped into a conical flask containing 5 ml of 4 % boric acid in a mixed indicator till the boric acid solution turned green. Titration was done in the receiver flask with 0.01 M hydrochloric acid (HCl) solution until the solution turned red (AOAC, 2006).

Determination of crude fibre

A 2 g weight of the pulverised leaves was used in triplicates for estimating the crude fibre by acid and alkaline digestion methods using 20 % H_2SO_4 and 20 % NaOH solutions (AOAC, 2006).

Carbohydrate determination

The carbohydrate content was calculated using the following formula: Available carbohydrate (%), = 100 – [protein (%) + Moisture (%) + Ash (%) + Fibre (%) + Crude Fat (%)] (Mathew *et al.*, 2014).

Scanning Electron Microscopy (SEM)

The *Cordia obliqua* gum was subjected to SEM analysis at magnification of X320 and X500 to obtain high resolution images of the powdered gum.

FT-IR Spectroscopy

The sample was blended with solid KBr (50 mg) and compressed into discs. The spectra were scanned from 400 - 4000 cm^{-1} under dry air at normal temperature with FTIR spectrometer (Azubuike *et al.*, 2017).

Solubility determination

A 2 g quantity each of the mucilage powders were poured into a test tube containing 5mL of acetone, 5 mL of distilled water and 5mL of 0.1N Hydrochloric acid separately and the solubility of each sample determined and recorded (B.P, 2009).

pH

The pH of 2 g of each sample in 100 mL of distilled water was determined using a pH meter and the result recorded. This was done in triplicate for each of the two samples and the mean and standard deviation calculated (B.P, 2009).

Bulkandtappeddensity

A 10 g quantity of the powder samples was placed in a 50 mL measuring cylinder and the volume V_0 occupied by the sample without tapping noted. After 50 manual taps, volume occupied V_{50} was determined. The bulk and tapped densities were calculated as the ratio of the weight to volume (V_0 and V_{50} respectively). This was also done in triplicate for the two samples and the mean and standard deviation calculated (Bakre and Ajala 2012).

Angle of repose

The angle of repose of each of the sample was determined using a glass funnel clamped on a retort stand which was 10 cm away from the flat surface of the bench. 50 g of the sample powder was placed into the funnel and allowed to flow freely forming a conical heap. This was done in triplicate for each the samples and the mean and standard deviation were calculated after calculating the angle of repose from the heap of each of the samples (Achor *et al.*, 2014).

Angle of repose, $tan\Theta = h/r$

Where h=height and r=radius of the circular heap

Carr'sIndex

TheCarr'sindexwascalculatedusingtheEquation

$C.I = [(TD - BD)/TD]$x100

WhereC.IisCarr'sindex,TD=tapped DensityandBD=bulkdensity(BakreandAjala, 2012).

Hausner'sRatio

Hausner's ratio was calculated using the following formula presented in Equation:

$$Hausner's\ ratio = TD/BD$$

Where TD = tapped density and BD = bulk density

True density

True density (TrD) of the two samples were determined by the liquid displacement method using Xylene as the immersion fluid as described by (Ohwoavworhua *et al.*, 2004) and computed using the Equation:

$$TrD = \frac{W}{(\{a + w) - b\}}\ x\ SG$$

Where W is the weight of the powder, SG is specific gravity of liquid, a is the weight of bottle + liquid and b is weight of bottle + solvent + powder

Powder porosity

This was calculated using the outcomes of both bulk and tapped densities similar to (Ohwoauvorhua *et al.,* 2004) using the Equation:

$$e = 1 - TrD/TDe$$

Where TD is the tapped density, TrD, is the true density and e is the porosity

Hydration capacity

A 1 g weight of each sample was weighed and poured into centrifuge tubes, 10 mL distilled water was added and stirred for 2 min. The mixture was centrifuged for 10 min at a velocity of 1000 rpm. The supernatant obtained was emptied and the deposit weighed (Muazu and Suleiman, 2014).

Swelling capacity

This was determined using the method of Ologunagba *et al*. (2017) and computed according to the Equation $S = V2 - V1/\mathrm{V1}$

Where S is the swelling capacity, V2 is the volume of the hydration or swollen material and V1 is the tapped volume of the material prior to hydration.

Moisture sorption capacity

The powdered sample (2 g) was kept in a desiccator containing distilled water (relative humidity 100 %) for 5 days which was reweighed daily. The moisture sorption capacity was calculated as the ratio of change in weight to the initial weight (Bakre and Ajala, 2012).

Formulation of Paracetamol Suspension
Suspensions of paracetamol were prepared according to the method of Ponnada, (2017), paracetamol powder was weighed and transferred into a dry mortar and triturated until a fine powder was obtained, and the suspending agent was added and then triturated again. Distilled water was added to the above mixture, glycerol was added and mixed to form uniform suspension. Finally, to this suspension, benzoic acid dissolved in few mL of distilled water was added. This was mixed thoroughly and transferred into a calibrated bottle. The volume was made up to 100 mL with distilled water.

Determination of pH of the Suspension

The pH of the suspension was determined using digital pH meter. This was done in triplicate for all the samples and the mean and standard deviation were recorded (Ponnada, 2017).

$$Flow\ rate\ =\ volume\ of\ pipette\ (ml)\ /flow\ time(s)$$

Re-dispersibility
Re-dispersibility was determined similar to the method used by Mohanan and Krishna (2018). A volume of 100 mL of each suspension were kept at room temperature for 7 days. Re-dispersion was recorded as the number of inversions required to re-disperse the sediment formed at the end of 7 days of storage of the formulations.

Viscosity

The viscosity (M.pa/sec) of the sample was determined using NDJ Electric Viscometer at 25 ^{o}C 6rpm using spindle size 3 similar to methods reported by Alalor and Obunezie (2020). All determinations were made in triplicate and the results obtained are expressed as the mean values.

Determination of sedimentation volume

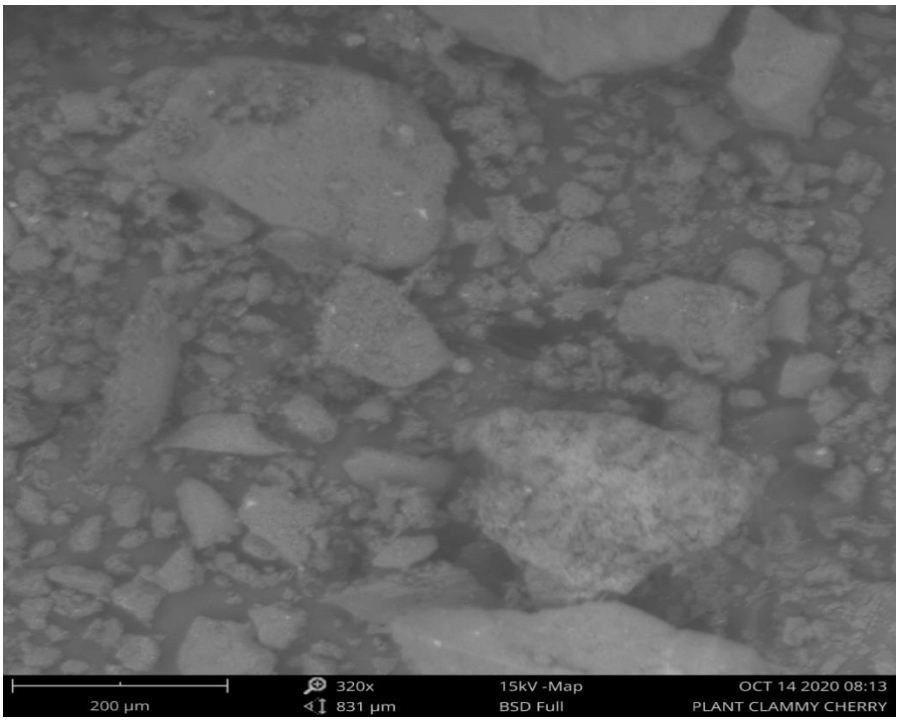
The suspension sample was transferred into a measuring cylinder and was kept aside without distorting, the height of the sediment was observed at regular time intervals of 0, 5, 10, 15, 20, 25, 30, 45, and 60 min. Thesedimentationvolumewasthencalculatedusingequation below

$$F = \frac{Hu}{Ho} \times 100$$

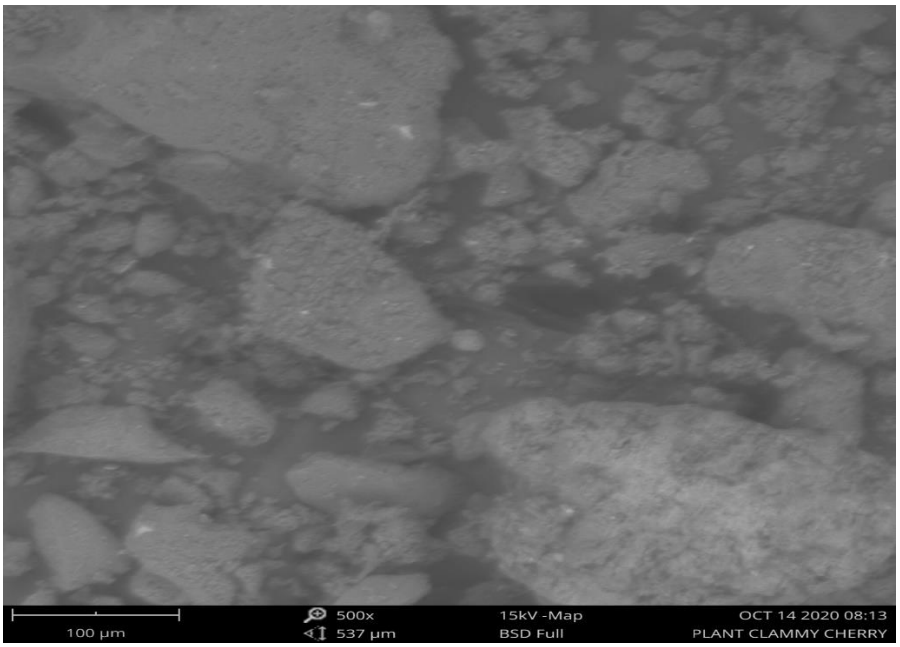
WhereHuandHoarefinalheightofsedimentandoriginalheightofsuspensionrespectively.
(Alalor and Obunezie, 2020).

Results
Table 1: Proximate analysis of *Cordia obliqua gum*

Parameters	Amount (%)
Carbohydrate	59.62
Protein	14.88
Crude fat	2.00
Fibre	10.0
Ash content	2.00
Moisture content	11.50



PlateI: Photomicrograph of *Cordiaobliquagum* (X320magnification)



PlateII: Photomicrograph of *Cordiaobliqua*gum (X500magnification)

Table 2: Solubility of *Cordia obliqua*, acacia and tragacanth gums

Parameters	CO	TG	AC
Solubility in cold water	Soluble	Insoluble	Soluble
Solubility in cold acetone	Insoluble	Insoluble	Insoluble
Solubility in cold Hcl	Soluble	Soluble	Soluble

Key: CO = *Cordia obliqua, AC = Acacia, TG = Tragacanth*

Table 3: Physicochemical Properties of *Cordia obliqua*, acacia and tragacanth gums

Key:CO = *Cordia obliqua, AC=acacia, TG=tragacanth*

Pa			
M			
Ar			7
Tr			
Sw			
Bu			
Ta			9
Ha			
Po			
Hy			
pH			

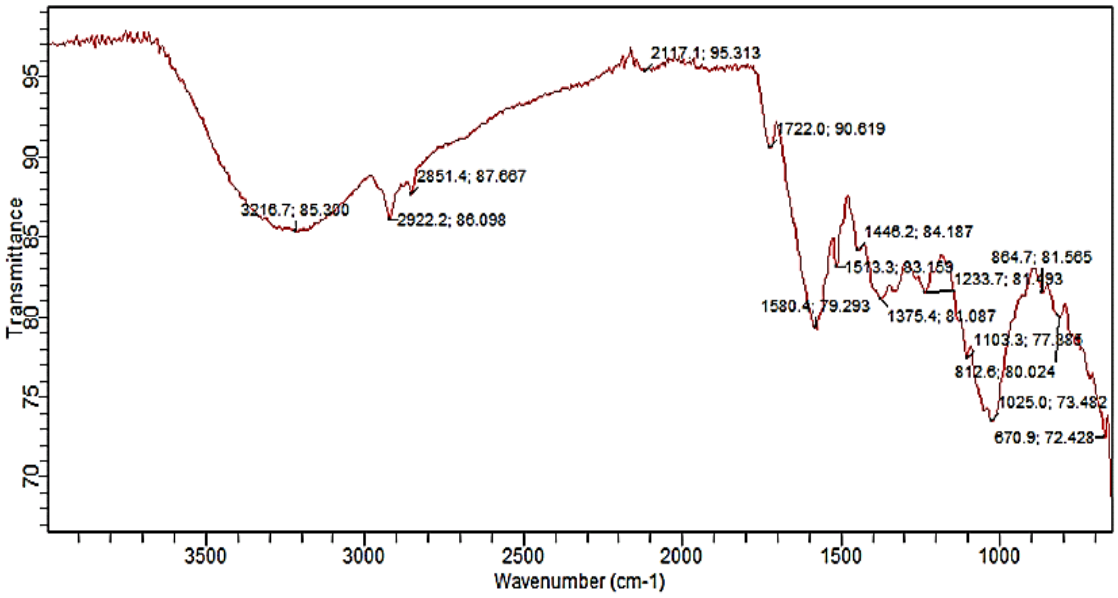


Figure 1: FT-IR Spectra of *Cordia obliqua* gum

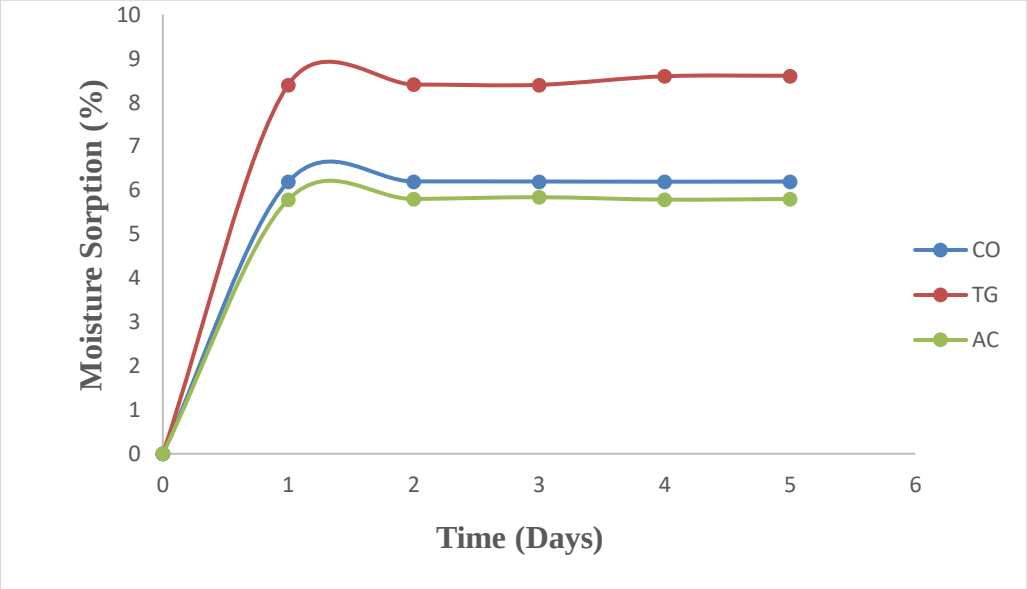


Figure2: Moisture sorptiongraph of *Cordiaobliqua*, acacia and tragacanthgums

Table4: Suspending properties of *Cordiaobliqua*, acacia and tragacanth suspensions

	Suspensions	pH	Flowrate(mL/sec)	Re-dispersibility	Viscosity(M.pa/sec)
CO	**0.1g**	9.5±0.00	0.95±0.01	15	**2595.30±3.47**
	0.2g	9.4±0.00	0.95±0.01	12	**2741.27±13.82**
	0.4g	9.2±0.00	0.94±0.01	10	**2958.20±7.38**
	0.8g	9.0±0.00	0.92±0.02	10	**3070.40±5.32**
	1.6g	9.0±0.00	0.92±0.04	10	**3080.50±6.42**
AC	**0.1g**	8.2±0.00	0.95±0.01	10	**1482±4.28**
	0.2g	8.4±0.00	0.94±0.03	9	**2783±11.68**
	0.4g	8.4±0.00	0.93±0.02	10	**3779±11.47**
	0.8g	8.6±0.00	0.91±0.05	8	**4150.87±20.83**
	1.6g	8.8±0.00	0.91±0.06	9	**4280.20±23.10**

TG	**0.1g**	6.8±0.00	0.95±0.20	9	**2086.53±4.51**
	0.2g	6.5±0.00	0.94±0.01	8	**2142.30±17.11**
	0.4g	6.3±0.00	0.92±0.03	8	**4329.80±23.41**
	0.8g	6.2±0.04	0.89±0.01	8	**4543.03±15.93**
	1.6g	6.5±0.00	0.89±0.02	8	**4543.20±17.10**

Key:CO=*Cordiaobliqua,*AC=acacia,TG=tragacanth

Table5: Sedimentation volume for *Cordiaobliqua*, acacia and tragacanth suspensions

TIME(MINS)		0	5	10	15	20	25	30	45	60
CO	**0.1g**	1	6.25	6.25	6.25	6.25	6.25	6.25	6.25	**6.25**
	0.2g	1	9.33	10.67	10.67	10.67	10.67	10.67	10.67	**10.67**
	0.4g	1	12.50	12.50	12.50	12.50	12.50	12.50	13.75	**13.75**
	0.8g	1	16.25	17.50	17.50	17.50	17.50	17.50	18.75	**18.75**
AC	**0.1g**	1	6.67	6.67	6.67	6.67	6.67	6.67	9.33	**9.33**
	0.2g	1	12.00	12.00	12.00	13.33	13.33	13.33	14.67	**14.67**
	0.4g	1	16.25	16.25	16.25	16.25	16.25	16.25	17.50	**17.50**
	0.8g	1	18.75	18.75	18.75	18.75	18.75	18.75	20.00	**20.00**
TG	**0.1g**	1	6.25	6.25	7.50	7.50	7.50	7.50	8.75	**8.75**
	0.2g	1	11.25	11.25	11.25	11.25	11.25	11.25	12.50	**12.50**
	0.4g	1	15.00	15.00	15.00	15.00	15.00	15.00	16.25	**16.25**
	0.8g	1	20.00	20.00	20.00	20.00	20.00	20.00	21.33	**21.33**

Key: CO=*Cordiaobliqua*,AC=acacia,TG=tragacanth

Discussion

The results of proximate analysis revealed the presence of carbohydrates, proteins, and fats in *Cordia oblique* (CO) gum, similar values of carbohydrate, fats and moisture content was reported for the gum from *Cordia myxa* by Mahmood *et al*.(2015). Similarly, Abdelgawad *et al*. (2021) reported that the fruits are rich in essential minerals, fats ash, carbohydrates and proteins.Thegum showed a moisture content of 11.5% which is as expected with natural gums and within limits for microbial stability,moisture content tends to affect powder mechanical strength and flow properties (Azubuike *etal*.,2011).The photomicrograph of *Cordia oblique* gum shows that most of the particles are less than100 µm in size and of irregular shapes and dimensions, irregular shapes of the particles may contribute to resistance to flow and lead to an increase in low shear viscosity (Ologunagba *et al*., 2017).

Fourier transform infra-red spectrometry is a physico-chemical analytical technique used to identify the functional groups of the components in a plant or other related materials based on the peak values in the region of infra-red radiation (Oladunmoye *et al*., 2018). The FT-IR spectra of CO gum revealed a broad peak at 3916 $cm^{-1}$of the functional group region, this suggests the presence of OH stretch or NH present, the peaks at 2922 and 2851 cm^{-1} indicates assymetric C-H stretch. Another peak was observed at 1722 cm^{-1} which suggests C=O possibly from an ester, aldehyde or ketone and the peak at 1580 cm^{-1} suggests C=C bonds, similar findings were reported by Dinda and Mukharjee, (2009) with the mucilage from *Cordia obliqua* with peaks at 3000, 1750 and 1500 cm^{-1} of the functional group region.

The solubility properties refer to the ability of a given substance, the solute, to dissolve in a solvent, acacia (AC) and *Cordia obliqua* (CO) gums are soluble in water and HCl and insoluble in acetone, while tragacanth (TG) is insoluble in water and acetone but soluble in HCl.

The bulk density of a powder is a characteristics of powder rather than individual particle while tap density is a measure of how well a powder can be packed in a confined space on repeated tapping (Bakre and Ajala, 2012; Nwandiogbi *et al.*, 2015). The bulk and tapped density of the acacia and CO gums were both 0.63 and 0.69 g/mL respectively while tragacanth had a bulk density of 0.55 g/mL and tapped densities were 0.62 g/mL.

According to Achor *et al. (*2014), the angle of repose, Carr's index and Hausner's ratio are considered indirect measurement of powder flowability which in turn, determines the suitability of a material as direct compression excipients. The angle of repose is the highest internal angle to the horizontal to which a powder can be heaped or piled without the material clumping or sliding. The angle of repose is high for a cohesive powder and low for a non-cohesive powder (Aulton, 2013). The values were observed to be 35.3 º for CO, 36.0 º for AC and 34.8 º for TG, which is indicative of a good flow for CO and TG and fair flow for AC. The Hausner's ratio is indicative of inter particulate friction between particles while the Carr's index indicates the tendency of a material to diminish in volume (Achor *et al.,* 2014). The Carr's index

further explains the flow of the samples as they all have a fair flow. The values of Hausner's ratio indicates a good flow property for all the powders (Aulton, 2013).

The true density according to Achor *et al.* (2014) is the density of the solid material excluding the volume of any open and closed pores and it also indicate how close a material is to a crystalline state. The true density for CO, AC and TG were 1.94, 1.94 and 2.32. Powder porosity determines the swelling capacity of a powder, the higher the porosity, the more inter particulate spaces for water to be absorbed (Achor *et al.,* 2014), from the results obtained the porosity of CO and AC was found to be 19.07 % having a slightly higher porosity value compared to TG this implies that they will likely have a higher swelling capacity than TG.Hydration capacity is the ability of a powder to be absorb and retain water, values of 8.8, 5.4 and 5.3 were observed for CO, AC and TG respectively, this shows that CO would absorb and retain more water compared to acacia and tragacanth, the value obtained is much higher than that of acacia and tragacanth it is relatively similar to the results reported by Achor *et al*. (2014) for microcrystalline cellulose obtained from the bark of *Lageriana siceraria*. Swelling capacity of CO is 87.5 %, AC 34.8 %, while tragacanth is 55.6 % this signifies that CO had a higher swelling capacity as compared to acacia and tragacanth, this suggest that it will potentially be a good disintegrant for

tablets. Moisture sorption is a measure of the moisture sensitivity of the material which will give an insight in to its susceptibility to microbial and fungal growth and other moisture induced distortion of its physicochemical (Bakre and Ajala, 2012). CO showed a higher value compared to AC and TG.

The evaluation of the CO, AC and TG suspensions reveal that the pH of all the three batches of the formulation were found to be within the range of 9.5 to 8.5 and 6.4. The flow rate of all the three batches were found to be decreasing as the concentration of the suspending agent increased and were within the range of 0.37 to 0.94 mL/sec, these are slightly higher compared to the findings of Azubuike *et al.* (2017) for *Corchorus olitorius* mucilage as a suspending agent for paracetamol with the range of 0.07 to 0.81 mL/sec. All suspensions were found to be easily re-dispersible after a maximum of 10 shakings after 5 days, this finding is the similar to the finding of Aremu and Oduyela (2015) for metronidazole suspension who obtained a maximum of 10 shakings for the sediments to re-disperse. The viscosity was found to be directly proportional to the concentration of the suspending agent for all the formulations and were found to be within the range of 1482 to 4543 m.Pas×sec, with 0.8 and 1.6 g from each batch being more viscous than the others within the batch. The sedimentation volumes of CO, AC and TG suspensions increased gradually with time and with increase in the concentration of the suspending agent from 5 to 60 min, these were found to be within the range of 6.25 to 21.33 %.

From the results obtained, it can be concluded *Cordia obliqua* gum has properties that are largely similar to acacia gum and tragacanth gum (to a relatively lesser extent). It can be used as an alternative suspending agent in pharmaceutical suspensions.

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