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A Cost-Effective Extraction Method for Improved Physicochemical, Rheological and Microbiological Properties of *Grewia mollis* gum

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ABSTRACT

Grewia gum is a versatile natural polymeric excipient and its preparation from *Grewia mollis* stem barks using chemicals and heat treatment may destroy useful phytochemical components and influence the physicochemical properties of the resultant product. There is a need to establish favourable processing and handling conditions to maintain the viscosity of *Grewia* mucilage throughout its shelf life. The study aimed to develop a simple and efficient technique to isolate *Grewia* gum from *Grewia mollis* stem barks and evaluate its organoleptic, physicochemical, microbial count and rheological profile. *Grewia* gum was prepared by aqueous maceration of stem barks of *Grewia mollis*.
The successful isolation of reddish-brown *Grewia* gum was confirmed using FT-IR. The product yield was 67.7±1.9% w/w. The irregular shaped and rough-textured particles did not compromise its flowability (CI 15.3 ± 1.7%, HR 1.18±0.02, and AOR 17.6 ± 0.7°). *Grewia* gum displayed swelling index: 8.3 ± 2.1%; moisture content: 8.8 ± 0.8%; total microbial count 65 ± 1 CFU/g and pathogenic organisms were absent. Phytochemical constituents include Saponins, flavonoids, proteins, amino acids, triterpenoids, phenols, and carbohydrates. The highest mucilage viscosity of 4458 ± 33 mPas was obtained at 25°C, 10 rpm while 1431 ± 20 mPas was the lowest viscosity recorded at 45°C, 60 rpm under acidic conditions. Its satisfactory swelling index suggested its usefulness to formulate controlled release dosage forms. A new and cost-effective technique has been identified to extract *Grewia* gum. *Grewia* gum is a valuable substitute to synthetic and semi-synthetic polymeric excipients for pharmaceutical applications.

Keywords: *Grewia mollis*, Extraction, Physicochemical, Rheology, Microbiological.

Introduction

Polymeric excipients are an integral part of any formulation as they enhance the loaded drug's stability, bioavailability, and controlled release profile.[1] They include natural (sodium alginate, gelatin, chitosan, *Grewia* gum), semi-synthetic (cellulose derivatives), synthetic (polyethylene glycols, poloxamers, polylactides, polyamides), and fermentation products (xanthan gum).[2]
Gums and mucilages are polysaccharides or complex carbohydrates derived from various plant parts, micro-organisms and marine algae.[3] Natural polysaccharide gums such as xanthan, tragacanth, acacia and *Grewia* gum are preferred over their synthetic counterparts because they are non-toxic, non-irritant, cost-effective, readily available and will not interact with the drug or other formulation and packaging components.[4]
Grewia mollis (Fam. Tiliaceae), is an edible plant grown wild or cultivated in the Northern and middle belt region of Nigeria and *Grewia* gum is typically extracted from its inner stem bark.[5] Structurally, the gum is composed of glucose (67.14%), arabinose (12.71%), galactose (9.61%), rhamnose (6.2%), and xylose (2.72%) as the major monosaccharide.[5,6] It is reported to have an average molecular weight of 5925 kDa and polydispersity of 1.6, which is obtainable with natural polysaccharide gums.[5] This molecular weight and weight distribution influences its physicochemical and rheological properties as well as its potential use in food and pharmaceutical industries.[7]
Grewia gum is increasingly relevant for pharmaceutical application because it can potentially be used as a binder in tablets,[8] bioadhesive for transmucosal drug delivery,[9] suspending agents in liquid dosage forms,[10] film-coating agent for sustained release formulations,[11] viscosity enhancer,[12] and emulsifier.[13] Potential phytochemical principles present in their aerial parts include flavonoids, saponins, tannins, cardiac glycosides and phenols with anti-tumour, anti-inflammatory, antimicrobial, cardioprotective and anti-oxidant properties.[14]
The moisture content of *Grewia* gum may be dependent on its method of preparation as well as its environmental conditions, and the moisture level of the gum should be minimized for the formulation of moisture sensitive drugs. Furthermore, the swelling index of *Grewia* gum provides information about their bioadhesive and sustained release potential and earlier report did not provide a quantitative swelling index for *Grewia mollis*.[15] Inclusion of additives, application of shear stress during processing and handling as well as environmental temperature variations may influence the viscosity and suitability of *Grewia* gum as polymeric excipient.
The study aimed to prepare *Grewia* gum using heat or chemical-free method and evaluate its organoleptic, physicochemical, rheological, and microbiological properties.

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Materials and Methods

Plant material
Grewia mollis stem barks were collected from Ibadan, Oyo State, Nigeria in June, 2019, by Mr. T.I. Adeleke of the Department of Pharmacognosy, University of Lagos, Nigeria. The stem barks were

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identified and authenticated at the Forestry Research Institute of Nigeria (FRIN) Herbarium, Ibadan FHI 112884. They were stored at room temperature prior to the extraction of *Grewia* gum.

Chemicals and Reagents
Ethanol (96%), hydrochloric acid solution (1%), and sodium hydroxide pellets were supplied by Sigma, Germany. All other chemicals and reagents were of analytical grade and obtained from commercial sources.

Preparation of Grewia gum and product yield determination
The mucilage was extracted from the inner stem bark of *Grewia mollis* by maceration in distilled water at room temperature. The inner stem barks (1700 g) of *Grewia mollis* plant were chopped into small portions and soaked in distilled water (8.5 L) for three~~five~~ days. The mucilage was collected by filtration through a sieve with mesh diameter of 2000 µm. The mucilage was dried using a vacuum desiccator at 40 ± 3°C for 6 h, and dried mucilage was pulverized and stored in an air tight container for subsequent use. The percent yield of mucilage was calculated:

$$\%\ \text{Yield of } Grewia\ gum = \frac{Weight\ of\ dried\ Grewia\ sample(g)}{Weight\ of\ the\ stem\ bark\ strips(g)} \times 100$$

Physicochemical characterization
Organoleptic
The organoleptic properties of *Grewia mollis* such as colour, odour, and texture were evaluated by visual examination and feel to the touch.

Microscopy for particle size and size distribution
The powdered sample was placed on a clean slide, stained with a drop of crystal violet, covered with slip and viewed under the BINO CXI compound microscope (Micron Instruments Ltd, USA), utilizing a calibrated slide. The particle size, size distributions, and shape were evaluated.

Fourier Transform-Infrared Spectroscopy (FT-IR)
The Tensor 27 Platinum ATR FT-IR spectrometer (Bruker, UK) was used to investigate the successful isolation of *Grewia* gum. The FT-IR spectra of solid samples of *Grewia* gum was obtained by scanning in the region of 4000 to 650 cm^{-1}, resolution of 4 cm^{-1}. Data was processed based on the average of 32 scans per spectrum generated by the instrument.

Estimation of Water Content
The hygroscopicity or moisture content of *Grewia* gum powder was determined using a previously reported method with modification.[16] The *Grewia mollis* gum powder (10 g) was dried in a laboratory oven (Uniscope SM 9053, Surgifriend Medicals, UK) and the weight of the powder was recorded every 30 min over a period of 3 h in order to evaluate the loss of weight of the powder upon drying, which correlates with its moisture content.

$$\%\ \text{Loss in weight on drying} = \frac{Initial\ weight\ of\ sample\ (g)\ -\ Final\ weight\ of\ sample}{Initial\ weight\ of\ sample(g)} \times 100$$

Flow properties
The flow properties of *Grewia* gum powder was evaluated in terms of its bulk density, tapped density, Carr's compressibility index (CI), Hausner ratio (HR), flow rate and angle of repose (AOR).

The bulk and tapped volumes of *Grewia* gum powder were determined according to the United States Pharmacopeia method. The *Grewia* gum powder (35 g) was filled into a graduated glass cylinder. The initial volume after transferring powder into the cylinder, bulk volume, was measured. The cylinder was tapped 500 times to obtain the tapped volume.[17] The Bulk density, Tapped density, Carr's index, and Hausner ratio were then determined using these formulae:

$$\text{Bulk density} = \frac{Weight\ of\ powder\ (g)}{Bulk\ volume\ (mL)}$$

$$\text{Tapped density} = \frac{Weight\ of\ powder\ (g)}{Tapped\ volume\ (mL)}$$

$$\%\ \text{Carr's compressibility index} = \frac{Tapped\ density\ (g/mL)\ -\ Bulk\ density(g/mL)}{Tapped\ density\ (g/mL)} \times 100$$

$$\text{Hausner ratio} = \frac{Tapped\ density}{Bulk\ density}$$

Flow Rate
The flow rate of *Grewia mollis* gum powder (35 g) was evaluated as a function of the duration of time during which the powder passes through the orifice of the flow meter. The time taken for the powder to flow through the orifice was noted. Flow rate was then calculated:

$$\text{Flow rate} = \frac{Weight\ of\ powder\ (g)}{Duration\ of\ flow\ (secs)}$$

Angle of Repose
The Angle of Repose for the *Grewia* powder was determined using the flow meter where the powdered sample (35 g) was poured through a funnel and it flows unto a horizontal surface, forming a conical heap. The angle of repose, θ was calculated, where h is the height of pile or heap, and r is the radius of the resultant cone-like base.

$$\theta = tan - 1\ \frac{h}{r}$$

Swelling Index
Grewia gum powder (2 g) was dispersed into a graduated cylinder containing 100 mL water. The initial volume of the resultant mucilage as well as its final volume after swelling for 24 h was noted and used to calculate the swelling index of the powder.

$$\%\ \text{swelling index} = \frac{Final\ mucilage\ level -\ Initial\ mucilage\ level}{Initial\ mucilage\ level} \times 100$$

Qualitative phytochemical determination
The *Grewia* gum powder or mucilage was investigated for the presence of carbohydrates, reducing sugars, proteins, amino acids, tannins, phenols, flavonoids, triterpenoids, alkaloids, and saponins using standard procedures.[18]

Ash content
The amount of ash contained in *Grewia* powder after incineration was calculated using the standard methods of the Association of Official Analytical Chemists.[19] The crucible was pre-heated in a furnace, cooled in a desiccator and weighed. *Grewia mollis* gum powder (2 g) was put in the crucible and heated at 700°C for 6 h in order to generate the white ash and the percent total ash calculated as follows:

$$\%\ Total\ ash = \frac{Ash\ weight\ (g)}{Original\ sample\ weight\ (g)} \times 100$$

Rheological Evaluation
Effect of temperature, pH and shear stress on the viscosity of Grewia mollis mucilage
The viscosity of *Grewia mollis* mucilage (2% w/v, 20 mL) was determined at both 25°C and 45°C using *DV-E* Digital Viscometer (Ametek Brookfield, UK) operated with spindle 4 at various speed settings from shear rate of 10 to 60 rpm. In addition, 1 M hydrochloric acid or 1 M sodium hydroxide was mixed with *Grewia mollis* gum mucilage at room temperature (25°C) to determine the impact of acidic or alkaline additives on the viscosity of *Grewia* gum mucilage at room temperature. The volume ratio of the mucilage to the acid or base was 100:1 (20 mL: 0.2 mL).

Influence of temperature on the pH of Grewia mollis mucilage
The effect of temperature on the pH of the mucilage was investigated by maintaining 20 mL mucilage (2% w/v) on a water bath at 25 and 45°C and the pH of the system determined using HI 2211 pH/ORP Bench top pH meter (Hanna Instruments, UK).

Microbial Limit Evaluation
The *Grewia mollis* mucilage was subjected to different microbiological tests - total aerobic viable count, total combined yeast count, and test for the presence of Gram negative pathogens like *Salmonella spps, Pseudomonas aeruginosa,* and *Escherichia coli.* One mL each of 1/10 and 1/100 dilutions of *G. mollis* mucilage in tween 80 (3%) was mixed with 19 mL of warmed Eosin methylene blue agar, trypticase soy agar, *Salmonella-Shigella* agar, and Sabouraud dextrose agar plates. These plates were incubated for 72 h at 37°C. Their microbial load was evaluated for conformity with limits recommended by the United States Pharmacopoeia for botanical fluid extracts.[20]

Statistical analysis
All experimental data were collected in triplicates and data expressed as mean ± standard deviation. A one-way ANOVA test, with Bonferroni post-hoc test was carried out using GraphPad Prism 8.4.3.686, with $p < 0.05$, depicting statistically significant differences between data sets.

Results and Discussion

Over the last decade, *Grewia* gum is being considered as a promising biocompatible pharmaceutical excipient to serve as a substitute or used in combination with synthetic polymeric excipients such as hydroxypropyl methylcellulose and sodium carboxymethylcellulose,[21] which are more expensive and require foreign exchange to procure them.[5]

Product yield
Grewia gum was successfully extracted from the stem barks of *Grewia mollis* by maceration in water at room temperature for 72 h. The product yield based on its dry weight was 67.7 ± 1.9% w/w, which was higher than 32.4% obtained by Nep and Conway (2010) that used heat and chemicals to treat their powdered stem barks.[5] Alobo and Arueya also obtained a lower yield of 18% for their *Grewia venusta* samples as mucilage was collected immediately after immersion of the stem barks in water without heat treatment.[22] Haile's group reported product yield of 11.96% for *Grewia ferruginea* after soaking the stem barks in water at room temperature for 48 h.[23] These findings revealed that the product yield may be dependent on the species of *Grewia* processed as well as the processing conditions.

Organoleptic characteristics
Grewia gum was reddish brown in colour, odourless, and rough in texture. This colour is in agreement to similar *Grewia* samples evaluated previously.[22] The powder particles may be cohesive in nature due to their rough texture, with greater tendency to interlock than particles with smooth surfaces and these organoleptic properties may affect their flowability.[24]

Microscopic evaluation of Grewia mollis powder
Grewia mollis powder is composed of two types of particle sizes, as shown on the microscopic image (Figure 1). The smaller particles were 105 ± 41 µm in diameter while the bigger ones were 474 ± 106 µm. Based on the particle size distribution, the presence of fines amongst the larger particles was minimal, which is desirable for improved homogeneity of the pharmaceutical dosage forms prepared using *Grewia* gum. Also, the particles were irregularly shaped, which is in good agreement with the particle shape reported earlier, for *Grewia* samples.[16]

Fourier-Transform Infrared Spectroscopy
The successful isolation of *Grewia* gum was evident from its FT-IR spectrum, with characteristic broad absorption bands (Figure 2) at 3276 cm^{-1} depicting OH stretch of the galacturonic and uronic residues. It also revealed alkyl C-H stretch (2930 cm^{-1}) depicting methyl groups of rhamnose; N-H bend of primary amines was evident around 1638 cm^{-1} while peak evident at 1409 cm^{-1} represented C-C stretch in ring of aromatic amines. The presence of C-N stretch of aliphatic amines was evident at 1249 cm^{-1}, The absence of water soluble groups accounts for the low aqueous solubility of *Grewia mollis* gum. These values are in good agreement with that obtained by Nep and Conway[5] and Haile *et. al*.[23]

Estimation of water content
The presence of residual water in *Grewia* powder makes it susceptible to microbial attack after storage. Moreover, hygroscopic pharmaceutical products absorb moisture, resulting in caking of such products, limiting their dispersibility during reconstitution.
Various researchers have isolated *Grewia* gum using different techniques which has resulted in the product exhibiting varied characteristics: Nep and Conway processed their powdered Grewia stem barks using chemicals, with product exhibiting 10.6% moisture content.[5] Alobo and Arueya did not allow the chopped stem barks of *Grewia venusta* to remain in contact with water used for maceration and this method increased their moisture content to 14%.[22]
The moisture content of our Grewia powder (8.8 ± 0.8%), was within the Pharmacopeial limit of ≤ 15%[25] and less than that of similar Grewia samples (10.6 ± 2.0%) previously studied.[5] Increased level of water in a powder mass increases the thickness of the adsorbed liquid layer as well as the strength of liquid bridges,[26] thereby enhancing the cohesiveness of the powder, and result in the formation of agglomerates which reduces powder flow properties.[27] Thus, Grewia gums are preferably stored in air-tight containers under low humidity. The removal of residual moisture content from Grewia powder was achieved within 2 h as shown on the drying curve (Figure 3).

Flow properties
Grewia mollis gum powder exhibited a bulk density and tapped density of 0.7 ± 0.1 and 0.8 ± 0.1 g/mL, respectively. These values are considered acceptable as a bulk density of 0.68 g/mL was reported for *Grewia venusta* while the tapped density of the Grewia sample was not evaluated.[22] Compressibility index and Hausner ratio are typically used to depict the flowability of powders. A free-flowing powder exhibits low compressibility index with improved stability and strength, with compressibility index [CI] values less than 20% depicting good powder flow.[24] *Grewia mollis* powder displayed a CI value of 15.3 ± 1.7%, suggesting good flow properties for the powder. Hausner ratio (HR) is a function of the inter-particulate interactions and a value less than 1.25 depicted good flow properties..[24] Grewia powder exhibited good flowability as its HR values was 1.18±0.02. The flowability of *Grewia mollis* powder was better than that of samples reported by Nep and Conway (2010), with the Grewia gum powder displaying passable flow, as CI and HR values were 25.2 ± 1.9% and 1.30 ± 0.02, respectively.[5] The flow rate of *Grewia mollis* powder was 8.2 ± 1.0 g/sec, which depicts the good flow of the powder. Also, its angle of repose was 17.6 ± 0.7°, and powders with angle of repose less than 30° usually have good flowability.[24] Overall, *Grewia* powder displayed excellent flow properties.

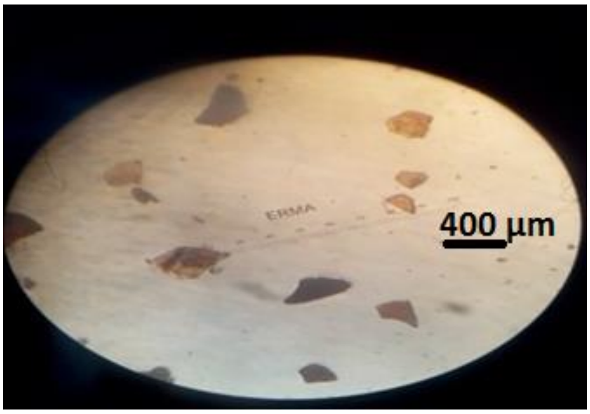

Figure 1: Photomicrographs of *Grewia mollis* powder showing irregularly shaped particles, scale bar = 400 µm.

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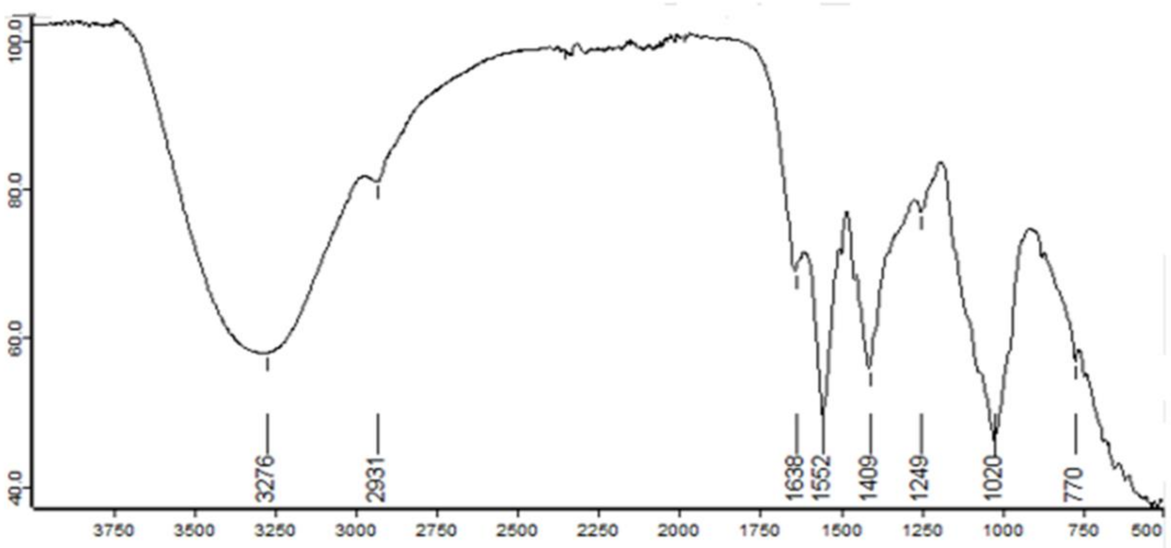

Figure 2: FT-IR spectrum of *Grewia mollis* powder

Swelling Index

The rapid swelling of polymers in water is associated with the breakage of intermolecular hydrogen bonds in the amorphous regions of their powder that allows irreversible and progressive water absorption.[28] Earlier studies revealed that polymers with high swelling index exhibited good bioadhesive and controlled release potential.[29,30] Nep and Conway carried out qualitative analysis of the swelling index for *Grewia mollis*, therefore comparison of their samples with ours, was not possible. The swelling index of our studied *Grewia mollis* gum powder was greater than that of *Grewia venusta* evaluated by Alobo and Arueya (2017) (8.3 ± 2.1% vs. 1.7 ± 0.1%).[22] HPMC, a semi-synthetic polymeric excipient with proven controlled release and bioadhesive properties exhibited lesser degree of swelling than our studied *Grewia mollis* gum (7.4 ± 0.1% vs. 8.3 ± 2.1%).[30] This finding revealed that *Grewia mollis* gum-containing dosage forms may exhibit superior bioadhesive and sustained drug release properties relative to HPMC.

Phytochemical characterization

Table 1 shows the phytochemical principles identified in the stem bark of *Grewia mollis*. Our finding was similar to that of Sambo and coworkers with respect to the presence of Saponins, flavonoids and phenols as well as absence of alkaloids.[14] However, tannins detected by Sambo *et. al*, was not detected in our samples, probably due to differences in our methods of Grewia gum preparation and phytochemical analysis. For example, Sambo and coworkers macerated their stem barks in water at 37^{o}C for 48 h and boiled diluted Grewia mucilage prior to inclusion of ferric chloride solution during investigation for the presence of tannins.[14] Alobo and Arueya also identified low levels of tannins in the liquid (0.01%) and dried mucilage (0.07%) of *Grewia venusta* stem barks.[22] Nevertheless, carbohydrate present in our samples has not been previously isolated from *Grewia mollis* stem barks. We also identified proteins but earlier studies did not investigate and identify proteins in their stem bark samples. We did not detect amino acids in our samples, probably due to the non-heat treatment of the stem barks. These findings indicated that the nutrient of interest may dictate the method of processing the stem barks.

The various phytochemical constituents identified in *Grewia mollis* powder may perform various pharmacological roles, for example, carbohydrates may serve as a viscosity-enhancer; proteins for skin regeneration; Saponins/triterpenoids glycosides exhibited antitumour, antimutagenic, and anti-hyperglycemic properties; flavonoids are anti-inflammatory, anti-diarrheal, anti-allergic, antitumour, and anti-oxidant in nature; and phenols with antimicrobial properties.[31,32]

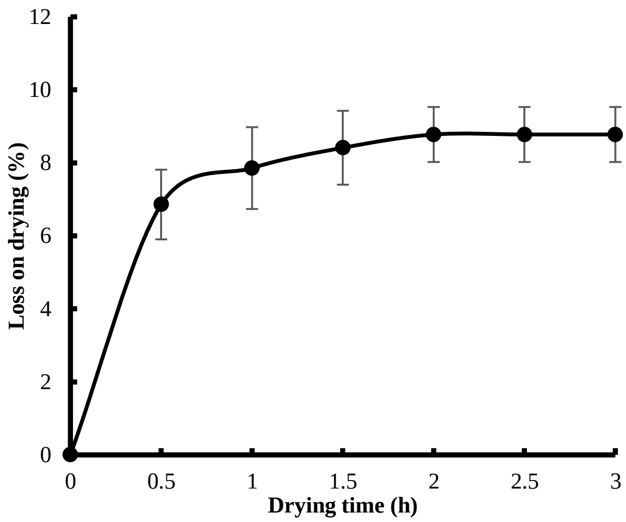

Figure 3: Drying curve of *Grewia mollis* powder over 3 h; mean ± SD, n = 3

Table 1: Qualitative Phytochemical constituents of *Grewia mollis* stem bark

Phytochemical	Inference
Carbohydrates	+
Reducing sugars	_
Proteins	+
Amino acids	-
Tannins	_
Phenols	+
Flavonoids	+
Triterpenoids	+
Alkaloids	_
Saponins	+

Key: + = Present; - = Absent

Total ash content

The ash generated from *Grewia mollis* powder after incineration, is typically used as a medicinal salt as it constitutes inorganic salts.[33] The amount of such ash obtained from our sample (7.7 ± 0.3% w/w) was satisfactory and within the British Pharmacopeial limit for related excipient, xanthan gum (6.5 - 16% w/w).[25]

Effect of shear rate, temperature and pH on the viscosity of Grewia mollis mucilage

Overall, *Grewia mollis* mucilage displayed a pseudoplastic shear-thinning flow pattern as previously reported[7,34] because its viscosity at 25°C decreased with increasing shear rate from 10 to 60 rpm. A similar trend was observed when the temperature of the system was increased to 45°C. Also, there was significant statistical differences between the viscosity of the mucilage at 25°C at shear rate of up to 40 rpm and that of the system maintained at 45°C (Figure 4). This behavior may be due to the structural changes in gum molecules, decreased chain entanglement in gum molecules as a result of the mucilage orientating itself more parallel to the surface, thereby decreasing resistance to spindle rotation and lowering viscosity.[35] However, there was no significant statistical difference between the viscosity of the *Grewia* sample at 50 rpm and 60 rpm when the mucilage was maintained at 25°C or 45°C. High energy pump system may be required to dislodge *Grewia* mucilage from containers at room temperature. Alternatively, the samples may be packed in wide-mouthed containers.

The *Grewia* sample exhibited its highest viscosity (4458 ± 33 mPas) at shear rate of 10 rpm, room temperature while the viscosity of 1431 ± 20 mPas was obtained for the sample at 60 rpm under acidic conditions, at 45°C, indicating that the presence of acidic components as well as application of increased shear stress in a *Grewia*-containing formulation may decrease the overall viscosity of the dosage form. Also, alkaline materials can be incorporated into *Grewia* containing formulations for viscosity enhancing effect.

Effect of temperature on the pH of Grewia mollis mucilage

Grewia mollis mucilage did not display temperature-dependent pH changes as the samples exhibited pH of 5.4 ± 0.1 and 5.0 ± 0.1 at 25 and 45°C, respectively. Nevertheless, these pH values were mildly acidic and comparable to that of *Grewia venusta* evaluated by Alobo and Arueya (2017),[22] with samples exhibiting pH between 5.17 and 5.80 at 25°C. These findings suggest that the pH value of *Grewia* mucilage would be relatively unchanged if samples are stored at 25 °C to 45 °C, which is desirable for pharmaceutical excipients that may be subjected to elevated temperatures during processing, handling or storage.

Microbial limit evaluation

The total viable microbial count contained in Grewia mollis mucilage was 65 ± 1 CFU/g. Salmonella spps, Pseudomonas aeruginosa and Escherichia coli as well as yeasts were absent in the Grewia mollis mucilage. These values were within the acceptable microbial limits recommended by USP for botanical fluid extracts (NMT 10^4 CFU/g of total viable organisms and absence of particular organisms).[20] These findings suggest that the Grewia mollis mucilage is suitable for use as an excipient in the manufacture of pharmaceutical solid, liquid and semi-solid dosage forms.

Conclusion

A novel, safe and cost-effective extraction method for *Grewia mollis* gum that result in high yield and acceptable moisture content, swelling index, rheological, phytochemical and microbial profiles has been developed. The phytonutrient of interest may dictate the method of extracting *Grewia* gum from *Grewia mollis* stem barks. The low moisture content of *Grewia* gum suggests that it could be a valuable delivery system for moisture sensitive drugs. The swelling index of *Grewia mollis* gum portends its use in bioadhesive and sustained release formulations.

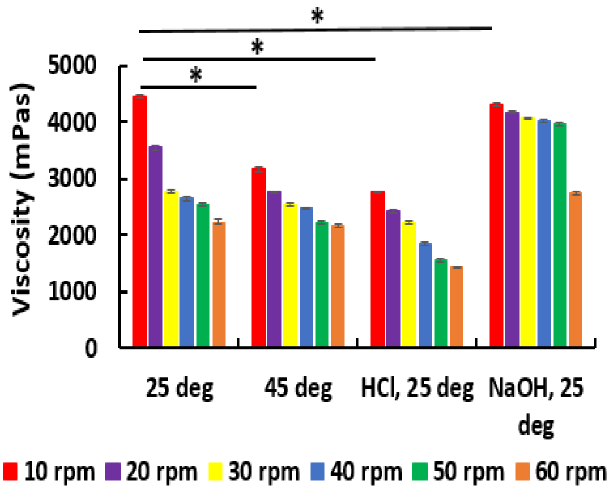

Figure 4: Effect of changes in temperature and pH on the viscosity of *Grewia mollis* mucilage (2% w/v), shear rate from 10 to 60 rpm. Data represent mean ± SD, n = 3; * indicates significant statistical differences between data sets with $p < 0.05$.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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