Isolation of *Cucurbita maxima* Duchesne. Fruit Mucilage and its Application as a Pharmaceutical Excipient

Kamatchi Sundara Saravanan[1*], Sharon Caroline Furtado[2*], Niveditha N[1], Harshita Gond[1], Shubha Shree M[1], Abdulbasith Bin Basheer PV[1], Afreen Makbul Shaikh[1]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, M S Ramaiah University of Applied Sciences, Bangalore - 560054, Karnataka, India*

[2]*Department of Pharmaceutics, Faculty of Pharmacy, M S Ramaiah University of Applied Sciences, Bangalore - 560054, Karnataka, India*

*Corresponding authors:

Kamatchi Sundara Saravanan
Department of Pharmacognosy,
Faculty of Pharmacy,
M S Ramaiah University of Applied Sciences,
Bangalore - 560 054, Karnataka, India

Sharon Caroline Furtado
Department of Pharmaceutics,
Faculty of Pharmacy,
M S Ramaiah University of Applied Sciences,
Bangalore - 560 054, Karnataka, India

Abstract:

The usefulness of mucilage from *Cucurbita maxima* Duchesne fruit as a tablet binder was evaluated in this study. Mucilage was isolated using solvent precipitation extraction process, and its physico-chemical characteristics were assessed. Isolated mucilage (4%, 5%, and 6% w/v) was used to prepare paracetamol granules through wet granulation process, whereas starch mucilage (5% w/v) served as standard binder for comparison testing. The granules were subjected to pre-compression tests followed by compression in a rotary punching machine to obtain tablets. Post compression, the tablets were evaluated for weight variation, hardness, thickness, friability, and disintegration. The percentage yield of the mucilage was 8.57% w/w. The mucilage colour was buff and had a characteristic odour and taste. It was slightly soluble in water exhibiting a pH of 5.96, and viscosity of 76.3 cP at 10 rpm. All tablets prepared passed the test for weigh variation. Tablets with starch as binder had a hardness of 4.66 kg/sq cm, whereas tablets with isolated mucilage had a hardness of 5.66, 5.33, and 5 kg/sq cm at 4, 5, and 6% w/v, respectively. The thickness range was observed to be from 4.14 to 4.33 mm. Percentage friability was 0.67 (with starch as binder) and 0.77, 0.80 and 0.59 (with 4, 5, 6 % isolated mucilage) respectively. Tablets prepared using 6% isolated mucilage and starch as binder passed the disintegration test.

Keywords: *Cucurbita maxima*, Mucilage, Excipient, Binding agent

Introduction

Excipients are the compounds or the substances, other than the pharmaceutical active ingredients and packaging components, that affect the quality of the pharmaceutically finished products and, in certain instances, constitute the entire formulation [1]. Plant derived gums, mucilages and their derivatives are extensively employed as pharmaceutical excipients besides its utilization in food, nutraceutical and cosmetic industries [2]. Mucilages are often large molecules and are composed of sugars and uronic acid residues [3]. They are regarded to be a pharmaceutically important component owing to its chemical nature with diverse range of applications that include binding, disintegrating, emulsifying, gelling, stabilizing, suspending and thickening agents. Additionally, these mucilages have received a lot of attention recently as matrices in sustained and controlled-release drug delivery systems [4].

There are numerous well-known plant-based polymers, including cellulose, gum karaya, starch, pectin, acacia etc., while many other mucilages of herbal origin have been evaluated and reported previously *viz*., *Linum ussitatissimum* [5], *Ocimum sanctum* [6], *Mimosa pudica* [2], *Lallemantia royleana* [7], *Hibisucs rosa-sinensis* [8], *Adansonia digitate* [9], *Ficus glomerata* [10], *Listea glutinosa* [11], *Trigonella foenum-graecum* [12], *Abelmoschus esculentus* [13], *Plantago ovata* [14], etc., However, there are still many plant sources with mucilage content that have not been investigated for its potential pharmaceutical applications.

Cucurbita maxima Duchesne (Cucurbitaceae), a most varied domesticated squash species is frequently used as food and has a long history of usage as a traditional remedy for a number of ailments. *C. maxima* can be identified by its perennial/annual nature and distributed across warm locations of the world. The bioactive substances in substantial concentrations throughout the plant include proteins, polysaccharides, sterols, fixed oils, and para-amino-benzoic acids [15]. Proteins, 13-hydroxy-9Z, phenolic glycosides, 11 E-octadecatrienoic acid, polysaccharides are reported to be present in leaves and seeds, whereas carotenoids and y-aminobutyric acid are reported to be present in fruits [16, 17]. Alkaloids, flavonoids, phenolics, carbohydrates, tannins, saponins, and terpenoids are the other phytobioactives reported from this species. These compounds have a variety of pharmacological activities such as anthelmintic, anti-microbial, anti-oxidant, anti-inflammatory, cytotoxicity; while reported to be useful in constipation, renal diseases, prostatitis, anaemia, wound healing, head and lung cancer and in the regulation of blood pressure and blood sugar levels [18-20]. Furthermore, a significant amount of mucilage and pectin have been reported to be present in this genus [19, 21].

Binders or binding agents are the substances that offers cohesion to the granules. These agents are helpful to achieve variable tablet mechanical strengths and drug release qualities for specific pharmaceutical uses. As a result, the tablet is guaranteed to stay intact during compression, and the formulation of granules with derived hardness and size further improves the flow properties. Synthetic binders can cause processing issues like rapid over-granulation, formulation hardness, and reduced formulation dissolution characteristics. Also, synthetic binders are typically expensive. Due to the usage of synthetic binders, formulation stability is negatively impacted, and film coating appearance is commonly seen on finished products [22]. As a result, efforts are always being made to find novel, improved, sustainable, and affordable excipients to fulfil the needs of the pharmaceutical formulation industry. Moreover, these natural polysaccharides are a preferred alternative to the existing synthetic polymers due to their availability, biodegradability, biocompatibility, non-toxicity, and cost [3,4]. In this context, the present study was aimed to isolate *C. maxima* fruit mucilage and to evaluate its potential application as a pharmaceutical excipient.

Materials and Methods

Fruit collection

Fresh *C. maxima* fruits were procured from Yeshwanthpur vegetable market, Bangalore during May 2022 and was authenticated by Dr. Noorunnisa Begum, Curator, FRLHT, Bangalore.

Mucilage isolation

Fruit mucilage was isolated following the method reported by Bahramsoltani R., *et al* [23]. Briefly, a weighed amount of fruit was thoroughly washed, cut into small pieces, and ground into a fine mixture using a blender. The mixture was transferred to a 500 ml beaker and 300 ml of 0.1N HCl was added. This was followed by heating for 5 min and filtered immediately. Thus, obtained filtrate was added to

an equal volume of ethanol with continuous stirring to precipitate mucilage. The mixture was refrigerated overnight for the mucilage to settle down. The settled mucilage was filtered through muslin cloth and transferred to a petri dish for drying. Dried mucilage was ground, and the percentage yield was calculated.

Evaluation of isolated mucilage

The isolated mucilage was assessed for its physico-chemical characteristics such as solubility, pH, viscosity, and chemical composition adopting standard procedures. Fourier Transform Infrared (FT-IR) spectra of the mucilage was also recorded using Shimadzu FT-IR-8400S spectrophotometer based on attenuated total reflectance to ascertain the functional groups.

Tablet preparation

Tablets with paracetamol as model active ingredient were formulated using starch mucilage (5% w/v) and isolated mucilage (4%, 5% and 6% w/v) as binder. The composition is given in table 1.

Table 1 Tablet composition

Ingredient	Quantity (for one tablet)			
	TF1	**TF2**	**TF3**	**TF4**
Paracetamol	500 mg	500 mg	500 mg	500 mg
Dicalcium phosphate	120 mg	120 mg	120 mg	120 mg
Starch powder	25 mg	25 mg	25 mg	25 mg
Starch Mucilage (5% w/v)	q.s.	-	-	-
Pumpkin mucilage (4% w/v)	-	q.s.	-	-
Pumpkin mucilage (4% w/v)	-	-	q.s.	-
Pumpkin mucilage (4% w/v)	-	-	-	q.s.

Briefly, weighed quantities of dicalcium phosphate and paracetamol were taken in a porcelain mortar and pestle. Aqueous solution of mucilage was added and triturated until a pliable dough like mixture was obtained. The mixture was passed through sieve number 18 to obtain granules followed by drying at 50^{o}C for 15 min in a tray dryer. The dried granules were sieved through sieve number 20. Purified talc and magnesium stearate were admixed, and the granules were subjected to pre-compression studies. They were then compressed into tablets using 10 mm punches in a rotary tablet compression machine (RIMEK, RSB-4 Minipress) [24].

Pre-compression tests

The prepared granules were assessed for bulk volume, tapped volume, bulk density, tapped density, Hausner's ratio, Carr's Compressibility Index, angle of repose and particle size in accordance with established procedures [25].

Post-compression tests

The following post-compression analysis were performed on the compressed tablets [25].

Weight variation

Weight variation test is an indirect measure of content uniformity. Twenty tablets from each batch were weighed individually and average weight was determined. Percentage deviation of individual weight from the average weight was calculated. Permissible upper and lower limit for tablet weight was calculated as follows:

Upper limit = average weight + 5% of average weight

Lower limit = average weight – 5% of average weight

The tablets were said to pass the test if not more than two tablet weights differed from the limit and no tablet differed by more than double the limit.

Thickness

Tablet thickness was determined using a screw gauge. Three tablets were measured and average thickness reported.

Hardness

Tablet hardness was determined using Monsanto hardness tester. Five tablets from each batch were subjected to hardness test and the average value was reported.

Friability

The tendency of tablets to powder, chip or fragment was determined using Roche's friabilator. Twenty tablets from each batch were weighed and placed in the friabilator. The instrument was set at 25 rpm for 4 min. The tablets were gently dusted and reweighed. Percentage friability was determined using the following equation:

Percentage friability = ((Initial weight - Final weight)/Initial weight) x 100

Disintegration

The first step towards dissolution of tablets is tablet disintegration. Pharmacopoeia specifies the disintegration medium for normal release of uncoated tablets as water and the time limit for dissolution is 15 min. One tablet was dropped into each tube of the disintegration apparatus (Koshiash Industries). A total of six tablets from each batch were tested. The disintegration time was calculated using the disintegration apparatus.

Results and Discussion

Total mucilage content and physico-chemical properties

The total mucilage content was calculated to be 8.57 % w/w (Figure 1). The physico-chemical properties like aqueous solubility, pH, and viscosity of the isolated mucilage are tabulated in Table 2.

Figure 1 Isolated mucilage (Inset-dry mucilage powder)

Table 2 Physico-chemical characters of isolated mucilage

Parameter	Character/value
Solubility	Forms a colloidal solution in water
pH	5.96 ± 0.03
Viscosity (1%)	76.3 cP at 10 rpm

Isolated mucilage tested positive with molisch reagent and ruthenium red reagent indicating the presence of carbohydrate and mucilage. FT-IR spectra of the isolated mucilage is represented in Figure 3. Some of the functional groups correlating with carbohydrates and mucilage were observed at 3434.68 cm^{-1} (-OH), 2925.53 cm^{-1} (C-H), and 1637.12 cm^{-1} (C=0) (Figure 2).

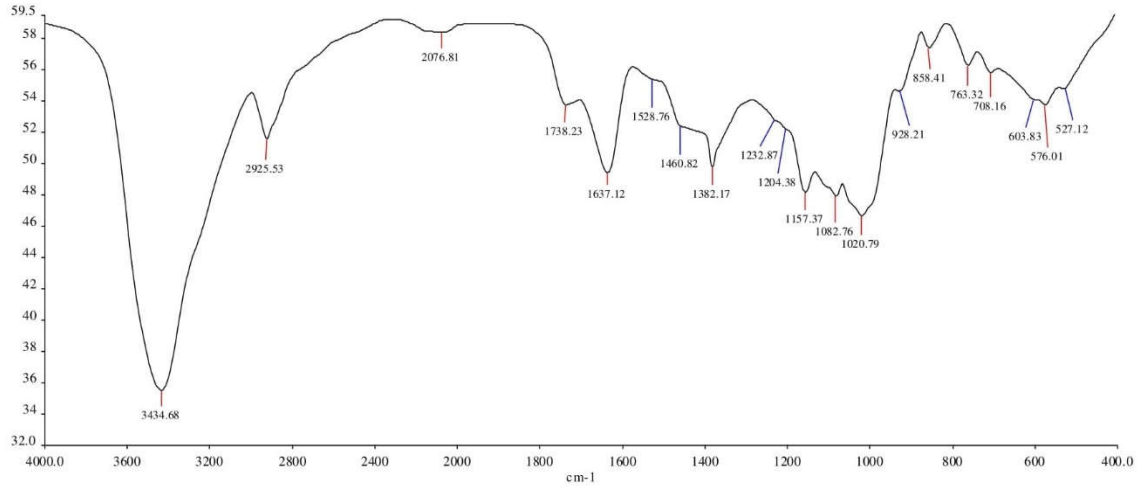

Figure 2 FT-IR spectra of isolated mucilage

Pre-compression evaluation

Granules were analysed for particle size by optical micrometry and results are reported in Table 3. The average granule size increased with increased percentage of mucilage. The size of granules prepared using starch mucilage was lower compared to granules prepared using pumpkin mucilage. The greater size may be attributed to better binding capacity of the pumpkin mucilage.

Table 3 Particle size of granules (µm)

Particle size	Granules with			
	Starch	Mucilage		
	(5%)	4%	5%	6%
Minimum	37.32	55.98	37.32	37.32
Average	147.41	152.26	170.36	185.85
Maximum	317.22	223.92	410.52	466.5

The results of bulk density, tapped density, Carr's index, Hausner's ratio and angle of repose are tabulated in Table 4.

Table 4 Pre-compression parameters of granules

Parameters / Binding agent	Starch (5%)	Mucilage		
		4%	5%	6%
Bulk density (g/ml)	0.59	0.55	0.53	0.53
Tapped Density (g/ml)	0.67	0.60	0.59	0.59
Hausner's Ratio	1.14	1.09	1.11	1.11
Carr's Index (%)	11.9	8.3	10.2	10.2
Angle of repose (θ)	23.6°	25.3°	24.45°	27.24°

Angle of repose values below 30º, indicating excellent flow property of all the granules. Carr's index and Hausner's ratio show that all granules have good to excellent flow property and hence have good compressibility and compactibility.

Post-compression evaluation

All tablets punched with mucilage and starch as binder passed the weight variation test (Table 5). Table 6 provides results of tablet hardness, thickness, and percentage of friability.

Table 5 Weight variation analysis

Parameter / Binder	Average weight (mg)	Permissible Weight range (mg)
Starch	656	632.20 - 688.80
4 % mucilage	656	632.20 - 688.80
5% mucilage	645	612.75 - 677.25
6% mucilage	635	603.25 - 666.75

Table 6 Results of post-compression analysis

Parameters/ Binding agent	Hardness (Kg/Sq.cm)	Thickness (mm)	Friability (%)	Disintegration time (min)
Starch (5%)	4.66	4.33	0.67	Within 15 min
4% mucilage	5.66	4.14	0.77	> 30 min
5% mucilage	5.33	4.14	0.80	> 30 min
6% mucilage	5.00	4.17	0.59	Within 15 min

All the tablets with starch as binding agent disintegrated within 15 min. Three tablets with 4% mucilage failed to disintegrate even after 30 min. One tablet with 5% of isolated mucilage as binding agent failed to disintegrate within 15 min. All tablets with 6% of isolated mucilage as binding agent disintegrated within 15 min. The results suggest good binding property of the isolated mucilage; however lower concentrations may require slightly higher amounts of disintegrating (both intra and inter granular) agents for achieving disintegration within the specified time limits.

Conclusion

The aforementioned results suggest that mucilage isolated form *C. maxima* Duchesne. fruit was able to produce tablets with desired hardness. The precompression and post compression parameters indicate the ability of the mucilage to produce tablets with properties that are comparable to tablets produced using starch mucilage. It was concluded that the extracted mucilage can be used as a novel binding agent in tablet formulations.

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