

ISOLATION AND CHARACTERIZATION OF MUCILAGE FROM SOME SELECTED SPECIES OF *ABELMOSCHUS* MEDIK. (MALVACEAE) AND THEIR APPLICATION IN PHARMACEUTICAL SUSPENSION PREPARATION

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ABSTRACT

Objective: The present study was concerned with the isolation and characterization of mucilage from five species of *Abelmoschus*; two cultivated (*A. esculentus*, *A. caillei*) and three wild (*A. manihot*, *A. angulosus* and *A. moschatus*). The study also includes an assessment of the efficacy of mucilages of two species, *A. esculentus* and *A. moschatus* in pharmaceutical suspension preparation.

Methods: The physical, physico-chemical and phytochemical properties of the mucilage of cultivated and wild species of *Abelmoschus* were analyzed using standard procedures. Suspending property is considered to be one of the most desirable qualities for pharmaceutical suspension preparation. Mucilage yield and quality was nearly equivalent in *A. esculentus* and *A. moschatus* and therefore the suspension properties of the mucilage of these two species was assessed using the suspending properties such as sedimentation volume, flow rate, particle size analysis, pH and viscosity.

Results: The results indicate the advantageous features of mucilages of all the five species of *Abelmoschus*. The suspending property of *A. moschatus* was noted to be at par with that of *A. esculentus* mucilage.

Conclusion: The results of the study suggest that the wild species, *A. moschatus* can be beneficially exploited for mucilage isolation.

Keywords: *Abelmoschus*, *A. esculentus*, *A. moschatus*, Mucilage, Suspension property.

INTRODUCTION

Mucilages are heterogenous in composition and are typically polysaccharide complexes formed from the sugars, arabinose, galactose, glucose, mannose, xylose and uronic acid units [1]. Several plant species such as *Aloe vera*, *Ceratonia siliqua*, *Opuntia ficusindica*, *Basella alba* and *Lepidium sativum* possess mucilage. Mucilages are noted to assume a multitude of physiological functions in plants and act primarily as energy reserves in the rhizomes, roots and seed endosperms. Foliar mucilages are reported to play a major role in wound responses [2], plant host pathogen interactions [3], water transport [4] and responses to abiotic stresses [5, 6]. Extracellular mucilages have been demonstrated to buffer leaf water status against environmental fluctuations and can also enable leaves to maintain low water potential when soil water deficits develop by acting as an apoplastic capacitor [7, 8].

Natural gums and mucilages possess a variety of pharmaceutical properties, which make them useful as additives in pharmaceutical preparations and nowadays, mucilages form vital components in such formulations. Among the different pharmaceutical properties, the suspension property finds application in the preparation of most pharmaceutical suspensions. The suspending agent actually reduces the rate of settling and permits easy redispersion of any settled particulate matter such as the associated drug [9, 10, 11]. Natural mucilages of *Acacia*, *Tragacanth*, *Khaya*, *Karaya* and *Cassia tora* are being used as suspending agents. There are also reports about the successful use of *Ocimum gratissimum*, *Butea monosperma*, *Albizia zygia* gum and *Laucaena leucocephala* seed gum as suspending agents [12]. The natural plant based materials are considered advantageous compared to synthetic polymers because of their natural origin, bioacceptance, edible nature, renewable nature, environment-friendly processing, low cost and local availability. With the increase in demand for natural mucilages, it has become necessary to explore novel and better sources of mucilage to meet the demands.

The present study is concerned with the detection, isolation and characterization of mucilages from the fruits of two cultivated and three wild species of *Abelmoschus*. Extraction and characterization of mucilage has been reported previously from only the cultivated species, *A. esculentus* [13, 14, 15, 16]. Therefore, the present study envisages a comparison of the mucilage yield in the cultivated and wild species of *Abelmoschus*. The study also intends to explore the suspension properties of mucilage from *A. esculentus* and *A. moschatus* in order to check whether the mucilage from wild species exhibits properties analogous to that in the cultivated species.

MATERIALS AND METHODS

The experimental materials for the present study include the two cultivated species, *Abelmoschus esculentus*, *A. caillei* and three wild species, *A. manihot*, *A. angulosus* and *A. moschatus*. The young seedlings or dried fruits of these species were collected from different localities in Kerala (Table 1). Plants were raised from the seeds, grown and maintained in a separate field in Department of Botany, University of Kerala, Thiruvananthapuram.

Table 1: Details of collection sites of species of *Abelmoschus*

S. No.	Name of species	Place of collection
1	<i>A. esculentus</i>	Agricultural University, Vellayani, Kerala
2	<i>A. caillei</i>	Guruvayoor, Thrissur, Kerala
3	<i>A. manihot</i>	Uduppi, Mangalore, Karnataka
4	<i>A. angulosus</i>	Periya, Calicut, Kerala
5	<i>A. moschatus</i>	Nedumangad, Thiruvananthapuram, Kerala

Experimental methods

Based on the number of days for fruits to mature, four time points were selected for mucilage quantification. The first sample was collected four day after flowering (4 D.A.F) while the subsequent samples were taken at intervals of four days such as, 8 D.A.F, 12 D.A.F and 16 D.A.F. Mucilage quantification was done until 16 days only since the fruits though still green were completely stiff and dry

Extraction and quantification of mucilage:

Fresh immature fruits of *Abelmoschus* were collected, washed and dried in an oven (105°C). The powdered material was soaked in water for 5-6 h and boiled for 30 minutes. Mucilage was extracted and quantified from this mixture [17, 18].

A. Physical characterization of mucilage: The isolated mucilage was evaluated for physical properties such as odour, appearance and solubility in hot and cold water, ethanol, acetone and chloroform.

B. Physicochemical characterization of mucilage: The physicochemical parameters such as loss on drying, swelling index; water and alcohol soluble extractives; total ash, water soluble ash and acid-insoluble ash and pH were carried out in accordance with the official monograph specifications [19].

C. Phytochemical screening of mucilage: The chemical constituents in the mucilage such as carbohydrates, proteins, tannins, phlobatannins, flavonoids, steroids, terpenoids, coumarins, saponins, alkaloids and reducing sugars were analyzed following standard biochemical procedures [20].

Suspension properties of *Abelmoschus* mucilage

Suspension properties are usually measured in terms of the sedimentation volume, flow rate, particle size analysis, pH, ability to redisperse in suspension and viscosity.

Preparation of mucilage suspension with paracetamol: Dried mucilage powder at different concentrations (0.5, 1, 1.5 and 2 g) and 5g of paracetamol were triturated together with 50ml distilled water to form a smooth paste. The mixture was then transferred to a 100 ml measuring cylinder, made upto the required volume with distilled water and then shaken vigorously for 2 min (thus making 2% w/v of the mucilage in the preparation). To the suspension, 0.1% w/v benzoic acid was added as a preservative.

*Paracetamol tablets (500mg tablets) purchased from a local medical shop (Safa Medicals Kallara).

Determination of sedimentation volume: Each suspension (50ml) was stored in a 50 ml measuring cylinder for 10 days at 35°C. Observations were made every 24 hr for four days. The sedimentation volume, F (%) was then calculated using the following equation.

$$F = \frac{V_u}{V_o} \times 100$$

Where, V_u = The ultimate volume of the test sample (mucilage + paracetamol) and V_o = The original volume of the suspension

Measurement of viscosity: The viscosity of prepared suspensions was determined using Ostwald's viscometer. The viscosity values were determined at 10, 20, 30, 40, 50, 60 and 100 rpm at 25°C. The viscosity of the suspension was calculated by the following formula.

$$\eta_2 = \frac{t_2 \rho_2 \eta_1}{t_1 \rho_1}$$

Where η_1 = Viscosity of water, η_2 = Viscosity of suspension, t_1 = Time of flow of water, t_2 = Time of flow of suspension, ρ_1 = Density of water, ρ_2 = Density of suspension

Determination of flow rate: The time required for each suspension sample to flow through a 10 ml pipette was determined and the apparent viscosity was calculated using the following equation.

$$\text{Flow rate } \eta_a = \frac{\text{Volume of solution in pipette (ml)}}{\text{Flow time (s)}}$$

Particle size analysis: The particle size and distribution in the suspension was determined with the help of an optical microscope.

The suspension was mixed thoroughly. A drop of the suspension was taken on a slide and spread into a thin film. A total of 100 particles were counted from ten different fields in the slide. The minimum, maximum and average particle sizes were determined from the observed fields.

pH determination: A 1% w/v dispersion of the sample was shaken in distilled water for 5 min and the pH determined [21].

Redispersibility: Fixed volume of each suspension (50 ml) was kept in calibrated tubes stored at room temperature for 20 days. At regular intervals of 5 days, one tube was removed and shaken vigorously to redistribute the sediment. Presence of deposits if any was recorded.

RESULTS

Mucilage quantification

Details of mucilage content in fruits of five species of *Abelmoschus* collected at different fruit developmental stages (4-16 D.A.F) are provided below.

Table 2: Mucilage content (g/g) in fruits of five species of *Abelmoschus*

Name of plant	4 D.A.F	8 D.A.F	12 D.A.F	16 D.A.F
<i>A.caillei</i>	0.229	0.216	0.237	0.228
<i>A.esculentus</i>	0.571	0.487	0.399	0.358
<i>A.angulosus</i>	0.194	0.161	0.108	0.102
<i>A.manihot</i>	0.382	0.252	0.178	0.125
<i>A.moschatus</i>	0.357	0.221	0.138	0.109

*The mean values of three observations are recorded.

Mucilage yield was highest in all the samples at 4D.A.F, decreasing subsequently (8D.A.F, 12D.A.F, 16D.A.F). The highest value was obtained for the cultivated, *A. esculentus* and slightly lower values were obtained for the wild species, *A. manihot* and *A. moschatus*. *Abelmoschus caillei*, had lower values at 4D.A.F, than the wild species but remained almost stable in the subsequent samples. *Abelmoschus angulosus* had the lowest mucilage yield.

The mucilage was subjected to both physical and physicochemical evaluation. The results are provided below

Physical characterization

The mucilage from all the species was brownish amorphous with a characteristic odour. It was completely soluble in hot and cold water but was partially soluble in organic solvents such as ethanol, acetone and chloroform.

Physicochemical characterization of mucilage

Physicochemical characterization involved the determination of the loss on drying, swelling index; water soluble and alcohol soluble extractives; total ash, water soluble ash and acid insoluble ash and pH (Tables 3 and 4).

Table 3: Results of physicochemical parameters

Name of the plant	Loss on drying (%)	Swelling index in (%)			Soluble- extractives (%)	
		Distilled water	0.1 N HCl	Phosphate buffer	water	Alcohol
<i>A.caillei</i>	9	26.1	14.0	8.0	94	18.4
<i>A.esculentus</i>	15	22.6	11.2	8.3	85	22.1
<i>A.angulosus</i>	13	26.5	15.0	10.0	92.8	22.4
<i>A.manihot</i>	12	25.4	12.0	8.0	85	25.6
<i>A.moschatus</i>	8	26.0	12.0	9.0	96	28

Table 4: Determination of Ash values and pH

Name of plants	Ash (%)	Water-soluble Ash (%)	Acid-insoluble Ash (%)	pH
<i>A.caillei</i>	5.6	2.7	0.32	5.4
<i>A.esculentus</i>	5.2	3.5	0.51	5.8
<i>A.angulosus</i>	6.3	2.8	0.44	6.4
<i>A.manihot</i>	5.6	3.1	0.38	5.2
<i>A.moschatus</i>	4.8	2.5	0.67	5.6

The moisture content was found to be relatively high in *A.esculentus*, and low in *A.moschatus*. Swelling index values were higher in distilled water compared to HCl (0.1N) and phosphate buffer. The yield of water soluble extractives in all the samples, were higher compared to the alcohol soluble extractives. The values were highest for *A. moschatus* followed by *A. caillei*.

The amount of water-soluble ash was highest in *A. esculentus* followed by *A. manihot*. The lowest value was for *A. moschatus*. The

acid-insoluble ash values were comparatively very low for all the species. All the samples showed pH values above 5. Near-neutral values were observed for *A. angulosus* which descended in the order - *A. esculentus* > *A. moschatus* > *A. caillei* > *A. manihot*.

Phytochemical screening of mucilage

Results of the chemical tests conducted in five species of *Abelmoschus* were similar.

Table 5: Details of qualitative phytochemical analysis

Tests	Observations
Test for carbohydrates	+
Test for reducing sugar	+
Test for proteins	-
Test for tannins	-
Test for phlobatannins	-
Test for flavonoids	+
Test for steroids and terpenoids	-
Test for coumarins	+
Test for saponins	+
Test for alkaloids	-

Carbohydrates and reducing reducing sugars were present in all the species studied, while proteins were absent. Among the secondary

metabolites analyzed, positive results were obtained for flavonoids, coumarins and saponins. Absence of tannins was noticed.

Suspension properties of mucilage in species of *Abelmoschus*

The suspension properties were measured and compared for two species of *Abelmoschus*, *A. esculentus* and *A. moschatus*. Though comparable mucilage yield was obtained for *A. manihot*, the species was comparatively rare in the collection spots. Keeping in view the large-scale availability of *A. moschatus*, it was chosen for the study.

The suspension properties were evaluated using the different parameters such as sedimentation volume, flow rate, particle size analysis, pH, re-dispersion values and also viscosity of the suspension (Tables 6-8).

The sedimentation volume and particle size were found to be directly proportional to the concentration of the suspending agent (mucilage of both species of *Abelmoschus*) while the flow rate was inversely proportional. Compared to *A. esculentus* suspension, larger-sized particles were observed in *A. moschatus* suspension. pH values were slightly acidic to near neutral. The redispersion values were not consistent in *A. esculentus* while they were directly proportional to the concentration in *A. moschatus* mucilage. The values, however were relatively similar for both *A. esculentus* and *A. moschatus* mucilage suspensions.

Table 6: Results of the suspension properties

Suspending agent	Concentration	Sedimentation volume (%)	Flow rate (mls ⁻¹)	Particle size (µm)	pH	Re-dispersion(ml)
<i>A. esculentus</i>	0.5	0.20	1.10	3.40	5.40	0.50
	1.0	0.28	1.03	3.81	4.66	0.70
	1.5	0.34	0.78	3.68	4.99	0.10
	2.0	0.38	0.13	4.03	5.30	0.14
<i>A. moschatus</i>	0.5	0.25	1.23	3.31	5.80	0.60
	1.0	0.28	1.01	4.11	5.50	0.65
	1.5	0.30	0.93	4.28	5.20	0.80
	2.0	0.32	0.62	3.47	5.40	0.90

Table 7: Measurement of viscosity of *A. esculentus* mucilage suspension

Concentration (% w/v)	Viscosity (Kgm ⁻¹ s ⁻¹)10 ³						
	10 rpm	20 rpm	30 rpm	40 rpm	50 rpm	60 rpm	100 rpm
0.5	1.41	1.02	0.54	0.71	0.54	0.49	0.43
1.0	1.7	1.53	1.05	0.85	0.67	0.53	0.5
1.5	2.1	1.62	1.73	1.58	1.34	0.80	0.68
2.0	2.35	1.75	1.70	1.53	1.48	0.88	0.73

*Viscosity was measured repeatedly (unless similar values were obtained) and recorded.

Table 8: Measurement of viscosity of *A. moschatus* mucilage suspension

Concentration (%w/v)	Viscosity (Kgm ⁻¹ s ⁻¹)10 ³						
	10 rpm	20 rpm	30 rpm	40 rpm	50 rpm	60 rpm	100 rpm
0.5	1.53	1.49	1.11	0.71	0.54	0.49	0.45
1.0	1.48	1.35	1.3	0.7	0.52	0.55	0.42
1.5	1.57	1.51	1.23	0.87	0.63	0.57	0.53
2.0	1.82	1.54	1.47	1.33	0.68	0.61	0.58

The viscosity of mucilage was found to be directly proportional to the concentration of the suspension. However, the viscosity values were found to decrease when the suspension was subjected to increased centrifugation speed (10-100 rpm). The rheological behavior of the suspension prepared with *A. esculentus* and *A. moschatus* reveal that the suspensions are pseudoplastic in their behavior.

DISCUSSION

The mucilage in *Abelmoschus* (Malvaceae) as in many other species is a sticky substance that has viscous colloidal dispersion properties in water [22]. Mucilage yield was observed to be the best in immature fruits (4D.A.F) in the present study. Mucilage yield is known to vary

depending upon the climatic conditions and age of the crop [23]. Variations in mucilage yield observed between different species of *Abelmoschus* presently may be associated with habitat preferences between the wild and cultivated species.

The isolated mucilage in all the species was brownish amorphous when powdered and exhibited properties analogous to that extracted from many other plant species as noted previously [24]. Physicochemical properties revealed the advantageous features of *Abelmoschus* mucilage. Swelling index was high and was directly proportional to mucilage concentration. Likewise the slightly acidic or neutral pH is considered beneficial [23]. The ash values reflect the mineral content in mucilage. The mucilages usually appear as

calcium salts which have a significant effect in the water holding capacity and other biophysical properties [25]. Presence of carbohydrates, flavonoids, saponins and coumarins probably indicate the edible nature and pharmaceutical utility of *Abelmoschus* mucilage. Previous reports also confirm these results. *Abelmoschus* mucilage possesses stomach cytoprotective activity and induces gastric reduction. Absence of tannins indicates the purity of mucilage [12].

Suspension properties of mucilage in species of *Abelmoschus*

The performance of natural gums and mucilages is evaluated on the basis of suspending ability and suspension stability, which is assessed in pharmaceutical formulations [12, 26]. The rate of sedimentation of the suspending agent provides an idea about the suitability of the suspending agent in medical formulations. The suspending agent is considered better if its sedimentation rate is less. Even if the suspension produces sediment on storage it must be readily dispersible so as to ensure the uniformity of the dose. If the sediment remains even after shaking vigorously for specified time, the system is described as caked and is considered disadvantageous.

Suspensions are routinely evaluated for their rate of separation which indicates its suspending property. The sedimentation volume profiles of the suspensions are prepared with fixed concentrations of the drug powder such as paracetamol and varying concentrations of the test mucilage. To evaluate the suspending properties of mucilage in the two species of *Abelmoschus*, suspensions were prepared with varying concentration of mucilage and fixed concentration of paracetamol (0.5-2.0%). The sedimentation volume of *A.moschatus* suspensions gave similar results as that with *A.esculentus*. Previous studies have suggested *A.esculentus* to be the better suspending agent compared to traditional suspending agents like tragacanth and sodium CMC [12]. Sedimentation volume was found to be directly proportional to the concentration of the suspending agent as noted earlier [27]. The prepared suspensions of both *A.esculentus* and *A.moschatus* were also assessed for flow rate, particle size, pH, re-dispersion and viscosity. The flow rate of the suspension was inversely proportional to the concentration of the mucilage. The pH of the suspension indicates the acidic or basic nature of suspensions. Change in pH should be considered when suspensions are prepared with drugs. Near-neutral pH of *A.esculentus* and *A.moschatus* suspensions shows their suitability. In a study conducted on the mucilage suspensions of *A.esculentus*, the particulate matter was found to be redispersible irrespective of the concentration of the mucilage [12].

The rheological behavior of the suspensions is actually a measure of the viscosity. Viscosity and particle size were found to be directly proportional to the concentration of suspending agents. The suspension prepared with *A.esculentus* and *A.moschatus* mucilage revealed that the suspensions are pseudoplastic. The viscosity of mucilage suspension decreases with increase in shear rate, which is an essential property in the formulation of suspension [12, 28].

Thus the study reveals that the extracted mucilage of both *A.esculentus* and *A.moschatus* are edible and may be used as potential pharmaceutical adjuvants even at low concentrations (0.5-2%w/v). In view of these properties, *A.esculentus* mucilage was previously suggested to be utilized as a stabilizer and thickener when high viscosity was desired, especially in cosmetic, pharmaceutical and food industries [12]. Mucilage of the *A.esculentus* pods has been reported to have binder potential for tablet formulations [29, 30]. Present work also indicates the suitability of *Abelmoschus* mucilage, (both *A.esculentus* and *A.moschatus*) as suspending agents. Further studies are required for generating a complete profile for *Abelmoschus* mucilage with information about disintegrating, gelling and emulsifying properties. Mucilage extraction from *Abelmoschus* fruits or other plant parts, of especially the wild species seems a profitable venture.

CONCLUSION

The present study revealed the advantageous physical, physico-chemical and phytochemical properties of mucilage of *Abelmoschus moschatus* and *A.esculentus*. The study also suggests the suitability

of both the species as potential suspending agents in pharmaceutical formulations. Similar properties in both species implicate that *A.moschatus* mucilage can be exploited beneficially after further studies.

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