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Isolation and Characterization of Mucilage Obtained from *Abroma augustum* Leaf

Sunitha P[1], Sathyanarayana N[1], Uma Sanker A[1], Balasundaram[1], Francis Stanley[2], Sobana R[1], Srilahari N[2]

[1]Faculty of Medicine, AIMST University, Semeling, Bedong, Malaysia.
[2]Faculty of Biomedical Engineering, NUS, Singapore.

Corresponding Author: Sunitha P

ABSTRACT

Plant Mucilage is pharmaceutically important polysaccharide with wide range of applications. There were no studies on isolation and characterization of mucilage of *Abroma augustum*. Hence, the present study was focused on isolation of mucilage from *A. augustum* leaf which belongs malvaceae family. *Abroma augustum* leaf mucilage morphological characteristics, identification, solubility, pH, swelling capacity or water binding capacity, oil binding capacity, swelling index, viscosity and FTIR was studied. Physicochemical results showed this mucilage may be useful for tablet base as well as a suspending agent.
The mucilage studied by various analytical techniques like water binding capacity 1.45ml, oil binding capacity 1.54ml. swelling index 1100. pH 2.93. The swelling properties and mucoadhesion properties of the mucilage were determined in-vitro and it was found that the mucilage had high swelling capacities along with appreciable mucoadhesive strength. Moreover, from the analytical studies, it was found that the mucilage is amorphous in nature. Thus, the mucilage obtained from *A. augustum* can be used as a natural mucoadhesive polymer for formulating various drug delivery systems.

Key Words: Polysaccharide, Polymer, *Abroma augustum* mucilage, FTIR, characterization.

INTRODUCTION

Plant mucilage is naturally available sticky and gummy hydrocolloid substance with high molecular weight 200,000. The polysaccharide complexes formed from uronic acid and sugar. [1] Mucilage obtained from plants, epiphytes, marine algae, microorganisms and animals. They are obtained mainly from seeds, leaf, stem, roots, flowers of the plants. [2]

The plant derived mucilage was widely used in pharmaceutical preparations such as emulsifying, protective colloids in suspensions, binding, thickeners in oral syrups. Stabilizing, disintegrating, gelling agents in gels and bases in suppositories. They are widely used in cosmetics, textiles, paints, and paper making industries.

The natural mucilage is an easily available, compatible, nontoxic, biodegradable, cost effective, emollient and nonirritant substances. [3,4,5]

The synthetic mucilage's have many disadvantages such as high cost, toxic, environmental pollution during synthesis, non-renewable sources, side effects, less compatible etc. They are used in many cosmetics industries, textiles, paints and papermaking. [6, 7] Hence the present study was planned to isolate and characterize mucilage of *A. augustum* leaf.

MATERIALS AND METHODS

The *A. augustum* fresh leaves were collected from Perak state, Malaysia. The plant was authenticated by plant biotechnologist, Faculty of applied sciences. AIMST University, Malaysia.

Isolation of mucilage from *A. augustum* leaf.

The *A. augustum* fresh leaves were collected, washed with water, dried and

powdered. The leaves were crushed and soaked in water for 6 hours, allowed to complete release of the mucilage into the water. The mucilage was extracted using a muslin cloth bag to remove the marc from the solution. 95% of ethanol (in the volumes of three times to the volume of filtrate) was added to precipitate the mucilage. The mucilage was separated, dried in an oven at 40°C, ground, passed through a # 80 sieve and stored in a desiccator at 30°C and 45% relative humidity till use (Figure 1). [8]

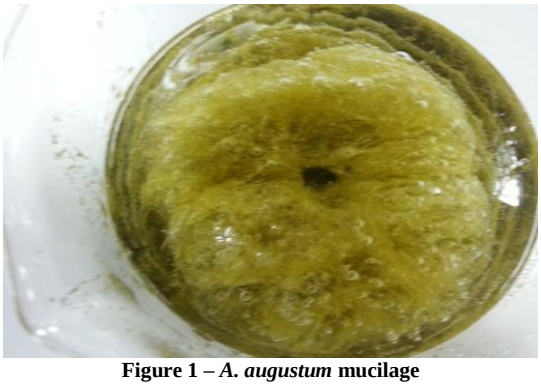

Figure 1 – *A. augustum* mucilage

Characterization of *A. augustum* mucilage.

1.Phytochemical screening

The Phytochemical screening of mucilage results showed Molisch's test for carbohydrates, Ninhydrin test for proteins, Wagner's test for alkaloids (Keller – Killaini test for glycosides, Ruthenium red test for mucilage (Figure no 2), Shinoda test for flavonoid, Ferric chloride test for Tannins (Table 3). [9]

2. Solubility of mucilage

The solubility of mucilage was studied by using solvents like water, acetone, polyethylene Glycol, Glycerin, Ethyl alcohol, methyl alcohol (Table 2).

3. Determination of the pH of mucilage.

Dispersion of 1% mucilage in 25 ml of distilled water by shaking for 5 minutes and determined the pH of mucilage solution using a digital pH meter (Merck, Mumbai) (Table 3).

4. Determination of the viscosity

A Brookfield Viscometer (Engineering labs, INC, Middle board, USA) was used to determine the intrinsic viscosity of 2% w/v mucilage at 28 ^{o} C. The spindle was inserted into the test solutions and viscometer was sheared at different speeds of 0.5, 2.5, 5, 10, 20, 50, 100 rpm at room temperature. The measured viscosities versus speed of rotation were plotted in graph (Figure 3). [10]

5. Water holding capacity

Water holding capacity of mucilage was determined using the modified method from chau and cheung (1998). The sample of 0.25 gr mucilage was dissolved in 25 ml distilled water in a centrifuge tube and heated for 30 minutes with continuous shaking at 80^{0}C. Thereafter, the suspension was centrifuged at 1000 rpm for 15 minutes, supernatant was removed, and the paste weight was noted. The swelling capacity was calculated by the ratio between the weight of paste and weight of dry mucilage (Table 3). [11]

Swelling capacity = wet sample weight - dry sample weight (g water/g dry sample weight)

6. Oil binding capacity

Oil binding capacity of mucilage powder determined by the oil absorption method. The samples were accurately weighed (0.5 g) into centrifuge tube, added with 10 ml sunflower oil mixed by vortex stirrer for 1 min kept at room temperature for 30 min and centrifuged at 10000 rpm for 30 min. Then the supernatant was removed, and the tube was kept upside down for 1 min. [12] Finally weighed the oil absorbed sample weight and calculated the oil absorption by the following equation: (Table 3).

Oil binding capacity = Oil absorbed sample weight - Dry sample weight (g oil/g dry sample weight)

7. Swelling index of mucilage

One gram of *A. augustum* mucilage powder was accurately weighed and transferred to a 100 ml stoppered measuring cylinder. The initial volume of the powder in the measuring cylinder was noted. The volume was made up to 100 ml with distilled water. The cylinder was stoppered, shaken gently and set aside for 24 h. The volume occupied by the mucilage was noted after 24 h. [13] Swelling index (SI) is expressed as a percentage and calculated

according to the following equation (Table 3).

Where X_O is the initial height of the powder in graduated cylinder and Xt denotes the height occupied by swollen mucilage after 24 h.

The content from the measuring cylinder from the above test were filtered through a muslin cloth and water was allowed to drain completely into a dry 100 ml graduated cylinder. The volume of water collected was noted and the difference between the original volume of the mucilage and the volume drained was taken as water retained by sample and was referred to as water retention capacity or water absorption capacity.

Swelling Index (SI) = $Xt\text{-}X_0/X_O$ X 100

8. Fourier transform infrared spectroscopy.

Fourier transform infrared (FTIR) spectrum of *A. augustum* dried mucilage was recorded on samples prepared in potassium bromide (KBr) disks using FTIR spectrophotometer (JASCO 4100, Japan). Samples were prepared in KBr disks and placed in sample holder. The samples spectral scanning range was 500–4000/cm, at resolution of 4cm-1, with scan speed of 1cm/sec. (Figure 2) (Table 4). [10]

RESULTS AND DISCUSSION

Physical properties of mucilage

The *Abroma augustum* mucilage was light brownish in colour, odourless, tasteless, irregular shape and hard nature (Table 1).

Table 1: Physical properties of mucilage.

S.No	Parameter	*Abroma augustum* mucilage
1.	Colour	Light Brownish white colour powder.
2.	Odour	No odour
3.	Taste	Tasteless
4.	Shape	Irregular
5.	Touch and Texture	Rough and Hard

Phytochemical screening

Phytochemical analysis of mucilage showed the presence of starch and absence of tannins, alkaloids, proteins, flavonoids, reducing sugars, glycosides (Table 2).

Table 2: Phytochemical properties of *Abroma augustum* mucilage.

S.no	Tests	Results
1.	Molisch's test (Carbohydrates)	Positive (+)
2.	Ferric chloride test (Tannins)	Negative (-)
3.	Wagner's test (Alkaloids)	Negative (-)
4.	Ninhydrin test (Proteins)	Negative (-)
5	Shinoda test (Flavonoid)	Negative (-)
6	Benedict test (Reducing sugar)	Negative (-)
7	Keller-Killaini test (Glycosides)	Negative (-)
8	Ruthenium red test (Mucilage)	Positive (+)
9.	Iodine test (Starch)	Positive (+)
	Positive (+)	Negative (-)

Physicochemical properties

The mucilage was studied for physicochemical parameters such as solubility, water binding capacity, oil binding capacity, swelling index, and viscosity. The swelling index was found to be 1100 and the pH of mucilage was found to be 2.93 which indicate that this mucilage will be less irritating to gastrointestinal tract and suitable for suspending agent in suspensions (Table 3,4).

Table 3: Solubility nature of *Abroma augustum* mucilage.

S. No.	Solvents	Solubility
1.	Cold water	Swell to form a gel
2.	Luck warm water	Soluble in luck warm water
2.	Hot water	Soluble
4.	Ethanol	Insoluble
5.	Methanol	Insoluble
5.	Benzene	Insoluble
6.	Acetone	Insoluble
7.	Ether	Insoluble

Table 4: Physicochemical Properties of Abroma *augustum* mucilage.

Parameters	Results
pH of mucilage	2.93
Swelling capacity or water binding capacity.	1.45ml
Oil binding capacity	1.54ml
Particle size	122.6
swelling index of mucilage	1100
Viscosity	0.8872cP

Fourier Transform Infrared Spectroscopy of mucilage.

FTIR is a useful for identification and purity of a compound. The principal absorption peaks of *A. augustum* mucilage were found at 3290cm-1(primary OH), 2950cm-1, 2850m-1(C-H symmetric stretching), 1740cm-1(C=O Stretching, aldehyde absorption), 1000cm-1(C-O-C, ether group absorbance), which indicates that mucilage was polysaccharide (Figure 2) (Table 5).

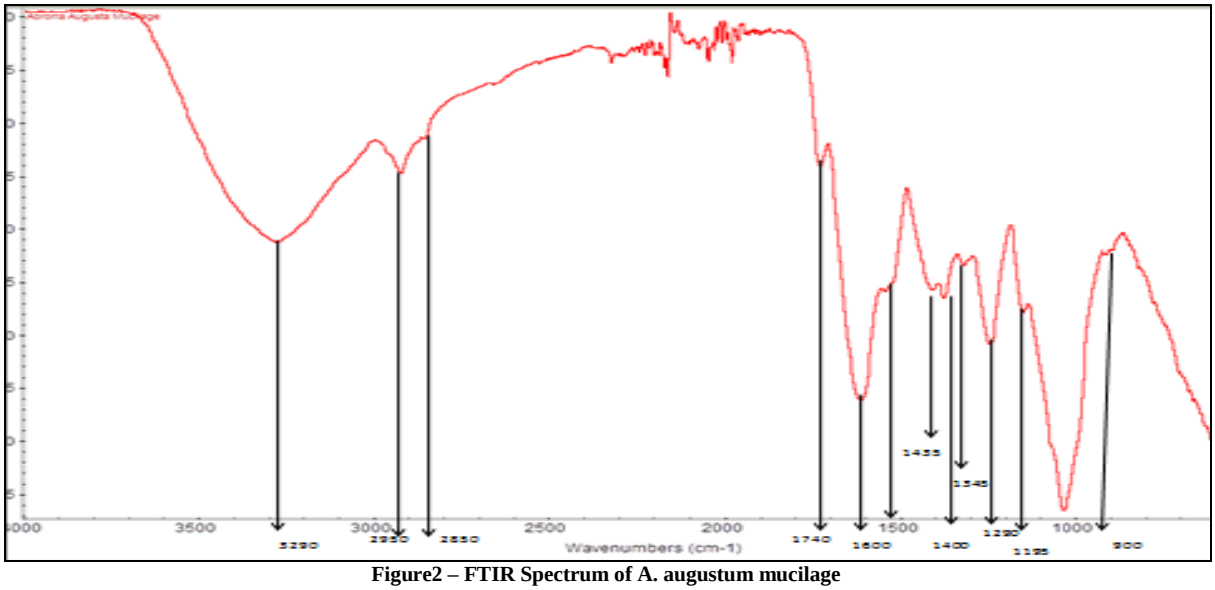

Figure2 – FTIR Spectrum of A. augustum mucilage

TABLE -5. FTIR results of *A. augustum mucilage*.		
S.no	Absorption peak value (cm^{-1})	Type of bond - Functional Groups
1	3290	O-H
2	2950	C-H symmatric stretching
3	2850	C-H symmatric stretching
4	1740	C=O Stretching
5	1600	Bending mode of H_2O
6	1500	C-H bending
7	1435	C-H bending
8	1400	-OH bending
9	1345	C=O Stretching vibration
10	1290	C=O stretching
11	1195	C=O asymmetric bridge stretching
12	1000	C-O-C Pyranose ring

The spectra of *A. augustum* mucilage composed of non-polar chemical groups such as alkyl groups like methyl and the hydrophilic characteristic of mucilage is due to presence of hydroxyl, carbonyl and amino groups. The mucilage exhibited the osmotic property of retaining water is due to its high concentration of hydroxyl groups.

Viscosity of *A. augustum* mucilage

A. augustum mucilage exerted the less oil binding capacity due to hydrophilic properties which formed colloidal emulsion and exerted good emulsifying agent. The measured viscosity of a fluid can be seen to behave in one of four ways: (a) Viscosity remains constant no matter what the shear rate (Newtonian behavior). (b) Viscosity decreases as shear rate is increased (shear thinning). (c) Viscosity increases as the shear rate is increased (shear thickening). (d) Viscosity appears to be infinite until a certain shear stress is achieved (Bingham plastic behavior). [14,15]

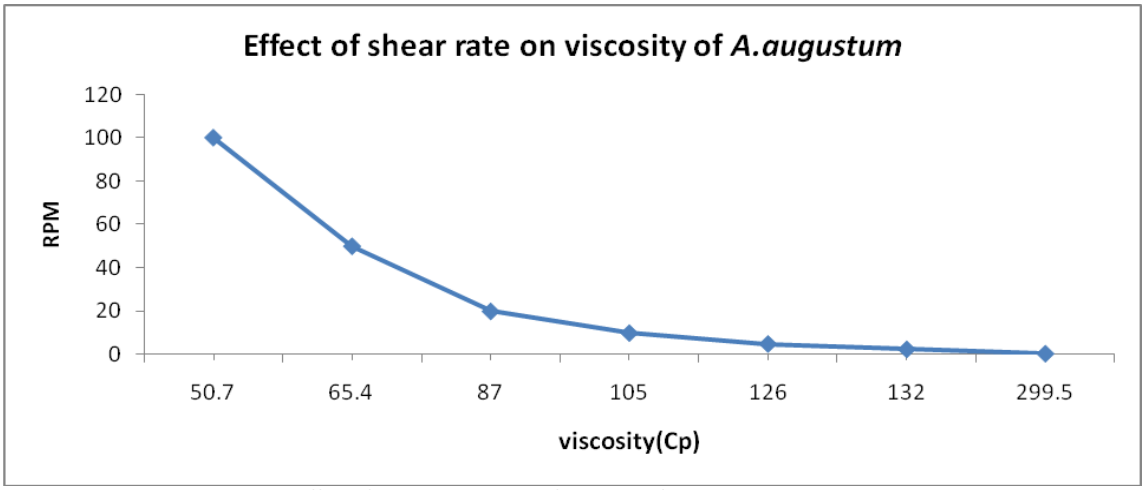

Figure 3 - Effect of shear rate / speed of viscosity of *A. augustum* 2%w/v mucilage.

Knowledge of the viscosity of a material like *A. augustum* mucilage at various shear rates is very important for processing, formulation and quality control. *A. augustum* mucilage showed shear thinning behavior which means the viscosity decreased as the shear rate increased. The mucilage probably orients itself more parallel to the spindle surface, thereby decreasing its resistance to spindle rotation. At faster spindle rotation more molecules formations are "destroyed" with the result that less of the molecules slide together leads to low viscosity. [14] In rheological studies, the relationship between shear stress and shear rate defines the flow characteristics of the material. The viscosity of the mucilage obtained was found to be directly proportional with the concentration of the suspension. It was also found that, with the increase in centrifugation speed of suspension solution, the viscosity of the mucilage gradually decreases. This proves that the suspension prepared from *A. augustum* is having shear thinning behavior. The knowledge of *A. augustum* mucilage helps for the development of new formulations in novel pharmaceutical excipient.

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