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Original Article

EXTRACTION, PURIFICATION AND PHYSICOCHEMICAL EVALUATION OF MUCILAGE OF *CHRYSOPHYLLUM LANCEOLATUM* (BLUME) DC FRUITS: AN INVESTIGATION FOR BIOADHESIVE PROPERTY

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ABSTRACT

Objective: The objective of the present investigation is to extract, purify and characterize the physicochemical properties of the mucilage obtained from the fruits of *Chrysophyllum lanceolatum* (Blume) DC and also to evaluate the bioadhesiveness of *Chrysophyllum lanceolatum* Mucilage (CLM) to be used as the biodegradable mucoadhesive agent.

Methods: The mucilage was extracted, purified and identified by the methods as described in the official books. Purified CLM was characterized for physicochemical properties, swelling capacity, loss on drying and flow properties. The CLM was further characterized by Fourier Transform Infrared (FTIR) spectroscopy, Differential Scanning Calorimetry (DSC) and High-Performance Liquid Chromatography (HPLC). The mucoadhesivity of the mucilage was assessed by shear stress method and falling sphere method using goat intestine as mucosal substrate and the results were compared with Hydroxy Propyl Methyl Cellulose (HPMC) and Carbopol 934P (CP). The acute oral toxicity study was also conducted in Swiss Albino mice.

Results: The nature of the CLM is confirmed as polysaccharide from the experimental data. The physicochemical property and toxicity study also shows its acceptability as bioadhesive excipient. The bioadhesive test of CLM showed a good adhesive strength to the biological membrane.

Conclusion: The present investigation showed better bioadhesive property of the isolated mucilage and hence may provide an alternative to conventional synthetic/semi-synthetic mucoadhesive agents.

Keywords: *Chrysophyllum lanceolatum,* Mucilage, Extraction, Physicochemical, Bioadhesivity, Mucosal.

INTRODUCTION

Mucoadhesion in drug delivery systems has recently gained interest among pharmaceutical scientists as a means of increasing gastric residence time of dosage forms as well as improving contact period with various biomembranes. Bioadhesion can be defined as the state in which two materials, at least one of which is biological in nature, are maintained together for a prolonged time period by means of interfacial forces [1]. A mucoadhesive controlled release system can improve the effectiveness of treatment by many ways. Firstly, by maintaining the drug concentration within the therapeutic level for a longer period of time. Secondly, the targeting and localization of a drug at a specific site can be achieved by inhibiting the dilution of the drug in the body fluids. Thirdly, the bioavailability of the drug with a smaller dose can be attained by reducing the frequency of administration [2, 3]. Mucoadhesive drug delivery systems utilize the property of bio-adhesion of certain water soluble polymers which become adhesive on hydration [4] and hence can be used for targeting a drug to a particular region of the body for an extended period of time [5]. The mucosal layers exist on a number of regions of the body including the GIT, the urogenital tract, the airway of the ear, nose and the eye. Hence, the mucoadhesive drug delivery systems can be classified as following types [6].

(a) Gastrointestinal mucoadhesive drug delivery system, and

(b) Vaginal, Ocular, Nasal, Rectal, Buccal and Sublingual muco-adhesive drug delivery systems.

Natural bio-adhesives have advantages in comparison to the synthetic and semi-synthetic polymers as they are non-toxic, easily available, sustainable, biodegradable, biocompatible and eco-friendly [7, 8]. Currently, the use of biodegradable polymers in dosage forms are preferred over non-biodegradable polymers because their breakdown products are biocompatible after releasing the drug from the matrices which in turn also overcome various toxicity problems of other polymers. The plant *Chrysophyllum lanceolatum* (Blume) DC belongs to the family Sapotaceae. The common name of the fruit is Star apple/Indian star apple. The local names in Assamese are Bonpitha and Pithogarkh. In Marathi and Bengali, it is called Tarsi and Petakara respectively. The plant is found in North east India and Western ghats up to 4000 ft. It is a tall evergreen tree about 70-120 ft high and bears insipid globose fruits of size 1.5-2.0 inch diameter with translucent whitish flesh surrounding 3-8 seeds [9]. The fruits have a firm, sticky, sweet pulp and are edible [10]. Fruits are berry rusty tomentose when young, yellow and soft when ripe and greedily eaten by the natives. The fruit pulp is tolerably firm but exceedingly clammy, adhering to the lips or knife with great tenacity [11-13]. The fruits contain amino acids such as aspartic acid, glutamic acid, proline and lysine and minerals [14].

The objective of the present investigation is to extract, purify and characterize the physicochemical property of the mucilage obtained from the fruits of *Chrysophyllum lanceolatum* (Blume) DC and also to evaluate the suitability of the mucilage as biodegradable muco-adhesive agent.

MATERIALS AND METHODS

Fruit pulp

The fruits of *Chrysophyllum lanceolatum* (Blume) DC were collected by hand picking from the forest near Dibrugarh, Assam (India) during the month of October-January. The fruit was identified and authenticated at the Botanical Survey of India, Shillong, Meghalaya, Vide No.: BSI/ERC/2012/Plant identification/518 dtd. 07.01.2013.

Chemicals and reagents

Acetone, Ethyl alcohol, Diethyl ether, Trichloroacetic acid (TCA), Sodium hydroxide, Hydrochloric acid, HPMC, CP, n-Octanol were purchased from E Merck India Ltd, Mumbai, India. D-galactose, D-galacturonic acid, Arabinose, Mannose, Glucose, Rhamnose and Fructose were purchased

from Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore, Karnataka, India. All other chemicals used were of analytical grade.

Apparatus and instruments

Electronic balance (Sartorius, Germany), Centrifuge machine (Remi, Model-R100, Mumbai, India), Magnetic stirrer (Remi), Brookfield viscometer (Model DV-E, Brookfield, USA), Hot air oven (Remi), Tapped density tester (ACM-157, Acmus Technocracy, New Delhi), pH meter (Multiparameter tester 35, EUTECH, OAKION, China), Differential Scanning Calorimeter (JADE DSC, Perkin Elmer, USA), Fourier-transformed Infrared Spectrophotometer (Perkin Elmer, USA), High Performance Liquid Chromatography (Waters, USA), UV-Visible Spectrophotometer (Jasco V-530).

Animals

Healthy male and female Swiss albino mice weighing 20-25 g (8 w) were supplied by M/S Chakraborty Enterprise, Kolkata, India. The experimental protocols were approved by the Dibrugarh university vide its Approval No. IAEC/DU/43 dtd. 24.9.13 and procedures followed were in accordance with the ethical committee's guidelines.

Extraction and purification of mucilage from the fruits of *Chrysophyllum lanceolatum* [15, 16]

Extraction

The fruit pulp was soaked in distilled water for 5-6 h, boiled for 30 min and then allowed to stand for 1h. The slurry was filtered through muslin cloth and the filtrate was centrifuged at 500 rpm for 20 min. Supernatant was concentrated at 60 °C to 1/3rd of its volume to which 3 volumes of acetone was added. The precipitate was separated by filtration and the mucilage so obtained was dried in an oven at 40 °C. It was ground, passed through 80 mesh sieve and stored the mucilage in a desiccator until used.

Purification[17, 18]

The 100 ml of crude mucilage (1.0% w/v in water) was homogenized with 10 ml of 5.0% w/v cold TCA solution. The solution was centrifuged at 3500 rpm for 20 min and neutralized with the dropwise addition of 5.0% w/v sodium hydroxide solution. The mucilage was precipitated from the supernatant by the addition of three parts by volume of 95% v/v ethanol, washed successively with ethanol, acetone and diethyl ether. The mucilage so obtained was dried under vacuum, passed through 80 mesh sieve and stored in a desiccator at 30±2 °C/45±5% relative humidity till its use.

Characterization of the Mucilage (CLM) [19-22]

Physical characterization

The purified mucilage was evaluated for physical characteristics viz., appearance, odour, taste, percentage yield, loss on drying, moisture content, total ash, acid insoluble ash, melting point and charring temperature according to the procedures as described in Indian pharmacopoeia [19]. All these values were tested in triplicate.

Loss on drying and moisture content

Loss on drying was determined by drying accurately weighed the quantity of 1 g of CLM at 105±5 °C in a hot air oven till a constant weight of the mucilage was obtained. Loss on drying and moisture content were calculated by the following formulae:

$$\%\text{Loss on drying} = \frac{\text{weight lost after drying}}{\text{total weight before drying}} \times 100 \quad (1)$$

$$\%\text{Moisture content} = \frac{\text{weight loss}}{\text{dry weight}} \times 100 \quad (2)$$

Ash value determination

Total ash content

1 g of CLM was accurately weighed and evenly distributed in the crucible, dried at 150 °C for 1 h and then ignited at 450 °C for 15 min in an incinerator. Total ash was calculated by the following formula:

$$\text{Total ash} = \frac{W_1 - W_2}{W_3} \times 100 \quad (3)$$

Where, W_1 =weight of crucible after ignition, W_2=Blank weight of crucible and W_3=weight of mucilage.

Acid-insoluble ash

The ash obtained above was boiled in 25 ml of 2M HCl for 5 min. The insoluble ash was collected on ashless filter paper and washed with hot water. The insoluble ash was transferred into a silica crucible, ignited and weighed. The procedure was repeated to get a constant weight. The % acid insoluble ash was calculated using the above formula.

pH value determination of CLM

The pH of 1% w/v solution of CLM in distilled water was determined using a digital pH meter at 25 °C.

Chemical characterization of CLM [19, 20]

The extracted mucilage was subjected for various identification tests for the presence of carbohydrate, tannins, alkaloids, amino acids, glycosides, etc.

Solubility study of CLM [21]

The mucilage was evaluated for solubility in different solvents in accordance with the specifications of India Pharmacopoeia.

Flow properties of CLM [34]

The dried mucilage was tested for the flow properties viz., angle of repose, bulk density, tapped density, compressibility index and Hausner's ratio.

Angle of repose of CLM

Dried mucilage, 10 g was poured into an open-ended glass cylinder with its bottom resting on a horizontal surface at it's base. On raising the cylinder vertically, the powder flowed out and formed a conical heap as a result of gravitational force balancing the inter-particulate forces. The side of the heap formed an angle with the horizontal base known as the angle of repose. The height of the cone was measured with the aid of a pair of dividers and a ruler. The angle of repose (θ) was calculated using the equation:

$$\theta = \frac{\tan^{-1} h}{r} \quad (4)$$

Where, h=height of conical powder heap, r=radius of the circular base.

Bulk density (BD)

The bulk density of the powder bed is simply the weight of the powder divided by the whole volume of the bed. The mucilage powder, 25 g was poured inside a measuring cylinder through a funnel at atmospheric pressure. The bulk volume (V_1) was recorded and the density determined in triplicate. The bulk density (BD) was determined using the following equation:

$$BD = \frac{M}{V_1 \pi r^2 h} \quad (5)$$

Where, M = weight of powder (g), V_1 = volume of the powder bed, r = radius of the cylinder (cm), h = height of the powder column in the cylinder.

Tapped density (TD)

Accurately weighed 25 g of mucilage powder sample was transferred to a 100 ml graduated cylinder. Tapped the cylinder containing the powder by raising the cylinder and allowed it to drop under its own weight using mechanically tapped density tester that provides a fixed drop of (14±2) mm at a nominal rate of 300 drops per min. The cylinder was tapped for 500 times initially and measured the tapped volume (V_1) to the nearest graduated units. Repeated the tapping for additional 250 times and measured the tapped volume (V_2) to the nearest graduated units. Ratios between the two volumes were calculated in g/ml by the following formula.

$$TD = \frac{\text{weight of powder}}{V_2} \quad (6)$$

Hausner's ratio

Hausner's ratio is a number that is correlated to the flowability of a powder and calculated as

$$\text{Hausner}'\text{s ratio} = \frac{TD}{BD} \quad (7)$$

Car's index

Compressibility of the powder was determined by Car's Compressibility index. It is a simple test to evaluate the BD and TD of a powder and the rate at which it packed down. The formula for Car's index is as follows:

$$\text{Car}'\text{s index (\%)} = \frac{[(TD - BD) \times 100]}{TD} \quad (8)$$

Swelling index and swelling capacity of CLM [16]

Accurately weighed 1 g of the purified mucilage was transferred to a 50 ml stoppered measuring cylinder. The initial volume of the powder in the measuring cylinder was noted. The volume was made up to 50 ml mark 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 (V_2). The ratio of the difference of the initial and final volume ($V_2 - V_1$) to the initial volume (V_1) is the swelling capacity.

$$\text{Swelling capacity} = \frac{V_2 - V_1}{V_1} \quad (9)$$

Swelling index (SI) is expressed as a percentage and calculated according to the following equation:

$$SI = \frac{V_2 - V_1}{V_1} \times 100\% \quad (10)$$

Apparent viscosity of CLM

The apparent viscosity of 1%, 2%, 5% and 10% w/v solution of the mucilage powder in water was determined using a Brookfield viscometer; spindle-64 at 50 rpm.

FT-IR spectroscopy of CLM

The presence of functional groups on the purified mucilage was analysed by infrared spectroscopy. The dried mucilage powder was mixed with KBr and pressed into pellets under mechanical pressure. Then the FT-IR spectra were recorded by scanning within the range of 450-4000 cm^{-1} and analyzed for the presence of different functional groups.

DSC of CLM

DSC was performed to observe the occurrence of exothermal and endothermal changes in the mucilage sample with an increase in temperature. DSC measures the heat loss or gain, resulting from physical or chemical changes within a sample as a function of temperature. It is extensively used to study phase transition of polymers because of its sensitivity and accuracy. DSC thermogram of the dried mucilage powder was recorded at a heating rate of 10 °C/min from 40 to 300 °C. Nitrogen gas purging was maintained at 20 ml/min. Empty aluminium pan was taken as a reference during the analysis.

Total carbohydrate content [23, 24]

The total carbohydrate content of CLM was determined by a phenol-sulphuric acid method taking D-galactose as standard. The uronic acid content was analyzed by carbazole test taking D-galacturonic as standard. The absorbance was measured on UV-Visible Spectro-photometer at 490 nm and 535 nm for total carbohydrate and uronic acid respectively.

Monosaccharide composition analysis by HPLC

CLM was analyzed for monosaccharide composition after its acid hydrolysis. The HPLC system used had 515 HPLC Pump (Waters, USA) which was equipped with a 20 µl sample injection loop linked with carbohydrate analysis column (3.9 × 300 mm, Waters) and a refractive index detector (2414 RI Detector, Waters, USA). Acetonitrile/water (80/20, v/v) was prepared and used as the mobile phase. The flow rate was maintained at 0.8 ml/min and the detector sensitivity was set at 128×. The analysis was performed at room temperature (30 °C). About 10 mg of the purified mucilage was taken in 5 ml of 2.5 M HCl and vortexed for few minutes to improve the solubility. It was then hydrolyzed at 100 °C for 3 h, cooled and neutralized with sodium carbonate solution until effervescence ceases. The volume was made up to 10 ml with deionized water and centrifuged. The supernatant was filtered through 0.22 µm syringe filter and 20 µl was injected into the system for each analysis.

***Ex-vivo* mucoadhesive property of CLM [25]**

Ex-vivo mucoadhesivity was determined by using shear stress measurement and Falling sphere method taking HPMC and CP as reference mucoadhesive polymer.

Shear stress measurement [26, 27]

The shear stress measures the force that causes a mucoadhesive to slide with respect to the mucus layer in a direction parallel to their place of contract of adhesion. The test was performed by using a different concentration of mucoadhesive agent's viz. 1%, 2%, and 3% w/v solution of CLM, HPMC and CP in water.

A specified amount of prepared solution was spread on a glass plate. Another clean slide was placed over the first plate and made to spread the polymer solution uniformly in between two glass plates by placing 100 g weight on the glass plates. It was allowed to remain undisturbed for 15, 30 and 60 min. Then one side of the glass plate was fixed on a hook and the other was connected to a string passing over a pulley and at the end the weight was attached. After that, an interval of 15, 30 and 60 min, the weight was placed in an increasing manner till the plates attached with polymer solutions got detached. The weight which just detached the glass plates was noted and the average values were tabulated for calculation of mucoadhesive force.

Falling sphere method [28, 29]

To determine the mucoadhesive strength, the falling sphere method was used. A clean burette was taken and filled with 10% mucus solution obtained from freshly excised goat intestine by scrapping the intestine. The burette was fixed on a burette stand. Mustard grains which retained on sieve size # 40 were taken and dipped in the polymer solutions (CLM, HPMC, and CP) of different concentrations viz. 0.5, 1.0 and 3.0% w/v solution in water. After few min they were strained out and dried. The dried grains were slowly placed at the top of the mucus solution and allowed to settle down. Time taken by the grain to fall 50 divisions in the burette was noted and the values were tabulated.

Acute oral toxicity study [30]

The acute oral toxicity study was conducted on healthy male and female Swiss albino mice (8 w) by following OECD guideline (OECD guideline for testing of chemicals 425, 2001). Mice were supplied by M/S Chakraborty Enterprise, Kolkata, India. The animals were housed in polypropylene cages and provided with bedding of clean paddy husk. The animals were acclimatized to laboratory conditions for one week prior to the experiment. The temperature of the animal house was maintained at 25±2 °C with a relative humidity of 70% and illumination cycle set to 12 h light and 12 h dark.

The mice were fed with standard laboratory pellet feed. All the mice were fasted overnight before experimentation and were allowed to take food 1 h after the experiment. The dried powder of the purified mucilage was administered orally at a test dose of 2000 mg/kg b.w. in distilled water. One animal was dosed at a time and observed for 48 h after which the second animal was dosed and observed for any mortality and morbidity (convulsions, tremors, grip strength and pupil dilatation). Accordingly six animals were tested from which results of LD_{50} was made based on the study. The study was approved by the Institutional Animal Ethical Committee of Deptt. of Pharmaceutical Sciences, Dibrugarh University, India (Reg. No, 1576/GO/a/11/CPCSEA, India).

RESULTS AND DISCUSSION

Characterization of CLM

Physical characterization of CLM

After isolation of mucilage from the fresh fruit pulp of *Chrysophyllum lanceolatum* by ethyl alcohol, the yield of purified mucilage was found to be 10.17% w/w. The results of laboratory analytical data for physicochemical characteristics of the dried mucilage are presented in table 1. The purified mucilage was reddish brown in colour with characteristic odour and sweetish sour in test.

The pH of 1% w/v solution of CLM in distilled water at 25 °C was found to be 4.7 which indicate that this mucilage would be less irritating in GIT and suitable for an uncoated tablet. The melting point was 156-158 °C with a colour changing point of 235 °C.

The swelling capacity of CLM was 2.50±0.50 which was slightly less than HPMC (7.43±0.08) and less than CP (17.90±0.05) suggesting its moderate swellability. Swelling is one of the primary characteristics for a polymer to be bioadhesive. But excessive swelling due to over hydration always leads to the formation a slippery surface. Hence, retention of a delivery system at the site of application is practically impossible. Further, it is not suitable for a solid adhesive dosage form because of the loss of mechanical strength and structural integrity due to excessive swelling [31].

The Pharmacopoeial limit for moisture content of natural gums and mucilages has been set at ≤15.0% [32]. The moisture content of CLM was found to be 14.50±0.04% which was also expressed as percentage loss on drying (12.32±0.53%) and is comparable with other fruit mucilages [32]. The total ash content is designed to measure the total amount of residual material remaining after ignition. This includes both physiological ash, which is derived plant tissue itself and non-physiological ash comprising extraneous matters such as sand and soil. The maximum limit for total ash for food and pharmaceutical quality gum acacia and tragacanth is set at 4.0% w/w [32]. The result showed that the total ash value for CLM is 4.00±0.03% which is within the range of other natural gum and mucilage like acacia and tragacanth.

Table 1: Physicochemical properties of the mucilage (CLM)

Parameter	*Chrysophyllum lanceolatum* mucilage (CLM)
Test for mucilage (ruthenium red)	Positive
Colour	Reddish brown
Odour	Characteristic
Taste	Sweetish sour
Swelling index	150.00±0.65 (in %)*
Swelling capacity	2.50±0.50
Moisture content	14.50±0.04 (in %)*
Loss on drying	12.32±0.53 (in %)*
Total ash	4.00±0.03 (in %)*
Acid insoluble ash	1.00±0.02 (in %)*
pH (at 25 °C)	4.7±0.05*
Melting point	156–158 °C
Charring temperature	235 °C

*Each value in the table is represented as mean±SD. (n=3)

Table 2: Preliminary phytochemical screening of the mucilage (CLM)

Active constituent	Test	Inference
Alkaloids	Dragodroff's, Mayer's and Wagner's test	Negative
Amino acids	Ninhydrin test	Negative
Carbohydrates	Molish's, Benedict, Barfoed's and Iodine test	Positive, Negative for Starch
Fats and Oils	Grease test	Negative
Flavonoids	Lead acetate test	Negative
Glycoside	Liebermann's and Brantrager's test	Negative
Saponin	Foam test	Negative
Protein	Biuret test	Negative
Tanins and Phenolic compounds	Lead acetate and Ferric chloride test	Positive

Chemical characterization of CLM

The isolated and purified mucilage gave a positive test for mucilage by red ruthenium test. Phytochemical investigation of isolated mucilage showed the presence of carbohydrate, tannins and phenolic compounds while the test for alkaloids and proteins found to be negative. The results obtained for phytochemical tests are tabulated in table 2.

Solubility of CLM

The result of solubility of the mucilage in different solvents is presented in table 3. From the results, it is evident that CLM is sparingly soluble in cold water, but forms a viscous colloidal dispersion in hot distilled water and insoluble in most of the organic solvents. The solubility of CLM in hot water forming a clear viscous solution may be due to the gelling properties of mucilage. Mucilage used in the tablets swells to form a gel layer, and drug release is retarded up to certain extent. At higher concentration mucilage used in the tablets, swells and drug release is increased due to burst effect.

Flow properties of CLM

The result of the observed flow properties of the mucilage are presented in table 4. The bulk and tapped densities signify the packing arrangement of the particles and the compaction behaviour of a material. The flow property of a powder is necessary to be considered for industrial application of excipients [33]. The Carr's index of CLM was found to be 25.03±0.05 indicating the mucilage has fairly good flow property and compressibility. The angle of repose of CLM was found to be 35.52±0.11 which also indicated that even though the mucilage is not free-flowing, it has acceptable flow property [34].

Apparent viscosity of CLM

The viscosity of CLM was found to be dependent on concentration. It increases with increase in mucilage concentration. The effect of concentrations of the mucilage on viscosity is shown in fig. 1. 1 to 10% w/v solutions of mucilage showed viscosity ranging from 145 to 850 cps.

Table 3: Solubility of the mucilage (CLM) in different solvents

Solvent	Inference
Cold distilled water (25 °C)	Sparingly soluble
Hot distilled water (60 °C)	Viscous colloidal solution
Acetone	Insoluble
Chloroform	Insoluble
Ethanol	Insoluble
Methanol	Insoluble
n-butanol	Insoluble
n-hexane	Insoluble
Dichloromethane	Insoluble
Diethyl ether	Insoluble

Table 4: Flow properties of the mucilage (CLM)

Parameter	Value
Bulk density (g/cm^3)	0.5±0.04
Tapped density (g/cm^3)	0.7±0.03
Hausner's ratio	1.42±0.06
Car's index (%)	25.03±0.05
Angle of repose (θ °)	35.52±0.11

Each value in the table is represented as mean±SD. (n=3)

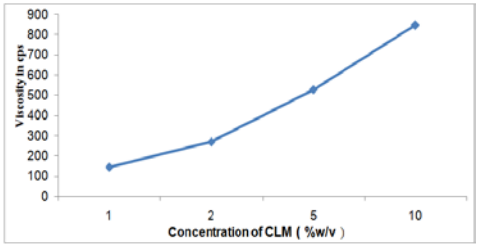

Fig. 1: Apparent viscosities (cps) at different concentrations of CLM FT-IR spectroscopy of CLM

The FT-IR spectrum is used to determine the major functional groups present in the structure. The recorded FT-IR spectrum of CLM is presented in fig. 2 which shows a characteristic spectrum for polysaccharides. The peak present at 3266 cm^{-1} (OH stretching) is attributed to hydroxyl groups, while the peaks at 1731 cm^{-1}, at 1631 cm^{-1} and at 1440 cm^{-1} (C=O stretching) are may be due to carboxyl groups [35]. Specifically the peaks at 1331 cm^{-1}, 1224 cm^{-1}, 1141 cm^{-1} (C-O-C),1073 cm^{-1} (C-H aromatic) and at 1011 cm^{-1} (C-O) indicate the presence of uronic acid and O-acetyl groups; and peaks at 951 cm^{-1}(O-H), at 920 cm^{-1} (OH) and at 830 cm^{-1}(C-H bending aromatic ring) are taken as evidence for β-and α-linkages in the molecule respectively[36].

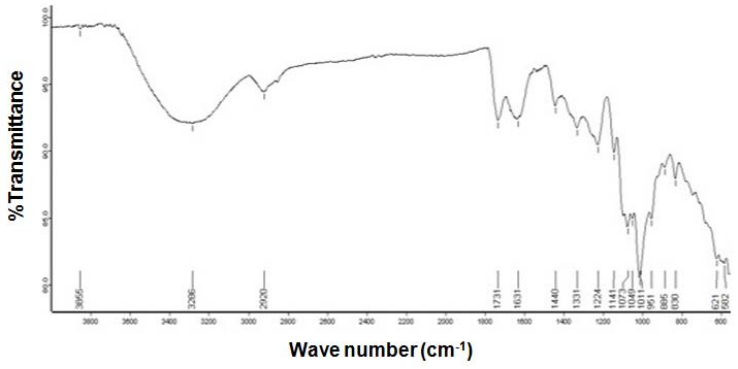

Fig. 2: FT-IR spectra of mucilage powder of CLM

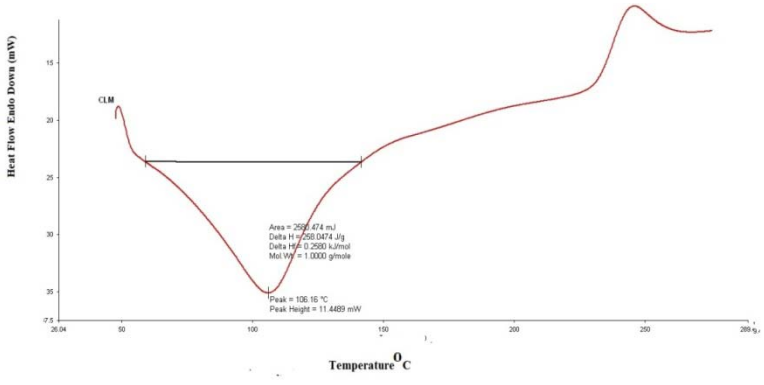

Fig. 3: DSC thermogram of powdered CLM

DSC study of CLM

The DSC thermogram of the mucilage is depicted in fig. 3 which presents a typical plot for polysaccharides. CLM exhibited an early endothermic peak at 106.16 °C that can be attributed to the loss of water present in the compound [37]. The onset temperature occurred at 55 °C and end state temperature at 145 °C. A sharp melting peak observed at around 106 °C with an enthalpy value of 258.0474 Jg^{-1}. Glass transition temperature, onset and melting peak are reported to be correlated with structural stability and crystallinity of the mucilages [35]. Lower brancing of the structure creates a higher binding energy between the backbone monosaccharides which results in a higher value of ΔH value. The observed enthalpy change for the thermal transition process of CLM was higher suggesting good thermal stability (fig. 3).

Monosaccharide composition of CLM

The polysaccharide characterization depends on the determination of its purity which is reflected by its chemical composition, including total carbohydrates, the amount of uronic acids, ash, moisture, etc. [22]. The total carbohydrates and amount of uronic acids of CLM were found to be 71.853±5.67% and 8.613±1.95% respectively.

The high carbohydrate content of the mucilage indicates its purity. The monosaccharide composition analysis of hydrolyzed CLM by HPLC is given in table 5. The main sugar components are rhamnose (48.288±1.09%) and fructose (22.6885±1.08%) with small amounts glucose, arabinose and mannose. The HPLC chromatogram of the acid hydrolysed mucilage is depicted in fig.

Table 5: Carbohydrate composition of CLM

Constituent sugar	% of constituent sugar present in CLM
Total carbohydrate	71.853±5.67
Uronic acids	8.613±1.95
Arabinose	0.1175±0.04
Fructose	22.6885±1.08
Galactose	Absent
Glucose	0.155±0.05
Mannose	0.604±0.08
Rhamnose	48.288±1.09

Each value in the table is represented as mean±SD. (n=3)

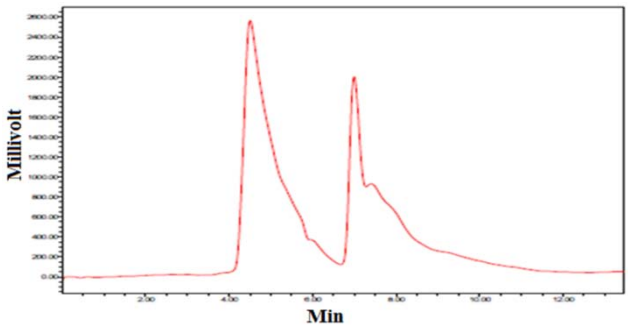

Fig. 4: HPLC Chromatogram of acid hydrolyzed CLM

Table 6: Mucoadhesive characterization of CLM by shear stress method

Polymer	Concentration of polymer (% w/v)	Weight required to detach glass plate (g) at the time interval		
		15 min	30 min	60 min
CLM	1	1.0	1.35	2.05
	2	1.0	1.39	1.95
	3	1.0	1.40	2.15
HPMC	1	1.0	1.40	2.05
	2	1.0	1.35	1.78
	3	1.0	1.35	1.80
CP	1	1.0	1.30	1.98
	2	1.0	1.30	1.75
	3	1.0	1.24	1.95

Each value in the table is represented as mean±SD. (n=3)

Table 7: Mucoadhesive characterization of CLM by falling sphere method

Polymer	Concentration of polymer (% w/v)	Average time taken (sec)
CLM	0.5	10.2
	1.0	10.5
	3.0	11.4
HPMC	0.5	10.2
	1.0	10.6
	3.0	11.2
CP	0.5	09.5
	1.0	09.9
	3.0	10.5

Each value in the table is represented as mean±SD. (n=3)

Acute oral toxicity study

Toxicity study of the mucilage revealed no behavioural changes in the animals. The LD_{50} was found to be higher than 2000 mg/kg b.w. after 48 h survival of all the animals. Since all the 6 animals tested survived after giving the oral dose of 2000 mg/kg b.w., the mucilage was considered to be safe.

CONCLUSION

From the studies conducted, the conclusion can be drawn that the isolated natural mucilage shows the promising inbuilt mucoadhesive property. Results also showed that the CLM exhibit properties typical of polysaccharides and found to be mainly composed of rhamnose and fructose. FT-IR study also confirms the identity and purity of the CLM. Since this mucoadhesive agent is obtained from edible fruit, it undergoes easy biodegradation and may provide an alternative to conventional synthetic/semi-synthetic mucoadhesive agents.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

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