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Extraction and characterization of mucilage from *Lepidium sativum* Linn. seeds

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ABSTRACT

Lepidium sativum is widely used as herbal medicine in India. It is widely available in market and has very low cost. Lepidium sativum have been widely used to treat a number of ailments in traditional system of medicine throughout India. Natural gums and mucilage as important part of formulation with the development of pharmaceutical dosage forms. Mucilages are generally normal products of metabolism, formed within the cell. Mucilage and gums have been used since ancient times for their medicinal uses. In the modern era also they are widely used in the pharmaceutical industries as thickeners, water retention agents, emulsion stabilizers, suspending agents, binders and film formers, disintegrants as well as sustaining agents in tablets and as gelling agents. A variety of parts of the plant namely; seeds, leaves and roots have been used in treating various human ailments. The seeds of Lepidium sativum contain the mucilage around the outer layer. The major problem in isolation of mucilage is that it swells but does not separate from the seeds. Because of this, general methods of separation of mucilage are not applicable to separate the seed mucilage and hence, different procedures were tried for the separation of mucilage by using various methods. Chemical, Fourier transforms infrared (FTIR) spectraland physicochemical characterization of Lepidium sativum mucilage were performed.

Keywords: *Lepidium sativum*, Botanical description, Extraction of mucilage, Mucilage characterization.

INTRODUCTION

The use of natural gums and mucilage as important part of formulation is with the development of pharmacy and different dosage forms. As general exicipients in tablets and capsules etc. The options are limited. The prospects of natural polymers are brighter but even here extensive testing will be required [1].

Mucilages are heterogenous in composition and are typically polysaccharide complexes formed from the sugars, arabinose, galactose, glucose, mannose, xylose and uronic acid units. Several plant species such as *Aloe vera, Ceratoniasiliqua, Opuntiaficusindica, Basellaalba* 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 mucilage's are reported to play a major role in wound responses, plant host pathogen interactions, water transport and responses to abiotic stresses. Extracellular mucilage's 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 [2]. Mucilages are most commonly

used adjuvant in pharmaceutical preparations. Plant mucilage's are pharmaceutically important polysaccharide with wide range of applications such as thickening gelling agent, binding, disintegrating, suspending, emulsifying, stabilizing and gelling agents [3].

Lepidium sativum (family: Cruciferae) is known as asaliyo and is widely used as herbal medicine in India. It is widely available in market and has very low cost [4]. Garden cress (*Lepidium sativum* Linn.) is an annual herb, belonging to Brassicaceae family. *Lepidium sativum* is a polymorphous species and believed to have originated primarily in the highland region of Ethiopia and Eritrea. Garden cress (GC) or pepper cress, is a fast growing edible plant [5]. Mucilage is found to be a brownish white powder which decomposes above 200^{o}C and have characteristic odour [6]. *Lepidium sativum* mucilage because of its colloidal nature and viscosity can be used to suspend insoluble substances in liquid and help in preventing sedimentation [7].

Botanical description [8]

Parts used

A variety of parts of the plant namely; seeds, leaves and roots have been used in treating various human ailments.

Minuscule

Seeds powder were creamish yellow in colour, microscopy of the seeds powder shows uniform thick walls, oily endosperm, number of reddish-brown fragments of seed coats and reddish colouring matter.

Morphology

It is a small, evergreen, glabrous and semi-parasitic tree with slender branches, attaining a height up to 18 m with dark grey or nearly black or reddish and rough bark. Sapwood is unscented and white but heartwood is scented and yellowish-brown or dark-brown. Leaves are opposite, ovate or ovate-lanceolate, glabrous, 1.5-8.0 to 1.5-3.0 cm or larger and thin. The flowers are brownish purple, violet or straw-coloured unscented and are borne in terminal and axillary paniculate cymes. Drupes, the fruits are globose, 1.2 cm across, and purple black with hard ribbed endocarp. Seeds are small, oval-shaped, pointed and triangular at one end, smooth, about 2-3 mm long, 1-1.5 mm wide, reddish brown, a furrow present on both surfaces extending up to two thirds downward, a slight wing like extension present on both the edges of seed, when soaked in water seed coat swells and gets covered with a transparent, colourless mucilage, taste, mucilaginous.

Habitual uses

Lepidium sativum have been widely used to treat a number of ailments in traditional system of medicine throughout India. Cold infusions of seeds are used to relieve hiccough. The seeds are used in chronic enlargement of liver and spleen and also used as carminative adjunct to purgatives. The bruised seeds, mixed with lime juice are used as local application for the relief of inflammatory and rheumatic pains. The seed are bitter, themogenic, depurative, rubefacient, galactagogue, emmenagogue, tonic, aphrodisiac and diuretic. They are useful as poultices for spraines, and in leprosy, skin diseases, dysentery, diarrhea, splenomegaland asthma. The leaves are mild stimulant and diuretic, useful in scorbutic diseases and in liver complaints. The roots are bitter, acrid and are useful in treatment of secondary syphilis and tenesmus and used as a condiment. The recovery was assessed with the disappearance of the symptoms like intermittent and incomplete evacuation, intermittent diarrhoea etc. in most of the cases. The response was good in 90% cases and patient preference was very high.

MATERIALS AND METHODS

Materials

Dried seeds of *Lepidium sativum* were procured from local market (Grocery shop) and other all the chemicals used were of analytical grade.

I. Extraction of mucilage from *Lepidium sativum*

The seeds of *Lepidium sativum* contain the mucilage around the outer layer. The major problem in isolation of mucilage is that it swells but does not separate from the seeds. Because of this, general methods of separation of mucilage are not applicable to separate the seed mucilage and hence, different procedures were tried for the separation of mucilage.

Method A

In the first method (method A) the seeds (100 g) were soaked for12 hour in distilled water (1litre). Then mucilage was separated by passing through vacuum pump. After that remaining particulate matter separated by passing through muslin cloth. Then separated clear material was treated with acetone. So as to get precipitated mucilage.

Drying was done at 45°C for 6 h. Then powder was passed through 80 # mesh sieve and weighed to calculate the yield.

Method B

In second method (method B) the seeds (100 g) were soaked for 12hour in distilled water (1litre). Then mucilage was separated by passing through vacuum pump. After that remaining particulate matter separated by passing through muslin cloth. Then separated clear material was treated with ethanol. So as to get precipitated mucilage. Drying was done at 45°C for 6 h. Then powder was passed through 80 # mesh sieve and weighed to calculate the yield.

Method C

In third method (method C) the seeds (100 g) were boiled with distilled water (1 litre) for 15 minute and the mass was filtered through Buckner funnel without filter paper. The retained residues were boiled with distilled water (0.5 litre) for 15 minute and the combined liquid was passed through eight folds of muslin cloth. The mucilage was precipitated from the filtrate by adding ethanol. The precipitated mucilage was dried in an oven at 45°C till it was completely dried. The powder was passed through 80 # mesh sieveand weighed to calculate the yield.

II. Characterization of mucilage [9]

A. Chemical characterization of *Lepidium sativum* mucilage

The presence of mucilage in extracted material was confirmed by performing Molisch's test and by treatment with ruthenium red. Both tests were positive for the presence of mucilage.

Molisch's test

To the test solution add few drops of alcoholic alpha napthol, then ad few drops of concentrated sulphuric acid through side of test tube, purple to violet color was obtained.

With ruthenium red

Test solution with ruthenium red solution, pink colour is obtained.

B. Physicochemical characterization of *Lepidium sativum* mucilage [9]

i. Loss on drying

In this method appropriate quantity of mucilage was weighed and dried at 105 °C for 2 hour. After 2hrs.weight was taken and weight loss on drying, percentage loss of moisture on drying was calculated. Weight loss on drying was determined by formula,

Initial weight - final weight= Weight loss

Percentage loss of moisture on drying was calculated using the formula.

LOD (%) = (Weight of water in sample/Weight of dry sample) ×100 Eq.(1)

If the weight loss on drying indicates the amount of moisture present in the material available to interact with other material.

ii. Particle size

The particle size of the dried-powder mucilage was determined by the microscopic method.

1. In this method microscope was adjusted for maximum light.
2. Then calibration of eyepiece was done by using micrometer scale.
3. Then sprinkle small amount of mucilage suspension on clean slide.
4. Place the slide on microscope and minimum of 300-500 particle.

iii. pH of solution

The pH of the 0.5% solution was measured with a pH meter. In
This 0.5% solution of mucilage prepare with distilled water.

iv. Charring

A few milligrams of dried mucilage were placed in a melting-point apparatus. The temperature was taken and recorded when the material started to char.

v. Swelling ratio

1. The study was carried out using a 100 ml stoppered graduated cylinder.
2. The initial bulk volume of 1 gm of dried mucilage was recorded.
3. Water was added in sufficient quantity to yield 100 ml of a uniform dispersion.
4. The sediment volume of the swollen mass was measured after 24 hour, stored at room temperature.
5. The swelling ratio was calculated by taking the ratio of the swollen volume to the initial bulk volume.

vi. Flow property

The flow properties and compressibility of the dried mucilage, including bulk and tapped density, Carr's index, the Hausner's ratio, and the Angle of repose.

a. Bulk and tapped density

It is a property of powders, granules and other "divided" solids, especially used in reference to mineral components , chemical substances, pharmaceutical ingredients, food stuff or any other masses of corpuscular or particulate matter. It is defined as the mass of many particles of the material divided by the total volume they occupy. The total volume includes particle volume, inter-particle void volume and internal pore volume. Bulk density is not an intrinsic property of a material; it can change depending on how the material is handled. Bulk density of powder is depend upon particle size distribution, particle shape and tendency of particle to adhere to one another.

For example, a powder poured in to a cylinder will have a particular bulk density; if the cylinder is disturbed, the powder particles will move and usually settle closer together, resulting in a higher bulk density. For this reason, the bulk density of powders is usually reported both as "freely settled" (or "poured" density) and "tapped" density (where the tapped density refers to the bulk density of the powder after a specified compaction process, usually involving vibration of the container.) Tapped density refers to the bulk density of the powder after a specified compaction process, usually involving tapping motion of the product cylinder.

A Pre-weighted, presieved quantity of dried mucilage was poured into a graduated cylinder, and the volume recorded. The cylinder was tapped until the powder-bed volume reached a minimum value and the tapped volume was recorded. The bulk and tapped densities were calculated by using Tapped or Bulk Density or Apparent Volume Test Instrument - Type PT-TD200. It is measured in gm/ml.

Bulk density = Mass/Bulk volume
Tapped density = Mass/Tapped volume
Hausner's ratio= Tapped density/ Bulk density Eq.(2)

b. Compressibility index

Compressibility index gives the important property of granules. It is also known as Carr's index. In which pre weighted, presieved quantity of dried mucilage was poured into a graduated cylinder, and the volume recorded. The cylinder was tapped until the powder-bed volume reached a minimum value and the tapped volume was recorded. Lower the compressibility value indicate better flow. The formula for Carr's Index is as below,

Compressibility index = Tapped density-Bulk density/ Tapped density Eq.(3)

% Compressibility index = Compressibility index×100.

c. Angle of repose

Good flow properties are critical for the development of any pharmaceutical powder formulation. It is essential that an accurate assessment of flow properties be made as early in development process as possible so that an optimum formulation can be identified. Interparticle forces or forces between particle as well as flow characteristics evaluated by angle of repose. Angle of repose is defined as the maximum angle possible between the surface of pile of sample and horizontal plane.

The fixed funnel and free-standing cone methods employ a funnel that is secured with its tip at given height, H, which was kept 2 cm, above graph paper that is placed on a flat horizontal

surface. With r, being the radius of base of conical pile.

$\tan \theta = h / r$ Eq.(4)

iv. Viscosity
Rheological studies of dried mucilage were carried out using varying concentrations (0.1–0.5% w/v) prepared in distilled water. The viscosities were measured using a Brookfield viscometer.

v. Fourier transforms infrared (FTIR) spectral studies
Fourier transform infrared (FTIR) spectral data were taken on a Shimadzu (model FTIR-8300) instrument to find out chemical stability of the excipients. FTIR spectra of pure drug, mucilage and mixture were obtained. All the samples were crushed with potassium bromide to get pellets at 1 ton/cm2. Spectral scanning was done in the range between 4000-400 cm^{-1} [10].

RESULT AND DISCUSSION

A. Chemical characterization of *Lepidium sativum* mucilage
The presence of mucilage in extracted material was confirmed by performing Molisch's test and by treatment with ruthenium red. Both tests positive for the presence of mucilage.

B. Physiochemical characterization of *Lepidium sativum* mucilage
i. Loss on drying
The weight loss on drying indicates the some amount of moisture was present in the material and which is available to interact with other material.

ii. Swelling ratio
The swelling ratio of mucilage, determined in measuring cylinder. It was found to be 2.8. There was a significant change in swelling after 24 which indicated that the mucilage had good swelling properties.

iii. Viscosity
The viscosity of 0.5% solution at 60 rpm was found to be 319.93 cps. It can be concluded that mucilage has a viscosity of such type that is suitable for formulation of gel, jellies, cream and other semisolid drug dosage forms .

iv. Flow property
The flow properties and compressibility of the dried mucilage, including bulk and tapped density, Carr's index, the Hausner's ratio, and the Angle of repose are shown in Table. It can be concluded that the dried mucilage has a good flow properties which is suitable for a direct compression formulation.

v. FTIR study
The FTIR a study was carried out for the mucilage (*Lepidium sativum)* as shown in Fig.1.

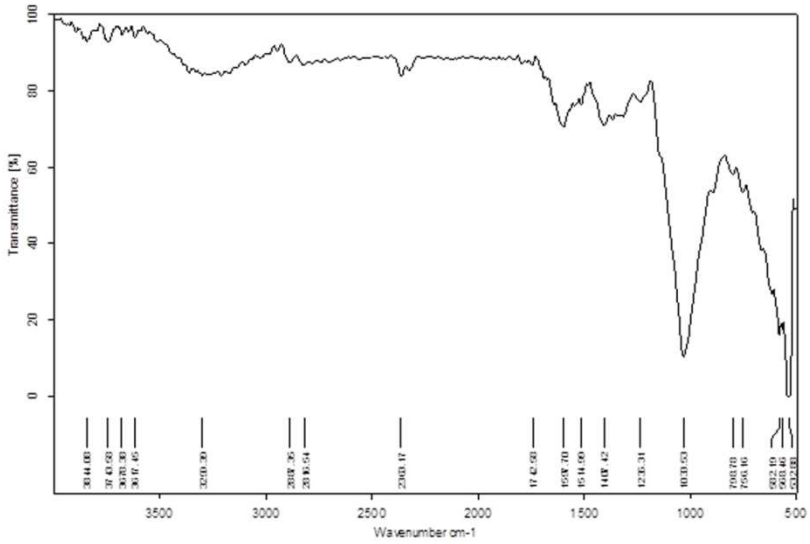

Figure 1. FTIR study for the *Lepidium sativum* mucilage

Current scenario for peak at wavelength 1033.53 $cm^{-1}$and 1597.70$cm^{-1}$are as shown in Table 1 and Table 2 respectively whereas physicochemical characterization of *Lepidium sativum* mucilage as shown in Table 3.

Table 1. Peak at Wavelength 1033.53cm^{-1}

Sr. No.	Bond	Type of compound	Frequency (in cm^{-1})	Mode
1.	C-O	Alcohol	900-1300	Streching
2.	C-C	Alkane	800-1200	Streching
3.	C-F	Fluorides	1000-1400	Streching
4.	C-N	Amines	1000-1350	Streching

Table 2. Peak at Wavelength 1597.70cm^{-1}

Sr. No.	Bond	Type of compound	Frequency (in cm^{-1})	Mode
1.	C=C	Alkene	1680-1600	Streching
2.		Aromatic	1600 & 1475	Streaching

Table 3. Physicochemical characterization of *Lepidium sativum* mucilage

Sr. No	Tests	Observations
1.	Description	Brownish white powder
2.	Odour	Characteristic
3.	Appearance	Lustous
4.	Identification Molisch's test Ruthenium red	 Solution shows violet colour. Solution shows pink colour.
5.	pH	7.8
6.	Loss on drying	19.3%
7.	Swelling index	2.8
8.	Angle of repose	40˚
9.	Bulk density	0.673 g/ml.
10.	Tapped density	0.7 gm/ml.
11.	Hausner's ratio	1.040
12.	Compressibility index	0.03857
13.	Viscosity	319.93 cps.
14.	Melting point	228˚

CONCLUSION

The major problem in isolation of mucilage is that it swells but does not separate from the seeds. Because of this, general methods of separation of mucilage are not applicable to separate the seed mucilage and hence, different procedures were tried for the separation of mucilage by using various methods. Mucilage in extracted material was confirmed by performing Molisch's test and by treatment with ruthenium red, both tests positive for the presence of mucilage. Also mucilage had good swelling properties and viscosity of suchtype that is suitable for formulation of gel, jellies, cream and other semisolid drug dosage forms. Also dried mucilage has a good flow properties which is suitable for a direct compression formulation. FTIR a study was indicates groups present, Type of compound and bonds present in mucilage from *Lepidium sativum* seeds.

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