RESEARCH ARTICLE

Development of effective extraction method for *Lepidium sativum* seed mucilage with higher yield

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ABSTRACT

In the present study, we are reporting a novel extraction method for production of *Lepidium sativum* seed mucilage maximizing the yield of the product with good organoleptic property and swelling index. Extraction method developed was aimed at simplifying the process in which less amount of solvent will be used. The developed method was compared with the other methods reported in the literature. Mucilage extracted by precipitation of soaked and blended seed in acetone gave maximum yield of 12%w/w as compared to the other extraction methods where yield was 3-5% respectively. Mucilage was found to have excellent swelling index (351.5) and also exhibited good Organoleptic characteristic and flow properties.

Keywords: Extraction method, *Lepidium sativum* mucilage, % yield, organoleptic property, swelling index, flow property.

INTRODUCTION

The plant derived gums and mucilages are hydrophilic and gel- forming in nature. They are normal product of metabolism formed within cell. [1] Recent trend towards the use of plant based and natural products demands the replacement of synthetic additives with natural ones [2]. These plant based polymers have been studied for their application in different pharmaceutical dosage forms like matrix controlled systems, film coating agents, buccal films, microspheres, nanoparticles, viscous liquid formulations like ophthalmic solutions, suspensions, implants and their applicability and efficacy has been proven. [3-5] These have also been utilized as viscosity enhancers, stabilizers, disintegrants, [6] solubilisers, emulsifiers, [7] suspending agents, [8] gelling agents [9] and bio-adhesives and binders [10] in the above mentioned dosage forms. [11] The plant derived gums and mucilage comply with many requirements of pharmaceutical excipients as they are non-toxic, stable, easily available, associated with less regulatory issues as compared to their synthetic counterpart and inexpensive; also these can be easily modified to meet the specific need. [12]

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Lepidium Sativum (Garden cress) is an annual herb, belonging to *Brassicaceae* family. In some regions garden cress is known as garden pepper cress, pepper grass or pepperwort. It is also known as Asalio or chandrasur in India and it is an important medicinal crop in India. It is a fast growing, edible plant botanically related to watercress and mustard and sharing their peppery, tangy flavor and aroma. The main character of *Lepidium sativum* is that it can grow in any type of climate and soil condition with few requirements. Seeds, leaves and roots are economically important, however, the crop is mainly cultivated for seeds. Garden cress is a perennial plant, and an important green vegetable consumed by human beings, most typically as a garnish or as a leafy vegetable. Generally fresh leaves are used as salads. [6] Seeds are small, oval- shaped about 2 to 3 mm long and 1 to 1.5 mm wide with reddish brown in color. Seeds when soaked in water swells and get covered with transparent, colorless mucilage. The *Lepidium sativum* plant have anti-asthmatic, anti scorbutic, aperients, diuretic, stimulant, chemo protective effects, anti-diabetic, anti-hypertensive, fracture healing property, hepatoprotective activity, pesticidal activity, antidiarrheal activity etc. [13, 14] Taxonomic classification of *Lepidium sativum* plant is

Kingdom= Plantae

Division= Angisospermae

Class= Dicotyeledonae
Subclass= Polypetalae
Series= Thalamiflorae
Order= Parietales
Family= Brassicaceae
Genus= Lepidium Linn
Species= *Lepidium sativum Linn sp.*

Several procedures have been reported for the isolation and extraction of seed mucilage from *Lepidium sativum* using different methods. [15, 16, 17] Extraction of mucilage is mainly done on powered seed, crushed seed and whole seed by using various solvent like alcohol, acetone in suitable proportion. Archana, kumar et al [17] extracted mucilage by using aqueous extract of powder seed in water and chloroform and precipitate by acetone. Divekar et al [15] and Kenneth Bailey [18] extracted mucilage from the seeds of *Lepidium sativum* using 95% ethanol. Hasegawa et al. extracted mucilage from garden cress by changing various solvents. [16] H. Karazhiyan, S. Razavi et al [19] extracted mucilage by altering pH of seed and swollen mucilage was scraped by passing through an extractor with rotating plate. The method reported in literature are not cost effective as it give less yield and solvent required in extraction procedure increase the production cost. *Lepidium sativum* mucilage has multiple uses and has shown promising result as disintegrants, [20, 17] suspending agent. [21] Therefore there is need to developed the extraction procedure which give quality product with less cost of production and higher yield. The fig 1(a) shows the *Lepidium sativum* plant, fig 1(b) *Lepidium sativum* seed and fig 1(c) *Lepidium sativum* swollen seed in water with thin layer of mucilage.

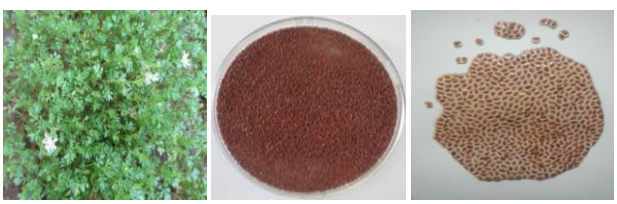

Fig 1: 1(a) *Lepidium sativum* plant; 1(b) *Lepidium sativum* seeds; 1(C) *Lepidium sativum* swollen seeds.

In present research work a simplified and effective method was developed and compared with the methods reported in the literature. Comparative studies were done with respect to time for extraction, steps involved, utilization of suitable solvent, quality and purity of mucilage as well as yield of mucilage.

MATERIALS AND METHODS

Seeds of *Lepidium sativum* were collected from the local market, Nagpur, Maharashtra. The plant material (seeds) was authenticated with the help of herbarium sheet by Dr. N. M. Dongarwar, Department of Botany, R. T. M. Nagpur University, Nagpur. The specimen numbers given to the authenticated herbarium sheet was 9890. Voucher specimen of the plant is deposited in the Department of Botany, R. T. M. Nagpur University, Nagpur. All the other solvents and reagents used in the study were of analytical grade.

Extraction of seed mucilage:

Extraction of seed mucilage was carried out by the method developed in our laboratory (Method 1) and the properties and yield of the obtained mucilage was compared with mucilage obtained by extraction methods reported in the literature (Method 2 and Method 3).

Method 1:

Precipitation of soaked and blended seed in acetone:

About 100 g of *Lepidium sativum* seeds were soaked in 800ml of distilled water for 12 hrs. The soaked seeds were blended for 15min at about 2000 rpm by using Phillips HR 1453 hand blender. The blended seeds were then filtered through muslin cloth. Additional 200ml water was added to the seeds and again blended and refiltered through muslin cloth to get maximum yield. To the filtrate (800 ml) equivalent amount of acetone was added to allow precipitation of mucilage. White supernatant coagulant mass separated after precipitation by acetone was filtered through the muslin cloth. Precipitated mucilage was then spread on glass slab and dried in tray dryer (Labotech) at temperature not exceeding 60°c for 16

hr. Dried mucilage easily separated in the form of flakes from glass slab by spraying acetone over dried mucilage. Mucilage flakes were further dried at 60°c for 5 min. Mucilage obtained was converted into powder by size reduction. Obtained powder was sieved using 80# sieve.

METHOD 2:

Precipitation of soaked seed in alcohol: [15, 18]

About 100 g of seeds were soaked in1000 ml distilled water and 5 ml of chloroform for 24 hr. The viscous solution obtained was filtered through muslin cloth. To the mucilaginous solution 1 L ethanol 95% was added to precipitate the mucilage, the precipitated Mucilage was collected and dried at temperature not exceeding 40 to 45°c till it was completely dried. The yield was calculated.

METHOD 3:

Precipitation of powdered seeds soaked and blended in acetone: [17]

About 100 g of seed powder was soaked in 1000ml distilled water and 5 ml of chloroform for 48 hrs. The extract was forced to pass through sieve. To the filtrate collected add 1 L acetone and allow precipitating. The precipitated mucilage was collected and kept in freezer for 8 hr and then dried in a freeze dryer.

Evaluation of Organoleptic properties of extracted seed mucilage by various methods:

The isolated mucilage was characterized for organoleptic properties such as color, odor, taste and texture.

Evaluation of Flow properties of extracted seed mucilage [22, 23, 24]

Bulk and Tapped Densities: A pre-weighed, pre-sieved 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.

Carr's Index and Hausner's Ratio: [25] Carr's index and Hausner's ratio were calculated from the bulk and tapped densities.

Angle of Repose: [26] The angle of repose was determined by the fixed height funnel method.

Determination of swelling index of seed mucilage: [27, 28] Swelling index of *Lepidium sativum* seed mucilage was determined by using modified method elsewhere reported. One gram of *Lepidium sativum* seed mucilage extracted was accurately weighed and transferred to a 100ml stopper measuring cylinder. The initial volume of the powder in the measuring cylinder was noted. The volume was made up to 100 ml mark with distilled water. The cylinder was shaken gently and set aside for 24 hr. The volume occupied by the gum sediment was noted after 24 hr. Swelling index (SI) was calculated according to the following equation:-

$$SI = xt - xo / xo$$

Where *xo* is the initial height of the powder in graduated cylinder and *xt* denotes the height occupied by swollen gum after 24 hr. The content from the measuring cylinder from the above test were filtered through a muslin cloth and the 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.

RESULT AND DISCUSSION

Extraction of seed mucilage:

Precipitation of soaked and blended seeds in acetone:

Seeds of *Lepidium sativum* contain mucilage around the outer layer. The major problem in isolation of mucilage is that it swells but does not separate easily from the seeds. [20] Therefore effective method was developed by using precipitation of soaked and blended seeds in acetone. Period of 12 hours was

sufficient for mucilage to swell completely and then swollen seeds were blended by using Phillips HR 1453 hand blander. The blades were designed in such a way that it scrapped out swollen mucilage layer without crushing the seed. Due to which pure mucilage filtrate can be obtained without affecting internal part of the seed and therefore yield of mucilage was also increased. The method developed was made cost effective by reusing distilled acetone from the filtrate which minimized the cost of solvent. Acetone increased the rate of precipitation and therefore less amount of solvent was required to precipitate the mucilage in larger quantity. Acetone being more volatile in nature was completely removed and no traces of solvent were found in dried the mucilage. The mucilage was dried at 60° c which reduced the time for extraction. The drying temperature did not affect the mucilage stability. [29] The total yield obtained was 12% w/w.

Precipitation of soaked and blended seed in alcohol:

In this method seeds were soaked for 24 hr in distilled water. Even after 24 hr time mucilage was not removed from the seeds completely which reduce the total yield of the mucilage. 95% Ethanol was used to precipitate mucilage which was required in larger quantity as compared to acetone. Mucilage was dried at temperature 45°C for 48 hours for complete drying to take place. The use of distilled water, quantity of alcohol required and drying time increased the extraction cost of mucilage. The total yield obtained was 5% w/w.

Precipitation of powdered seeds soaked and blended in acetone:

In this method seeds were powdered and soaked in 900 ml of water and 1 ml of chloroform for 24 hr. After total soaking time the filtrate become very viscous, with foul smell and foam was observed in filtrate. The viscous filtrate was unable to pass through the muslin cloth. It was therefore forcefully passed through sieve 80#. The filtrate obtained was not pure and contained traces of seed parts. Precipitated mucilage obtained by using acetone was kept in freezer for 8 hr and then dried using lyophilizer. Thus increase the drying time of mucilage. The yield obtained was 3 % w/w.

In Method 1 soaking of seeds for 12 hr was sufficient to swell mucilage, where as Method 2 and Method 3 required 24 hour soaking time so that mucilage could dissolve in filtrate. The soaking time in other two methods increased the extraction time of mucilage. Also in Method 3 seeds were powdered and then soaked which increased steps of extraction process. In Method 1 seed were blended by Phillips HR 1453 blender which is important step in extraction which increases percentage yield of mucilage. Solvent used in Method 1 and Method 3 was acetone whereas in Method 2 mucilage was extracted by ethanol. Method 2 and Method 3 required more solvent as compared to other methods, moreover solvent cost in Method 1 was reduced by reusing distilled acetone from filtrate. Drying time for mucilage in Method 1 was 16 hours whereas in Method 2 and Method 3 was up to 24 hours. The drying time for other two methods was comparatively more than developed Method 1. Mucilage in Method 1 and Method 2 was dried in simple tray drier whereas Method 3 required freezing and lyophilizing extracted mucilage which made drying of mucilage complicated.

Evaluation of Organoleptic properties of seed mucilage.

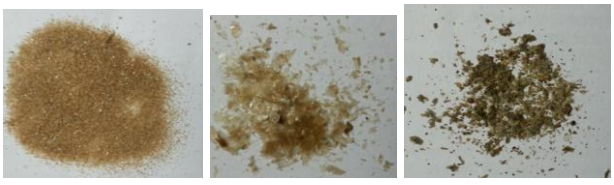

Fig 2: Images of extracted mucilage by method 1 (a), method 2 (b), and method 3 (c).

Figure 2a, 2b, 2c shows the images of extracted mucilage obtained from Method 1, Method 2, and Method 3 respectively. Organoleptic characteristic of mucilage are tabulated in Table 1. From the results it was observed that Quality of the mucilage obtained from Method 1 was very good in terms of color,

texture, odor and yield as compared to other two methods.

Table 1: Organoleptic characteristics of extracted mucilage

Properties	Method 1	Method 2	Method 3
Appearance	Lustrous amorphous powder	Small flakes	Amorphous powder
Color	Brown	Brown	Almost black
Odor	Odorless	Odorless	Foul smell
Taste	Tasteless	Tasteless	Taste less
yield	12%w/w	5% w/w	3%w/w

Evaluation of Flow properties of extracted seed mucilage:

The flow properties of dried mucilage are shown in Table 2. Carr's index, Hausner's ratio and angle of repose were selected as flow indicating parameters. They reflect the particle size, surface characters and moisture content of the mucilage. [30] Flow properties of extracted mucilage are tabulated in Table 2. From the result it was observed that mucilage extracted from the Method 1 exhibited good flow properties as compared to the other extracted mucilage. Therefore mucilage extracted from Method 1 can be used in direct compression formulation. [31]

Table 2: Flow properties of extracted mucilage.

Flow properties	Method 1	Method 2	Method 3
Carr's index	11.5±0.2	17.34±0.22	14.63±0.06
Hausner ratio	1.16±0.01	1.27±0.21	1.28±0.02
Angle of repose	31.54±0.04	47.32±0.04	44.61±0.03

Determination of swelling index of seed mucilage:

Mucilage on coming in contact with water hydrates and swells forming thick and viscous solution. [32] Swelling index of extracted mucilage from method 1, method 2 and method 3 were compared and results obtained are tabulated in Table 3. The particle size of mucilage obtained from method was reduced by passing it through 80# sieve. Due to its small particle size each particle swell to greater extent which results in higher swelling index. Also the water retention capacity was less which indicates that mucilage extracted from Method 1 swell to greater extent as compared to mucilage extracted from other methods. The mucilage with higher swelling index swell to greater extend forming sticky and viscous solution and can be used as gelling, suspending, binding and disintegrating agent in different pharmaceutical formulations.

Table 3: swelling index and water retention capacity of extracted mucilage

Properties	Method 1	Method 2	Method 3
Swelling index	351.5	314.8	259.1
Water retention capacity	10.5 ml/gm	16.3ml/gm	20.8ml/gm

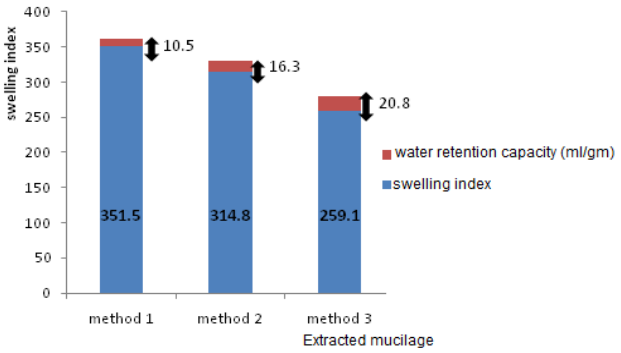

Fig 2: Effect of extracted mucilage on swelling index of mucilage

CONCLUSION

From the above investigations, it can be concluded that good quality *Lepidium Sativum* seeds mucilage can be effectively extracted by precipitation of soaked and blended seed in acetone (Method 1) to get better yield. The developed Method 1 is simple with minimum steps of extraction. It is cost effective method as it consumes less volatile solvent, requires very less time for extraction and gives highest yield as compared to other methods reported in the literature. Mucilage extracted from Method 1 showed good organoleptic properties, flow properties and excellent swelling index which are some essential parameters for mucilage to be used as a pharmaceutical excipient. However further studies are required to be undertaken to establish it as an excellent excipient and also the feasibility of isolation of mucilage at pilot scale needs to be emphasized in future.

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CONFLICT OF INTEREST

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

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