

## RESEARCH ARTICLE

STUDIES ON ISOLATION AND EVALUATION OF *OCIMUM TENUIFLORUM* Linn SEED MUCILAGE

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## ABSTRACT

Plant products serve as an alternative to synthetic products because of local accessibility, eco-friendly nature and lower prices compared to important synthetic products. Natural gums and mucilages have been widely explored as pharmaceutical excipient. The present study was undertaken to isolate and evaluate mucilage from the seeds of *Ocimum Tenuiflorum* Linn and explore its use as a pharmaceutical excipient. Various methods of mucilage extraction were tried and simple, economical and optimum method was developed. Physicochemical properties of *Ocimum* seed mucilage such as pH, Swelling index, Bulk and Tapped Densities, Carr's Index and Hausner's Ratio, Viscosity, Angle of Repose were studied.

**Keywords:** *Ocimum Tenuiflorum* Linn., Mucilage, Natural excipient.

## INTRODUCTION

After reviewing literature, it was found that the plant *Ocimum Tenuiflorum* Linn was not studied for its seed mucilage properties. So this plant was selected for the research work. The plant seeds were studied for isolation of the mucilage by different extraction methods.

The flowering bud of the plant was selected for the authentication. The plant sample was sent to Botanical Survey of India (B.S.I.), Pune. The plant was authenticated as *Ocimum Tenuiflorum* Linn, belonging to family Lamiaceae.

Today, we have a number of plant-based pharmaceutical excipients. A number of researchers have explored the utility of plant-based materials as pharmaceutical excipients. Majority of investigations on natural polymers in drug delivery systems are centered on polysaccharides and proteins, due to their ability to produce a wide range of materials and properties based on their molecular structures.

Natural ingredients, either active or inactive, are in high demand for their drug delivery applications because of their versatile availability, low cost as compared to synthetic and semi-synthetic products, and their biocompatible and biodegradable nature. Novel drug delivery systems are aimed to produce effective drug therapy with better patient compliance, efficacy and safety<sup>1</sup>.

Mucilages and gums are well known for their medicinal use. In recent times, increasing attention has been given to the application of gums of various sources as pharmaceutical excipients. Plant gums and exudates are getting screened for their use as pharmaceutical adjuvant. Mucilages are used for their binding, thickening, stabilizing, humidifying, disintegrating and release controlling properties in medicines<sup>2</sup>.

## MATERIALS AND METHODS

## Materials

The *Ocimum* seeds were procured from the local market. Petroleum ether was purchased from Loba Chemicals Pvt. Ltd. Mumbai. All other solvents and reagents used were of analytical grade.

## Methods

## Isolation of the Mucilage

Mucilage can be isolated by different methods. A number of methods were used to isolate the *Ocimum* seeds mucilage. The mucilage isolated from the different methods was compared for the yield of the mucilage. The procedure giving maximum yield of mucilage was used for isolation. The various isolation methods used to isolate the *Ocimum* seed mucilage are given below.

- A. General methods of mucilage Isolation
  - a. Precipitation of mucilage in alcohol
    1. Boiled extract of seeds was precipitated in alcohol.
    2. Boiled extract of soaked and crushed seeds were precipitated in alcohol.
  - b. Precipitation of mucilage in acetone
    1. Boiled extract of soaked and crushed seeds were precipitated in acetone
    2. Precipitation of soaked and blended seeds in acetone
- B. Microwave oven extraction
- C. By defatting with petroleum ether
  - a. Defatting by 12 hrs. Shaking with petroleum Ether.
  - b. Defatting by Soxhlet apparatus with petroleum Ether.

**A. General Methods of Mucilage Isolation<sup>3</sup>**

Two general methods were followed for the isolation of the Ocimum seed mucilage.

**a. Precipitation of Mucilage in Alcohol****1. Boiled extract of seeds was precipitated in alcohol**

100 g of Ocimum seeds were taken in a beaker and 1 liter of distilled water was added to it. Then it was boiled for 15 min. and was filtered by Buchner funnel without filter paper. The residue was again boiled with 500 ml of distilled water and filtered. The combined filtrate was then passed through muslin cloth with 8 folds. The filtrate was then precipitated in ethanol and the dried in the oven. The yield was recorded.

**2. Boiled extract of soaked and crushed seeds were precipitated in alcohol**

100 g of Ocimum seeds were soaked in the distilled water for 12 h. The soaked seeds were then blended for the 15 min. The blended seeds were boiled for 15 min. The boiled seed mass were filtered by the muslin cloth with 8 folds. The filtrate was then precipitated using ethanol. The mucilage was separated and dried in the oven. The yield was recorded.

**b. Precipitation of Mucilage in Acetone****1. Boiled extract of soaked and crushed seeds were precipitated in acetone**

100 g Ocimum seeds were boiled in 1 liter of distilled water. The boiled seed mass was then filtered by the muslin cloth with 8 folds. The filtrate was then precipitated using 1000 ml of acetone. The precipitated mucilage was separated and dried in the oven. The weight of obtained mucilage was recorded.

**2. Precipitation of soaked and blended seeds in acetone**

100 g of Ocimum seeds were soaked in the distilled water. The soaked seeds were then blended for 15 min. the blended seeds were then passed through the 8 folds of the muslin cloth. The filtrate was then precipitated in the equal amount of the acetone. The precipitated mucilage was then separated and dried in the oven. The yield was reported.

**B. Microwave oven extraction method<sup>4</sup>**

Ocimum seeds were blended in the mechanical blender for 5 min and soaked in the distilled water (150 ml) for 24 h in 1000 ml beaker. It was kept in the microwave oven along with the glass tube inside to prevent bumping. It was subjected to microwave irradiation at 800W intensity for 3 min. The beaker was removed and kept aside for 2 h for the release of mucilage into the water. The material was filter through the muslin bag and hot water (25 ml) was added through the sides of the marc and squeezed well in order to remove the mucilage completely. The equal amount of ethanol was added to the filtrate to precipitate the mucilage and kept inside a refrigerator for one day for effective settling. It was filtered and dried completely in an incubator at 37<sup>0</sup> C.

**C. By defatting with petroleum ether<sup>5</sup>****a. Defatting by 12 hrs. shaking with petroleum ether**

The Ocimum seeds were blended and kept in contact with petroleum ether in a conical flask for 12 h. The flask was kept on the electrical shaker for the continuous shaking. The material was then filtered out and dried at room temperature for complete removal of petroleum ether.

The blended seed powder was then soaked in distilled water. The swollen wet mass was then spread on a glass tray and dried at 60<sup>0</sup>C. The dried material was then passed through mesh #30. The material was winnowed and again passed through mesh #60. The weight of mucilage obtained was recorded.

**b. Defatting by Soxhlet apparatus with petroleum ether**

The Ocimum seeds were blended and kept in contact with petroleum ether in Soxhlet apparatus. The cycles of petroleum ether were run till complete defatting was obtained. The defatted material was then dried at room temperature for complete removal of petroleum ether.

The dried defatted seed powder was then soaked in distilled water. The swollen wet mass was then spread on the glass tray and dried at 60<sup>0</sup>C. The dried material was then passed through mesh #30. The material was winnowed and again passed through mesh #60. The weight of mucilage obtained was recorded.

The method yielding maximum amount of mucilage was utilized further to obtain required quantity of mucilage and used in further studies.

**EVALUATION****Physicochemical properties of Ocimum seed mucilage**

The dried mucilage was studied for percentage yield, appearance, solubility, viscosity, pH, swelling index, bulk and tapped densities, angle of repose, compression properties.

**pH of solution:** The pH of the 1% w/v aqueous mucilage solution was measured with a pH meter<sup>6</sup>. (Equip-Tronics, EQ-610)

**Determination of swelling index of Ocimum seed mucilage:**

Swelling characteristics of the Ocimum seed mucilage was tested in distilled water. The Swelling index is the volume in ml occupied by 1g of the substance. The Swelling index of the mucilage powder was determined by according to British Pharmacopoeia method 6. The test was performed by taking 1gm of the mucilage powder in a 50.0ml ground glass stoppered cylinder graduated over a height of 120 to 130mm in 0.5 divisions. To this 25ml of distilled water was added and this was shaken vigorously every 10min for 1hour and then allowed to stand for 24hours. The volume occupied by the mucilage powder was measured<sup>7</sup>.

**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<sup>8</sup>.

**Carr's Index and Hausner's Ratio:** Carr's index and Hausner's ratio were calculated from the bulk and tapped densities<sup>9</sup>.

**Angle of Repose:** The angle of repose was determined by the fixed height funnel method<sup>10</sup>.

**Microbial Count:** The microbial count of the dried mucilage was performed for total aerobic microbial count of bacteria and fungi using the plate count method<sup>11</sup>. The limit for colony forming units (CFU) for bacteria is 300 and for fungi is 100.

### Effect of pH on the Ocimum seed mucilage swelling<sup>12</sup>

The Ocimum seed mucilage was tested for its swelling characteristics at acidic and basic pH. The mucilage was tested with 0.1 N HCl, 0.1 N NaOH.

**Effect of temperature on the Ocimum seed mucilage viscosity<sup>13</sup>:** To study the effect of temperature on the viscosity of the mucilage, the solutions of different concentrations at different temperature were tested keeping rpm constant. (Brookfield Viscometer, DV-II+ Pro). The results are given below.

Table 1 Comparison among the mucilage extracted by different methods

Sr. no.	Method of isolation of mucilage	Yield (%) (Approx.)
1	Boiled extract of seeds precipitated using alcohol	5
2	Boiled extract of soaked and crushed seeds precipitated using alcohol	7
3	Boiled extract of soaked and crushed seeds precipitated using acetone	5
4	Precipitation of soaked and blended seeds using acetone	6
5	Microwave oven extraction	3
6	Defatting by 12 hrs. shaking with petroleum Ether	30
7	Defatting by Soxhlet apparatus with petroleum Ether	35

## RESULT AND DISCUSSION

*Ocimum Tenuiflorum* Linn seed mucilage is isolated by defatting by Soxhlet apparatus with petroleum ether and dried at room temperature. This method is selected because it gives highest total yield of mucilage as compared to other methods of mucilage isolation (table 1).

The results of physicochemical testing of *Ocimum Tenuiflorum* Linn seed mucilage are shown in table 2.

Ocimum seed mucilage is brown powder with no characteristic odor and amorphous in nature. It is soluble in water and insoluble in ether, acetone, methanol and chloroform. Mucilage is having good swelling potential with swelling index 20. Mucilage is studied for pH, Swelling Index, Bulk and Tapped density, Carr's index and Hausner's ratio, Angle of repose indicated that powder is having good flow.

Table 2 Physicochemical Characterization of Ocimum Mucilage

S. N.	Parameters	Results
1	Appearance	Amorphous powder
2	Odor	No characteristic
3	Color	Brown
4	Swelling index	20
5	pH(1% w/v)	6.2
6	Test for carbohydrates	Present
7	Microbial test	Passes the test for bacteria and fungi
8	Solubility	Soluble in water, Insoluble in ether, Acetone, methanol, chloroform.
9	Angle of repose	28° 21'
10	Bulk density	0.5 g/cc
11	Tapped density	0.66 g/cc
12	Compressibility index	24.24 %

Table 3: Effect of Temperature on the Viscosity of the Mucilage

Mucilage %	Viscosities at various temperature			
	Viscosity at 45°C	Viscosity at 37°C	Viscosity at 25°C	Viscosity at 15°C
At 100 rpm				
2.5 %	35	24	20	20
5%	90	72	40	37
7.5%	156	150	133	80
10%	280	252	190	150

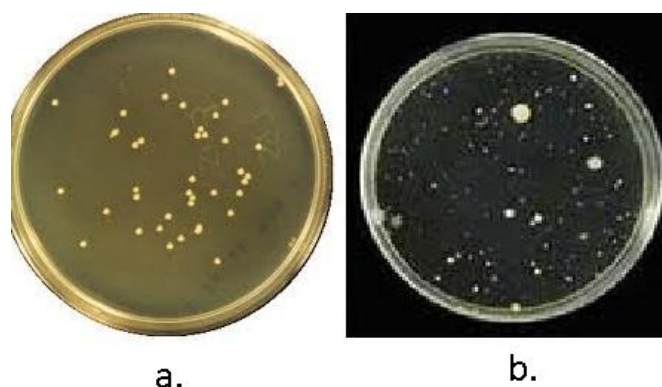


Figure 1: Petri plates showing a. bacterial and b. fungal growth

The microbiological properties of *Ocimum* seed mucilage were studied to estimate number of viable aerobic microorganisms present, the result shown in table 2 and figure 1 indicate that the mucilage under study passes the microbial load test. The seed mucilage is further evaluated for effect of pH on the *Ocimum* mucilage swelling and effect of temperature on the viscosity of the mucilage. Result shows that mucilage slightly swelled in the 0.1N HCl and did not swell in 0.1N NaOH. As the temperature increases there is a rise in the viscosity and as temperature decreases there is a decrease in the viscosity of the *Ocimum* mucilage (table 3).

## CONCLUSION

From the above study, we conclude that *Ocimum Tenuiflorum* Linn seed mucilage can be used as excipient in various pharmaceutical formulations. The method employed for isolation of mucilage, i.e. defatting by Soxhlet apparatus with petroleum ether is simple, economic and gives maximum total yield as compared to other methods of mucilage isolation. The physicochemical properties show the potential of the mucilage as disintegrant, binder and drug release retardant in tablets. However further study is essential to determine its category as excipient in tablets.

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