Research Article

Isolation and Characterisation of Natural Mucillage from Cucumis melo L.

E. VADIVEL AND SWATI SUBASH NAIK
Postgraduate Chemistry Department, Dnyanprassarak Mandal`s College and Research Centre, Assagao-Bardez, Goa, India
email: velpharmamsu@gmail.com, (m): 09561641736
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ABSTRACT
Mucilages are found as common ingredients in cosmetic, pharmaceutical, food and non-food industries due to their low cost compared to the synthetic polymers. The synthetic polymer such as excipients suffer from many disadvantages such as high cost, toxicity, non-biodegradability and environmental pollution caused during their synthesis. In the present study mucilage was isolated from the fruits of Cucumis melo(Muskmelon). The mucilage was extracted using distilled water and isolated by two methods such as precipitation with ethanol and acetone.The isolated mucilage was characterized by physicochemical properties such as solubility, pH, swelling index, Loss on drying, preliminary phytochemical studies and FTIR. The micromeritic properties such as bulk and tapped densities, Carr's index, Hausner's ratio and angle of response were also evaluated. The results show that only carbohydrates and amino acids were present in isolated mucilage. These tests indicate the purity of the mucilage. Extracted mucilage was soluble in warm water while insoluble in organic solvents. This showed that this can be safely used in dosage form without causing any adverse effect.

Keywords: Cucumis melo, Muskmelon, Isolation, Mucilage.

INTRODUCTION

Formulation of an active pharmaceutical ingredient into desired dosage forms are rarely possible without the addition of excipients. They are vital part of medicinal compounds, which may be also a major portion of medicinal product. These are inert molecules that play a very important role in designing of dosage form. Today, we have a number of plant-based pharmaceutical excipients, which may be selected and optimized based on the properties of the drug, requirements on the dosage form and its site of action. Apart from its common functions like serving as inert vehicle for the administration of right volume of active pharmaceutical ingredient with consistency in weight, excipients also fulfil multifunctional roles such as release retardants, solubility enhancers, viscosity modifiers, etc. In addition to this they offer significant advantages in ease of manufacturing, enhancement of patient compliance, improved bioavailability, reproducibility, targeted delivery etc. [1]. The plant based polymers have been studied for their application in different pharmaceutical dosage forms like matrix controlled system, film coating agents, buccal films, microspheres, nanoparticles, viscous liquid formulations like ophthalmic solutions, suspensions and implants. Their applicability and efficacy has been proven. These have also been utilized as viscosity enhancers, stabilizers, disintegrants, solubilisers, emulsifiers, suspending agents, gelling agents, bioadhesives and binders in the above mentioned dosage forms[2].Polysaccharide hydrocolloids including mucilages, gums and glucans are abundant in nature and commonly found in many higher plants. These polysaccharides constitute a structurally diverse class of biological macromolecules with a broad range of physicochemical properties which are widely used for various applications in pharmacy and medicine. Gums are considered to be pathological products formed following injury to the plant or owing to unfavorable conditions, such as a drought, by a breakdown of cell walls (extra cellular formation, gummosis).Mucilage's are generally normal products of metabolism, formed within the cell (intracellular formation) and/or are produced without injury to the plant. Gums readily dissolve in water, whereas, mucilage forms slimy masses [3].

Mucilages are polysaccharide macromolecules that dissolve more or less upon contact with water and form colloidal solutions. Mucilages and gums are well known since ancient times for their medicinal value. In recent years, plant gums and mucilages have evoked tremendous interest due to their diverse application in pharmacy in the formulation of both solid and liquid dosage forms as thickeners, water retention agents, emulsion, stabilizers, suspending agents, binders and film formers. Apart from its use in finished medicines, newer uses have been found in the preparation of cosmetics, textiles, paint and paper. Hence the demand for these substances is

increasing and new sources are getting tapped. Vast application of plant mucilages and gums in various industries is because of low cost, ready availability and important properties which they confer on products. With the increase in demand for natural mucilages, it has become necessary to explore the newer sources of mucilage to meet the industrial demands. Muskmelon (Cucumis melo) is a vining plant in the Cucurbitaceae family. This warm-season crop is sensitive to cold temperatures and requires a fairly long growing season from seed to marketable fruit. Muskmelon is a beautiful, juicy, tasty and delicious fruit popular for its nutritive and medicinal properties. Musk melon is recommended for the treatment of cardiovascular disorders, as a diuretic, stomachic, antitussive and as a vermifuge. Its seeds are used to treat tuberculosis. They have high levels of potassium. Muskmelons are considered diuretics due to their high water content. It has been researched that Muskmelons possess the ability to lower the risk of cancer [4].However there are no reports on isolation and characterization of mucilage of Cucumis melo(Muskmelon). Hence, the present study is planned to isolate and characterize mucilage of Cucumis melo.

Materials & Methods

Musk melon was available from the local markets of Goa and Karnataka. All the other solvents, reagents and chemicals used were of analytical grade.

Isolation of mucilage

Precipitation with Alcohol

100g of musk melon pulp was taken and cut into small pieces. This was then soaked in 1 litter distilled water for 12hrs. The soaked pulp was then blended and was boiled for 15mins. It was then filtered using a muslin cloth with 8 folds. The filtrate so obtained was precipitated using ethanol. The mucilage was separated using many methods like filtration using separating funnel and then evaporating. The obtained mucilage was dried and stored.

Table 1: Physical characters of the mucilage

Physical characters	Observations
Percentage yield of ethanol	3%
Percentage yield of acetone	5%
pH	6.9-7.5
Appearence	orange in color
LOD	1.921%.
Swelling Index	33.1%

Precipitation with acetone

100g of musk melon was taken and cut in to small pieces and it was boiled in 1litre of distilled water. The boiled pulp was then filtered using muslin cloth with 8 folds. The filtrate was then precipitated using acetone. The precipitated mucilage was separated and dried

Characterization of the isolated mucilage:

Physical characterization:

The dried mucilage was studied for percentage yield, appearance, solubility, pH, swelling index.

Solubility:The mucilage was tested for solubility using various solvents.

pH: 1% w/v solution of mucilage was prepared in distilled water and the pH was determined using pH meter (Equip-Tronics. EQ610).

Table 2 : Preliminary phytochemical analysis

Test	Observations
Carbohydrates	+
Polysaccharides	+
Mucilage	+
Amino acids	+
Phenolic compounds	–
Alkaloids	–
Flavanoids	–
Fat and oil	–
Reducing sugars	–

(+) Present, (-) Absent

Swelling Index: 500mg of isolated mucilage was taken in a Petri dish and then 10ml of distilled water was added and the mixture was shaken and allowed to stand for 1hr. After one hour the remaining water in the petridish was discarded and the weight increase of the isolated mucilage was determined [5].

$$\text{Swelling Index (SI)} = \frac{W_2 - W_1}{W} \times 100$$

Where, W_1 = weight at time "0";
W_2 = weight at time "t"

Loss on drying: 500mg of mucilage was taken in the watch glass and weighed (W1). Then the watch glass was kept in the oven at 105° C, for 3hours. After 3 hours the watch glass was cooled and weighed again (W2) till the weight was constant.

$$\% \text{ of Loss on drying} = \frac{W_2 - W_1}{W} \times 100$$

Where, W_2 is the weight of the crucible after ignition; W_1 is the weight of the empty crucible; W is the weight of the substance.

Chemical characterization:

The mucilage was tested for preliminary phytochemicals such as carbohydrates, alkaloids, oils & fats, glycosides, phenolic compounds, mucilage, proteins, polysaccharides etc as per standard procedures.

FT-IR Spectral analysis:

The isolated mucilage were screened for FTIR analysis to find the stretching frequency using IR Affinity-1, Shimadzu, Japan in the range of 400-4000cm^{-1}.

Thin Layer Chromatography studies:

The TLC of the isolated mucilage was studied using silica gel as stationary phase and pet ether: ethyla acetate (80:20) was as mobile phase. Lactose, sucrose, glucose, dextrose were used as References.

Micromeritic evaluation

Bulk density (D_b)

Bulk density was determined by taking accurately weighted quantity of the dried mucilage in a measuring cylinder with the help of a large funnel and recording the volume and weight of the dried mucilage. It is expressed in gm/ml.

$$\text{Bulk density (Db)} = M/Vb$$

Where, M= mass of the particles, V_b= total volume of packing

Table. 3: Micrometric Analysis of the isolated mucilage

Property	Observation
Bulk density (D_b)	0.92622gm/cc
Tapped density (D_t)	0.95268gm/cc
Carr's Index	2.856%
Hausner's ratio	1.0285
Angle of repose	$38^0 48^{"}$

Tapped density (D_t)

Tapped density was determined by taking accurately weighed quantity of the dried mucilage in a measuring cylinder and recording the volume of granules after 300 tapping and weight of the total granules. Ti was expressed in g/ml.

$$\text{Tapped density (Dt)} = M/V$$

Where, M= weight of the granules (g), V= tapped volume of granules (ml).

Carr's Index

Carr's Index is an important measure that can be obtained from the bulk and tapped densities.

$$\text{Carr's index}(\%) = (Db - Dt)/Dt$$

Hausner's ratio

It indicates the flow properties of the powder and is measure by the ratio of tapped density to the bulk density.

$$\text{Hausner's ratio} = Dt/Db$$

Angle of repose

The funnel was fixed with its tip at a given height (h), above a flat horizontal surface on which a paper was placed. The powder was taken in the funnel and the test sample was allowed to flow smoothly, till the apex of the conical pile just touches the tip of the funnel. The height and the diameter of the powder cone was measured and angle of respose (θ) was calculated by using the following equation,

$$\text{Tan}(\theta) = h/r$$

Where, θ= angle of respose, h= height of the heap of powder, r = radius of the base of the powder.

Result & Discussion

Isolation of mucilage:

From the two methods employed such as acetone precipitation and ethanol precipitation method. Ethanol precipitation method was preferred since ethanol is easily available and is a green solvent.

Characterization of the isolated mucilage:

Physicochemical characterization:

Appearance:

The mucilage appeared to be orange in color (Figure.1).

Percentage Yield:

The percentage yield of the isolated mucilage was found to be 3% using ethanol and 5% using acetone precipitation method.

pH:

The pH of 1% w/v solution of the mucilage was found to be in the range of 6.9-7.5. This indicates neutral pH. Therefore the mucilage can be used in the various formulations, and hence will not alter the pH of the dosage form.

Solubility:

The solubility test was performed using various solvents. The mucilage was slightly soluble in chloroform, pet ether, diethyl ether and ethyl acetate. It was insoluble in ethanol and acetone. The results of the physicochemical properties were presented in Table.1

LOD of the mucilage was found to be 1.921%. This shows the moisture content of the mucilage and its hygroscopic nature.

Swelling index:

The swelling index of the isolated mucilage was found to be 33.1%. As the time increases, the swelling also increased. This indicates that the mucilage has good water absorbing capacity and it can retain water for a longer time. As the concentration of the mucilage increased the swelling index also increased.

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Chemical characterization:

Preliminary phytochemical analysis

The mucilage was tested for carbohydrates, alkaloids, glycosides, phenolic compounds, mucilage, amino acids, polysaccharides and oils & fats, etc. The preliminary phytoconstituents studies showed the presence of carbohydrates, mucilage, polysaccharides and amino acid in the isolated mucilage, which further conforms the presence of mucilage (Table.2).

FT-IR Spectral analysis

The spectra of the mucilage were taken using FTIR (IR Affinity-1 Shimadzu) in the range of 400-4000cm^{-1}.

The spectra of the mucilage (Figure.2) showed peaks at, 3286, 2927, 1649cm^{-1}these could be due to O-H streching, aliphatic C-H stretching, C=O stretching respectively.

Thin Layer Chromatographic studies:

The compared TLC plates of the mucilage and the reference showed the spot for presence of lactose. This suggests the presence of lactose in the isolated mucilage.

Micrometric evaluation:

The micrometric parameters such as Bulk density (D_b), Tapped density (D_t),Carr's Index, Hausner's ratio, Angle of repose of isolated mucilage are presented in Table.3.The angle of repose was found to be $38^0 48^{"}$, which indicates good flow property of the mucilage.

CONCLUSION

From the above study, we conclude that the evaluated parameters showed that Muskmelon The swelling index of the isolated mucilage was found to be 33.1% As the time increases, the swelling also increased. This indicates that the mucilage has good water absorbing capacity and it can retain water for a longer time. As the concentration of the mucilage increased the swelling index also increased.

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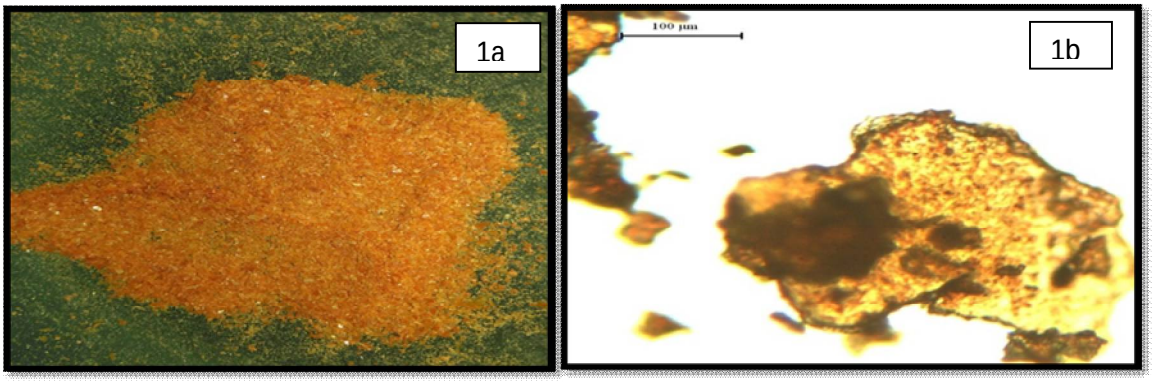

Figure 1: a) Mucilage powder; b) Microscopic examination of Mucilage

Figure 2: FT-IR spectra of the mucilage.