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Research Article

ISOLATION AND CHARACTERIZATION OF MUCILAGE FROM THE FRUIT PULP OF *FERONIA LIMONIA*

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ABSTRACT

Development of new excipients is time consuming, involves tedious procedures and is highly expensive. Instead, identification of new uses for the existing substances is relatively inexpensive and less time consuming. The intention of present study was designed for isolation and characterization of mucilage from the fruit pulp of *Feronia limonia* and explores its use as pharmaceutical excipient. Mucilage was isolated by maceration technique using distilled water and precipitated with acetone as a non-solvent. The isolated mucilage was investigated for purity by carrying out chemical tests for different phytochemical constituents and only carbohydrates were found to be present. The mucilage was further characterized for physical and flow properties. It had good swelling index (19.16) and pH was found to be 4.25. The mucilage powder had poor flow property and loose packing arrangement. The Fourier transform infrared (FT-IR) spectroscopic study revealed that the mucilage has characteristic peaks. From the study, it indicates that *Feronia limonia* mucilage powder has satisfactory pH and physicochemical properties, which can be used as pharmaceutical excipient in formulating various dosage forms.

Key Words: *Feronia limonia,* mucilage, isolation, characterization.

INTRODUCTION

The traditional concept of the excipients as any component other than the active substance has undergone a substantial evolution from an inert and cheap vehicle to an essential constituent of the formulation.[1]Excipients are generally considered as inert substances. But, with the advent of novel drug delivery systems, this concept no longer exists. As per International Pharmaceutical Excipients Council, USA (IPEC), pharmaceutical excipient can be defined as any substance other than the active drug or pro-drug, which has been appropriately evaluated for safety and is included in a drug delivery system to either aid processing of the system during manufacture; to protect, support, or enhance stability, bioavailability or patient acceptability; to assist in product identification; to enhance any other attribute of the overall safety and effectiveness of the drug product during storage or use.[2]

New and improved excipients continue to be developed to meet the needs of advanced drug delivery systems. Both synthetic and natural polymers have been investigated extensively. The synthetic polymers have certain disadvantages such as high cost, toxicity, environmental pollution during synthesis, non-renewable sources, side effects, and poor patient compliance. However the use of natural polymers for pharmaceutical applications is attractive because they are economical, readily available, low cost, non-toxic and capable of chemical modifications, potentially biodegradable and with few exceptions and also biocompatible. These can also be modified in different ways to obtain tailor made materials for drug delivery systems and thus can compete with the available synthetic excipients. Moreover, the tremendous orientation of pharma world towards these naturally derived polymers has become a subject of increasing interest to discover, extract and purify such compounds from the natural origin. Proteins, enzymes, muscle fibers, polysaccharides and gummy exudates are the natural polymers being used effectively in pharmaceutical dosage forms. Among these gums and mucilages are potent candidate to be used in various pharmaceutical formulations as a potential candidate for novel drug delivery system (NDDS).[3, 4]

Gums are pathological products, whereas mucilages are physiological products. Acacia, tragacanth, and guar gum are examples of gums while mucilages are often found in different parts of plants. For example, in the epidermal cells of leaves (senna), in seed coats (linseed, psyllium), roots (marshmallow), barks (slippery elm) and middle lamella (aloe).[5]

Feronia limonia (Wood apple) belongs to the family Rutaceae. In Hindi, it is known as Kaitha. It is a deciduous, glabrous tree with strong, sharp, straight, axillary thorns, found throughout the plains of India, Siwalik range and forests, at base of Himalayas up to an elevation of 450 m: often cultivated in many parts of India. Fruit pulp occurs mostly in broken pieces and sometimes entire, measuring about 4-5 cm in diameter; semicircular, rough, hard, having longitudinal ridges and furrows; reddish brown; odour, aromatic; taste, sour. It shows irregular, thin walled, parenchymatous cells; numerous idioblast cells filled with reddish-brown content; stone cells, slightly triangular and oval, with concentric striations and narrow lumen, found in groups; a few fibro-vascular bundles distributed in the pulp; xylem vessels having spiral thickenings. It consists of citric acid and mucilage.[6] The present study deals with the isolation of mucilage from the riped pulp of *Feronia limonia* fruit and its characterization as pharmaceutical excipient.

MATERIALS AND METHODS

Fruits of *Feronia limonia* were collected from Tadipatri, Anantapur District. The plant and fruits were authenticated at the Botany Department, Sri Venketaswara University, Tirupati, India. Acetone was procured from SL Scientifics, Anantapur. All other chemicals

used were of AR grade and distilled water was used throughout the experiments.

Isolation of Mucilage from the Fruit Pulp

The fruit pulp was collected from the fruits of *Feronia limonia* and were cut into small pieces.100g was weighed and soaked in water (500 ml) for 12 hours, and the material was crushed in a blender. The crushed material was warmed for 45 minutes with continuous stirring. After it is cooled, the material was passed through 8 folds of muslin cloth. To the filtrate, 1000ml of acetone was added to precipitate the mucilage. The mucilage was washed with acetone several times for purification. The coagulated mass was dried in oven at 40-45°C, and then it was crushed and passed through sieve number 80 and stored in a desiccator for further studies.[7-9]

Characterization of Mucilage

Chemical Characterization

The extracted mucilage was tested for chemical characteristics for identification to determine the purity of mucilage, tests for different phytochemical constituents such as carbohydrates, starch, proteins, alkaloids, glycosides, saponins, flavanoids, steroids and tannins and phenol compounds were carried out. [10]

Physical Characterization

Percentage Yield

The percentage yield was calculated based on the amount of kapitha fruit pulp used for isolation process and amount of dry powder obtained. The percentage yield was calculated using the formula mentioned below[11],

Percentage yield = Wt of dried mucilage obtained / Wt of fruit pulp used × 100

Organoleptic Evaluation

Mucilage isolated was characterized for various parameters like color, odor, taste, texture and fracture[12].

Solubility

One gram of mucilage was dissolved in 2 ml of distilled water, hot distilled water, acetone, methanol, chloroform, ether and dimethyl sulfoxide and the solubility profile was determined and recorded[13].

Loss on Drying

500mg of mucilage powder was weighed and placed in a clean and neat china dish. It was kept in hot air oven at 105°C for 1 hr. The china dish was removed from the oven and again the weight of the mucilage powder was determined until a constant weight was achieved. Loss on drying can be calculated by the following equation[14]

%LOD = initial weight – final weight / initial weight × 100

Swelling Index

The test was performed by taking 1 g of the mucilage powder in a 50.0 ml ground glass stoppered cylinder graduated over a height of 120 to 130 mm in 0.5 divisions. To this 25 ml of distilled water was added and this was shaken vigorously every 10 min for 1 hour and then allowed to stand for 3 hours. The volume occupied by the mucilage powder was recorded. Swelling index was calculated using the formula given as,

Swelling Index = [(Final volume – Initial volume)/Initial volume]

Melting Point

The powdered mucilage was transferred into a capillary tube and by using melting point apparatus melting point was determined. [8]

pH (1%w/v)

Extracted mucilage was weighed to prepare 1%w/v solution in water. pH of the solution was determined using digital pH meter. [13]

Micromeritic Properties Characterization[15]

Bulk Density (D_b)

Bulk density was determined by taking accurately weighed 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 and is given by,

$$D_b = M / V_b$$

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

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 100 tapping and weight of the total granules. It was expressed in g/ml and is given by,

$$D_t = M / V$$

Where, Dt = Tapped density (g/ml), M = weight of 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.

$$\text{Carr's Index (\%)} = D_b - D_t / D_t$$

Hausner's Ratio

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

Hausner's ratio = (Tapped density)/ (Bulk density)

Angle of Repose

A funnel was fixed with its tip at a given height (h), above a flat horizontal surface on which a graph paper was placed. The powder (10 g) 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 funnel. The height and diameter of the powder cone was measured and angle of repose (θ) was calculated by using the following equation,

$$\tan \theta = h / r$$

Where, θ = angle of repose; h = height of the heap of powder; r = radius of the base of the powder

Particle Size Distribution

Particle size distribution of the mucilage was determined by optical microscopy method. Standard stage micrometer is used to calibrate the eye- piece micrometer. A small portion of the mucilage powder

was transferred on a clean glass slide, one or two drops of liquid paraffin was added and dispersed uniformly with the help of a brush. So that particles should be independent and their distribution should be uniform. A cover-slip was placed carefully on the glass slide avoiding entrapment of air bubbles. Excess liquid was drained off with a blotting paper. The slide was placed on the stage of the microscope to focus in high power (45X). A total of 300 particles were measured for size distribution. [16]

Spectral Studies by FTIR

The Fourier transform infra-red analysis was conducted for the structure characterization. FTIR spectra of the isolated mucilage fruit pulp powder were recorded by using FT-IR spectroscopy (Bruker ATR, Model –Alpha).

Table 1: Phytochemical screening of mucilage

Tests for Phytoconstituents	Result
Carbohydrates	+
Starch	_
Proteins	_
Alkaloids	_
Glycosides	_
Saponins	_
Flavonoids	
Tannins and phenolic compounds	

+ Present; _ absent

Table 2: Organoleptic properties of isolated mucilage

Property	Inference
Color	Brown color
Odor	Characteristic
Taste	Tasteless
Touch	Hard
Texture	Rough

Table 3: Particle size distribution of isolated mucilage

Size (µm)	Number of particles
0-5	14
5-10	40
10-15	26
15-20	19
20-25	1

Figure 1: Isolated dried mucilage from the fruit pulp of *Feronia limonia*

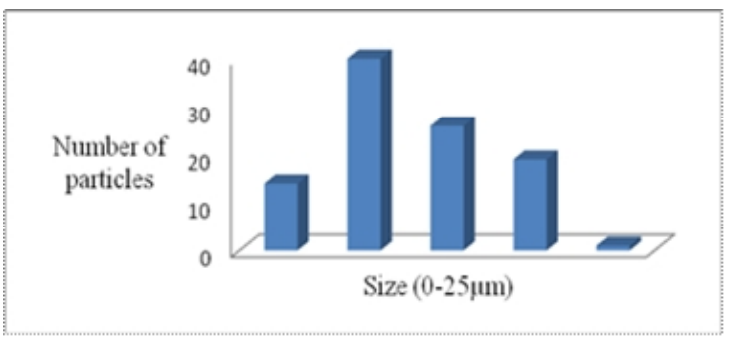

Figure 2: Particle size distribution for isolated mucilage

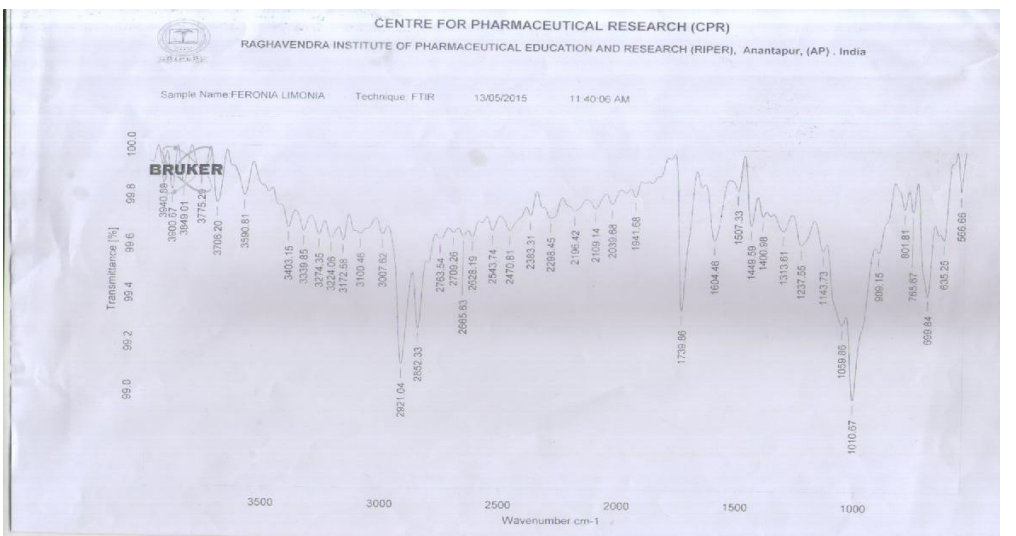

Figure 3: FTIR spectrum of the isolated mucilage

RESULTS AND DISCUSSION

Chemical Characterization

On treatment of mucilage with ruthenium red, it showed red colour confirming the obtained product as mucilage. A violet ring was formed at the junction of two liquids on reaction with Molisch's reagent indicating the presence of carbohydrates. The results of Phytochemical screening of mucilage was summarized in Table 1.

Physical Characterization

The percentage yield of the mucilage was found to be 4.38, with respect to dry weight of fruit pulp. The image of isolated dried mucilage powder from the fruit pulp of *Feronia limonia* was shown in Figure 1.

The organoleptic properties of isolated mucilage are shown in Table 2.

The solubility behavior of the isolated mucilage indicates that it forms viscous colloidal solution in distilled water, sparingly soluble in cold water, where as insoluble in methanol, acetone, chloroform, ether and dimethyl sulfoxide.

Loss on drying of isolated mucilage was found to be 0.171%. It gives information about the hygroscopic nature of the excipient, which can influence the manufacturing, packaging and storage of excipient as well as finished product.

Swelling index of isolated mucilage was found to be 19.16, indicating that mucilage might have good water uptake capacity and, hence, can form a hydrated three dimensional network from which the drug release might follow diffusion.

Melting point was found to be 115-120ºC.

The pH of the 1% mucilage solution was found to be 4.38, which was slightly acidic. The pH of mucilage indicated that adjustment of pH might be required in the formulation of oral and buccal drug delivery systems.

Micromeritic Properties Characterization

The Bulk density and tapped density was found to be 0.5263 g/cc, 0.7142 g/cc, respectively. These properties indicated about the packing arrangement of the particles. The result of these properties indicates the loose packing of the particles.

The Carr's index, Hausner's ratio and angle of repose were found to be 26.3 %, 1.36 and 83° 04'' respectively. The value of Carr's consolidation index indicated that mucilage was cohesive in nature. The results of Hausner's ratio and angle of repose indicated poor flow property of mucilage powder, which can be improved by the addition of glidants.

Particle size distribution for mucilage powder was given in Table 3. The Geometric mean was found to be 0.94 µm. The graphical representation of particle size distribution of the isolated mucilage was shown in Figure 2.

Spectral Studies by FTIR

The FTIR spectrum of isolated mucilage showed strong peaks at 2921.04, 1739.86, 1010.0 and medium peaks at 3706.2, 3590.81, 2852.33, 1604.46, 1059.86 and 699.84 respectively. The FTIR spectral values and probable bonds present were represented in shown in Figure 3.

CONCLUSION

From the results of the present work, it can be concluded that the isolated mucilage from fruit pulp of *Feronia limonia* shows the potential to be used as excipient in pharmaceutical formulation. The characterization studies indicated multifunctional property of mucilage. It may be used as binder in tablets, sustaining agent in matrix tablets, mucoadhesive material in buccal tablets, due to its swelling property. However, to establish the true potential, the above mentioned formulations should be prepared and evaluated.

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