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# EXTRACTION AND CHARACTERISATION OF MUCILAGE FROM THE LEAVES OF *HIBISCUS ROSA-SINENSIS* LINN. (MALVACEAE)

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**Keywords:**

*Hibiscus rosa-sinensis*, Mucilage extraction, Physico-chemical characterization

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**ABSTRACT:** Plant mucilages have found wide application in food, pharmaceutical and cosmetic industries. Hence, it would be worthwhile to characterise the physical and chemical attributes of mucilages from commonly available plants for their beneficial utilization. *Hibiscus rosa-sinensis* Linn. (Family Malvaceae), widely grown as an ornamental throughout the tropics and subtropicsis a good source of mucilage. The present study focuses on the extraction of the mucilage from the leaves of *Hibiscus rosa-sinensis*, its subsequent quantification and physico-chemical characterisation. A protocol was presently standardized for the yield optimization of mucilage from Hibiscus through detection of an appropriate maceration temperature and precipitation solvent. Further analyses revealed ideal pH value, swelling index, flow properties and particle size of Hibiscus mucilage. Moreover, chemical, FTIR spectral and electrokinetic data of Hibiscus mucilage revealed that it can be considered as a suitable blending ingredient in composites with potential industrial applications.

**INTRODUCTION:** Mucilages are normal metabolic products formed from sugars and uronic acid units in plant cells. Hydrophilic nature of mucilagenous plant parts becomes evident from the viscous and slimy mass it forms when dissolved in water [1]. Some of the mucilage rich plants are *Aloe vera, Abelmoschus esculentus, Trigonella foenum-graceum, Asparagus racemosus, Lepidium sativum, Salvia hispanica, Althaea officinalis* and *Hibiscus rosa-sinensis* [2, 3]. Generally, mucilages are merely considered as carbohydrate reserves in plant cells. However, reports show that they provide additional physiological benefits to plants including frost tolerance, water transport, wound response, plant host-pathogen interaction and maintenance of ionic balance of plant cells [4, 5].

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Moreover, mucilages find use as tablet binders, disintegrants, emulsifiers, suspending, gelling, stabilizing, thickening and film forming agents in transdermal and periodontal films in pharmaceutics [6, 7]. Further, they also serve as buccal tablets as well as sustaining agents in matrix tablets and coating agents in microcapsules including those used for protein delivery [8]. Thus the quantity and quality of mucilage in plants determines its eventual utility.

Presence of mucilage is a characteristic feature of the family Malvaceae [9]. One of the most commonly available ornamental plants belonging to Hibisceae tribe of the family Malvaceae is *Hibiscus rosa-sinensis* Linn. It is a tropical species of *Hibiscus* known colloquially as Chinese Hibiscus, China rose or shoeblack plant and is native to China, but is now widely grown as an ornamental throughout the tropics and subtropics [10, 11]. The mucilage of some plants are found to possess biologically active principles and are reported to have healing properties on burns, wounds, ulcers, external and internal inflammations

and irritations, diarrhoea and dysentery [4]. Extraction of mucilage from the leaves of *Hibiscus rosa-sinensis* and its use as natural shampoos is an age-old practise in India [12]. The present study is concerned with standardising the method for the extraction of leaf mucilage from *Hibiscus rosa-sinensis*, its subsequent quantification and physicochemical characterization.

## MATERIALS AND METHODS:

**Collection of the Plant Material:** Leaves of *Hibiscus rosa-sinensis* Linn. were collected from a home garden at Karamana (Kerala, India) in the month of November - December, 2015. The plant was authenticated by the herbarium curator, Department of Botany, Kerala University, Thiruvananthapuram and the voucher specimen was deposited in the Department Herbarium (KUBH 6035).

**Extraction of Mucilage:** The fresh young leaves of *Hibiscus rosa-sinensis* were collected, washed with water to remove dirt and debris, and shade dried for 25 days. The dried leaves were ground to a fine powder and the leaf powder was soaked in water for different time periods in order to identify the time taken for the complete release of mucilage.

The method of extraction of mucilage was a modified version of the method adopted by Baveja *et al*., [13] Extraction of mucilage involves, maceration and precipitation wherein, the mucilage released into the water is precipitated using a specific solvent.

In the present experiment, two temperatures (25 °C - considered here as the room temperature and 50 °C - achieved by keeping in hot air oven) and two solvents (acetone and ethyl alcohol) were tried for the maceration and subsequent precipitation to identify the difference in yield potential.

The leaf powder (10 gm) soaked in 100 ml water was squeezed through an eight - fold muslin cloth bag to remove the marc from the solution. The resultant filtrate was collected separately in two small beakers. Acetone and ethyl alcohol (150 ml each) were added separately to these two beakers in a quantity three times the volume of the total filtrate. The precipitated mucilage from both the beakers was taken out by gently spooling it out with a glass rod and collected separately in two different petri-dishes. The mucilage was then dried by keeping the dishes in an oven (at 50 °C). The dried mucilage powder was scraped out and grounded using a mortar and pestle and weighed. The powder so obtained was then stored in a desiccator.

**Quantification of Mucilage:** The percentage yield of extracted mucilage was calculated based on the amount of powdered leaf used for the extraction process and the amount of dry mucilage obtained individually depending upon solvents and temperature and expressed as percentage (%) [14].

% Yield = wt. of dried mucilage obtained × 100 / wt. of leaf powder taken

**Identification Test:** About 2 ml of aqueous extract of the mucilage was prepared and about 2.5 ml of absolute alcohol added to it [15]. The presence of mucilage in the extracted material was confirmed by performing Molisch's and Ruthenium red [16].

- **Molisch's Test:** Added a few drops of alcoholic alpha napthol, followed by a few drops of concentrated sulphuric acid through side of test tube.
- **Ruthenium Red Test:** Added a few drops of Ruthenium red to the test solution.

**Physicochemical Characterization of Isolated Mucilage:** Isolated mucilage of *Hibiscus rosa-sinensis* leaves were subjected to different physico-chemical analyses. The physical characters considered in the present study included organoleptic parameters (colour, odour and texture), solubility, loss on drying, flow properties bulk and tapped density, Carr's index, angle of repose, viscosity [17], particle size and zeta potential [18]. Chemical characters included preliminary phytochemical screening [19], estimation of total polysaccharide [20], total flavonoid [21] and phenolic contents [21]. FTIR spectroscopic studies were also carried out [17].

## RESULTS:

**Extraction of Mucilage:** The dried leaf powder was soaked in distilled water for different time durations in order to identify the time taken for the complete release of mucilage. After a few attempts, it was noticed that, the maximum release of mucilage could be achieved in about five hours of soaking in water.

**Quantification of the Mucilage:** The mucilage yield at two different temperatures used for maceration and precipitation (using two different solvents) are provided below **Table 1**. A relatively higher mucilage yield (21.41%) was obtained when the maceration was done at 50 ºC followed by acetone precipitation.

**TABLE 1: PERCENT YIELD VALUES OF THE ISOLATED MUCILAGE**

| S. no. | Solvent | Temperature | % yield |
|---|---|---|---|
| 1 | Ethyl alcohol | 25 ºC | 13.9% |
| 2 | Ethyl alcohol | 50 ºC | 18.72% |
| 3 | Acetone | 25 ºC | 15.78% |
| 4 | Acetone | 50 ºC | 21.41% |

**Physicochemical Characterisation of the Isolated Mucilage:**

**i) Organoleptic Characterisation of Isolated Mucilage:** The organoleptic features of the isolated mucilage as observed are given in **Table 2**.

**ii) Identification Test:** Appearance of a white cloudy precipitate at the junction on alcohol treatment indicates the presence of mucilage. Appearance of Purple to violet color with Molisch's test and a pink colour with ruthenium red confirms the presence of mucilage.

**iii) Solubility of Mucilage:** The solubility of the isolated mucilage in various solvents are given in **Table 3**.

**iv) Loss on Drying:** The weight loss on drying was found to be 12.01%.

**v) pH of Solution:** The pH value of Hibiscus mucilage (1% solution) was recorded as 6.2.

**vi) Swelling Ratio:** The swelling ratio of mucilage, determined in neutral, acidic and alkaline conditions are as follows **Table 4**.

**TABLE 2: ORGANOLEPTIC CHARACTERISATION OF THE ISOLATED MUCILAGE**

| S. no. | Tests | Observations |
|---|---|---|
| 1 | Colour | Brownish creamy |
| 2 | Odour | Characteristic |
| 3 | Texture | Amorphous |

**TABLE 3: SOLUBILITY OF THE ISOLATED MUCILAGE**

| S. no. | Solvent | Observation |
|---|---|---|
| 1 | Cold water | Swells to form a gel |
| 2 | Hot water | Soluble |
| 3 | Ethanol | Insoluble |
| 4 | Acetone | Insoluble |
| 5 | Chloroform | Insoluble |

**TABLE 4: SWELLING RATIO OF THE ISOLATED MUCILAGE**

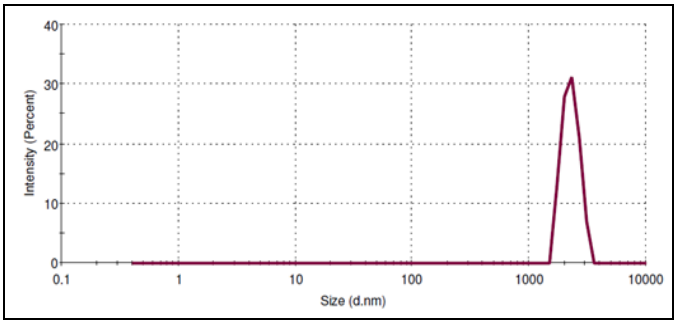
| S. no. | Solvent | Observations |
|---|---|---|
| 1 | Distilled water | 5.13 |
| 2 | 0.1N HCl | 4.86 |
| 3 | 0.1N NaOH | 4.83 |

**vii) Flow Properties:** The flow properties and compressibility of the dried mucilage, including bulk and tapped density, Carr's index, the Hausner's ratio, the Angle of repose and viscosity are as shown in **Table 5**.

**TABLE 5: FLOW PROPERTIES OF THE ISOLATED MUCILAGE**

| S. no. | Tests | Observations |
|---|---|---|
| 1 | Bulk density | 0.5 g/cc |
| 2 | Tapped density | 0.55 g/cc |
| 3 | Hausner's ratio | 1.1 |
| 4 | Compressibility index(Carr's index) | 9% |
| 5 | Angle of repose (ɵ) | 24.56º |
| 6 | Viscosity (1% solution) | 2.1435 poise |

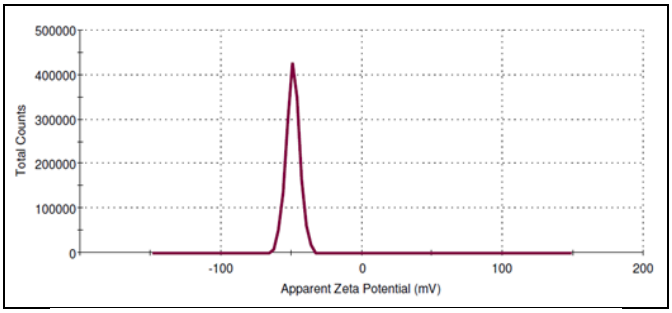
**viii) Particle Size:** The particle size was found to vary for the isolated mucilage. Maximum number of mucilage particles had a z-average value of 2273 ± 376.8 d.nm **Graph 1**.




**GRAPH 1: SIZE DISTRIBUTION BY INTENSITY**

**ix) Electrokinetic Studies:**

**Zeta Potential:** Electrokinetic studies revealed that the isolated mucilage particles have a negative surface charge. The zeta potential of maximum number of particles of *Hibiscus* mucilage was found to be -48.8 mV with a standard deviation of 4.86 mV **Graph 2**.




**GRAPH 2: ZETA POTENTIAL DISTRIBUTION**

**x) Preliminary Phytochemical Screening of the Isolated Mucilage Table 6:**

**TABLE 6: PHYTOCHEMICALS DETECTED IN THE ISOLATED MUCILAGE**

| S. no. | Tests | Observation |
|---|---|---|
| 1 | Test for carbohydrates | + |
| 2 | Test for reducing sugar | + |
| 3 | Test for proteins | _ |
| 4 | Test for fat | _ |
| 5 | Test for alkaloids | _ |
| 6 | Test for phenolics | + |
| 7 | Test for saponins | _ |
| 8 | Test for flavonoids | + |

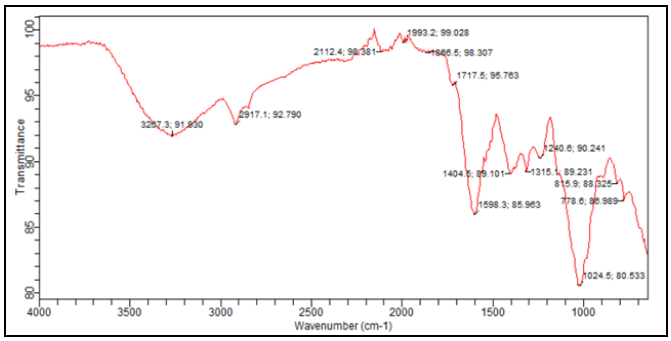
**xi) Quantitative Estimation:** The total polysaccharides, phenolics and flavonoids were quantified as described below.

**a. Determination of Total Polysaccharide:** The calibration curve for different concentrations of glucose was constructed and was found to be linear in the range of 50 - 100 µg/ml. The total polysaccharide content of Hibiscus leaf mucilage was found from the corresponding absorbance value in the calibration curve. The total polysaccharide content was found to be 64.13% (w/v).

**b. Determination of Total Phenolics:** The calibration curve for different concentrations of gallic acid was constructed and was found to be linear in the range of 10 - 60 µg/ml. The total phenolic content of Hibiscus leaf mucilage was found from the corresponding absorbance value in the calibration curve. The total phenolic content was found to be 1.64 % (w/v).

**c. Determination of Total Flavonoids:** The calibration curve for different concentrations of gallic acid was constructed as above. The total flavonoid content of Hibiscus leaf mucilage was found from the corresponding absorbance value in the calibration curve. The total flavonoid content was found to be 0.834 % (w/v).

**xii) Fourier Transform Infrared (FT-IR) Spectral Studies:** FTIR spectroscopy was used to characterise the functional groups in Hibiscus mucilage. The FTIR spectra of mucilage obtained from Hibiscus is presented in **Graph 3**. The FTIR spectrum of the mucilage showed sharp and characteristic peaks at 3267.3, 2917.1, 2319.3, 2112.4, 1598.3, 1404.5, 1315.1, 1240.6, 1024.5cm$^{-1}$ **Table 7**.




**GRAPH 3: FTIR SPECTRA OF THE ISOLATED *HIBISCUS ROSA-SINENSIS* MUCILAGE**

**TABLE 7: FUNCTIONAL GROUPS AND PEAK FREQUENCIES IN THE FTIR SPECTRUM**

| S. no. | Peak (frequency) (cm$^{-1}$) | Functional group |
|---|---|---|
| 1 | 3267.3 | Alcohol O-H stretch |
| 2 | 2917.1 | Alkyl C-H stretch |
| 3 | 2319.3 | C-H stretch |
| 4 | 2112.4 | Alkynyl C=C stretch |
| 5 | 1598.3 | Aromatic C=C bending |
| 6 | 1404.5 | C-H bend |
| 7 | 1315.1 | C-H rock |
| 8 | 1240.6 | C-O bend, aromatic |
| 9 | 1024.5 | C-O stretch |

## DISCUSSION:

**Extraction of Mucilage:** Generally all mucilage extraction methods consist of two successive processes namely maceration and precipitation. The most commonly used solvent for maceration is water but Phosphate buffer and NaOH solutions have also been used [22, 23].

A further refined method was defatting (petroleum ether for 12 hrs) prior to maceration [24]. Both percolation and soxhlet have been used for defatting. Microwave extraction was adopted by Kamble *et al.*,[25] However, precipitation is usually done using either ethanol [26] or acetone [27, 28].

In the present study, maceration was done using distilled water followed by ethanol or acetone precipitation. On addition of the solvent (three times the volume of the filtrate), a light brownish cream coloured precipitate was seen floating on the surface.

**Quantification of the Mucilage:** The percentage yield of mucilage was tested in two different temperatures and precipitated using acetone and ethyl alcohol.

The yield was found to be about 5% more when extraction was carried out at 50 ºC than at room temperature (25 ºC). This was probably due to an increase in the permeability of the cell membranes of mucilage canal cells whereby, a rise in temperature resulted in the release of mucilage. In addition, the increase in yield with temperature might be due to the decrease in viscosity of the mucilage which makes it less sticky and can be effectively released under high temperature [29].

The yield was found to be 2 - 3% higher when precipitated with acetone compared to ethyl alcohol. The yield in the present study (21.41%with acetone 18.72% with ethanol) was slightly higher compared to earlier reports (17% with acetone 14.7% with ethanol) by Shah *et al*., [27] and rabhakaran *et al.,* [22] respectively.

Thus from the present experiment, it may be said that acetone is the suitable solvent for extraction of mucilage from *Hibiscusrosa - sinensis* plants Further, hot extraction (carried out at 50 ºC) yielded better results than cold extraction.

**Physicochemical Characterisation of the Isolated Mucilage:** Physicochemical characterisation studies of isolated mucilage are relevant as they could serve as useful data for enabling the utilisation of Hibiscus mucilage for food and pharmaceutical industry.

Organoleptic characterisation of isolated mucilage showed that, it is a greenish brown powder, with characteristic odour and amorphous nature. When dissolved in water, it gave a neutral, greenish brown solution which is slimy and colloidal in nature. Shah *et al*., [27] and Prabhakaran *et al.,* [22] reported Hibiscus mucilage as cream coloured while Gupta *et al.,* [28] reported it as green coloured.

The identity of mucilage was evident by the appearance of a white cloudy precipitate [15] and was confirmed by the positive results with Molisch's test and Ruthenium red test [16].

The mucilage was found to swell in cold water and subsequently dissolved on vigorous agitation. It readily dissolved in hot water but was found insoluble in ethanol, acetone and chloroform. The property of being insoluble in these solvents is probably put to use in its precipitation during the extraction process. Since the weight loss on drying was found to be relatively high (12.01%), it appears that some amount of moisture was still available in the mucilage and could help in interactions with other materials.

pH (of 1% solution) of mucilage was determined as 6.2, which is nearly neutral, indicating that it may be less irritating to the mucous membrane and gastrointestinal tract when ingested orally [30]. Mucilage from the root of *Althaea officinalis*, another member of Malvaceae family is known to relieve irritation of mucous membranes by forming a protective film and has been traditionally used as a cough medicine [31].

Swelling of mucilage was found to be highest in distilled water with a swelling index of 5.13 followed by more or less similar swelling indices in acidic (0.1N HCl- 4.86) and alkaline media (0.1N NaOH- 4.83). Since there was an appreciable change in swelling after 24 hrs in all three media, the mucilage appears to have good swelling properties.

The Bulk and tapped density values were considered for calculating compressibility index or Carr index. Since the difference between the bulk and tapped density was low (0.05), the Carr index was also found to be low (9%). Powders with Carr index less than 15% are considered to have good compressibility properties [32]. Lower the value for Carr index, lower the inter-particle interactions, which in turn reflect a better flow property of the mucilage powder [33].

The angle of repose of a powder provides an insight into the magnitude of the cohesiveness of the powder and hence its flowability. The values for angle of repose and Hausner ratio for dried mucilage powder were 24.56º (<35º) and 1.1 (<1.25) respectively. It could be inferred that being less cohesive the mucilage has superior flow property [34].

The viscosity of 1% solution was found to be 2.1435 poise. It can be concluded that the mucilage has a viscosity that is suitable for formulation of gel, jellies, and films [17]. The size of the particles in a suspension has importance, as the size determines the surface area available for the interaction with other components of the suspension [35].

Particle size measurements not only gives a direct idea of the space occupied by the particles of the substance but also provides information regarding properties like potential to dissolve, packing density, sedimentation and product appearance like gloss [36]. Smaller the particles, larger the light scattering ability and hence increased gloss [37].

During particle size analysis, the particle size of *Hibiscus* mucilage was found to vary. However, particles with Z-average value of 2273 ± 376.8 d.nm were found in higher intensity. The Z-Average size or Z-Average mean used in dynamic light scattering is a parameter also known as the cumulant mean. It is the primary and most stable parameter produced by the technique [38]. The Z-Average mean is the best value to report when used in a quality control setting as it is defined in ISO 13321 and more recently ISO 22412 which defines this mean as the 'harmonic intensity averaged particle diameter' [39, 40].

The zeta potential is a key indicator of the stability of colloidal dispersions. The zeta potential is caused by the net electrical charge contained within the region bounded by the slipping plane [41]. The mucilage particles were found to have a negative charge on their surface. The electrokinetic studies revealed that the maximum number of *Hibiscus rosa-sinensis* mucilage particles had a zeta potential value of -48.8 mV with a standard deviation of 4.86 mV. Since the measured value of zeta potential for Hibiscus mucilage lies within the range of ± 40 mV to ± 60 mV, it is supposed to have good stability in water [42]. Here, the electrostatic repulsion between adjacent, similarly charged particles in dispersion is strong enough so that the solution resists aggregation and subsequent flocculation, thereby remaining uniformly distributed in the solvent (water).

*Hibiscus rosa-sinensis* mucilage gave positive results for carbohydrates specifically reducing sugars, phenolics and flavonoids in the present study. Phytoconstituents such as proteins, fats, alkaloids and saponins were absent in the isolated mucilage. Mucilage of *Hibiscus rosa-sinensis* has been earlier reported to contain reducing sugars like L-rhamnose, D-galactose, and acidic polysaccharides like D-galactouronic acid and D-glucuronic acid [43]. The total polysaccharide content in the isolated mucilage was found to be 64.13% (w/v) which was however lower than previous reports (72%) [44]. Trace amounts of phenolics (1.64%) and flavonoids (0.83%) could also be detected in the isolated mucilage.

The infra-red (IR) spectrum of a given compound is always unique and characteristic. IR spectroscopy is a quick and relatively cheap technique for identifying compounds. The different peaks in the IR spectra exhibit vibrations that indicate the nature of chemical bonds in the molecules of the different compounds [45]. FTIR spectra of *Hibiscus rosa-sinensis* is mucilage is characterized by eight strong peaks, with major functional and structural groups such as polymeric hydroxyl groups (3500 - 3200 $cm^{-1}$) and alkanes (3000 - 2850 $cm^{-1}$) and thereby ensures the presence of polymeric chains in the mucilage.

FTIR spectra show a characteristic peak in fingerprint region at 1024.5 $cm^{-1}$ to C–O stretching vibration. The peak at 1598.3 $cm^{-1}$ indicates presence of aromatic C=C bending vibration. Absence of characteristic sharp peaks at 1650 $cm^{-1}$ to 1700 $cm^{-1}$ indicates a modest degree of cross-linking in the molecule. The sharp band at 2917.1 $cm^{-1}$ is characteristic of methyl C–H stretching associated with aromatic ring system. The thick band at 3267.3 $cm^{-1}$ is due to intra- and inter-molecular hydroxyl groups that constitutes the gross structure of polymeric carbohydrates. All these characteristic bands confirmed it to be a polysaccharide with modest degree of cross-linking. The different bonds and functional groups characterize the molecules of the different compounds [46]. All the peaks in the IR spectrum of mucilages were consistent with a polysaccharide structure.

**CONCLUSION:** An extraction protocol was presently developed for yield optimization of *Hibiscus* mucilage. The extracted material was confirmed as mucilage by positive results with Molisch's and ruthenium red tests. The polysaccharide rich Hibiscus mucilage was found to possess excellent physicochemical properties such as neutral pH, higher swelling index, good flow properties and smaller particle size. Spectral studies revealed the presence of functional groups

which confer interactive stability for the mucilage. Also, electrokinetic studies indicated good stability properties. In view of the results obtained, mucilage of Hibiscus can be considered as a potential ingredient for food and pharmaceutical industry.

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