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Extraction and Characterization of Mucilage from *Cassia fistula* Seeds

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Abstract: In the present era, polymers from natural sources become a better alternate for delivery of various kinds of drugs. The compliance and use of polymers obtained from natural origin are increasing day by day in the pharmaceutical industries due to a fewer side effects. Mucilages obtained from natural sources are a better alternative for designing and development of mucoadhesive drug delivery system. Due to the mucoadhesive property, the mucilage may also be used for various purposes such as emulsifying agent, suspending agent, bio-adhesive agent, matrix forming agent and controlled release. Some properties of mucilage like biodegradable in nature, nontoxic, less expensive and free availability make them better excipients for different dosage form. The modifications in the mucilage make them to compete with the synthetic products that are commercially available in the market. In the present research work, we have carried out the extraction and characterization of mucilage obtained from *Cassia fistula* seeds. Further we have also carried out organoleptic parameters, Micromeritic study, particle size, swelling index etc for the extract. On the basis of these parameters we can conclude that *Cassia fistula* seed extract will be a better alternative for designing and development a mucoadhesive dosage form.

Key words: Mucoadhesive · Natural Polymer · Mucilage · Pharmaceutical Excipients · Cassia Fistula

INTRODUCTION

Commonly the mucilage is used as *an* adjuvant in the variety of pharmaceutical preparations. Now a day the researchers are giving special focus on the extraction of the mucilage from various natural sources. The natural mucilage has various advantages over synthetic materials like they are non-toxic, non-irritating and emollient are available at low cost [1]. The mucilage extracted from the plant has great importance in the pharmaceutical industry due to the presence of polysaccharide. Mucilage may be use for various properties like gelling agent, suspending agent, disintegrating agent, stabilizing agents in different types of pharmaceutical formulation etc. The mucoadhesive agents also can be used to provide the sustained and controlled release action for the formulations. Commonly the method used for the fabricating drugs in controlled release pharmaceutical formulation is by incorporating them with the matrix having hydrophilic rate controlling natural polymers. Presently many researches are going on the use and application of naturally occurring biocompatible polymeric material for the preparation and designing of various dosage form for controlled release drug delivery system. Mostly the naturally extracted gums are biodegradable and non- toxic, which posses a property of hydration and get swell when they come on contact with the aqueous media, this makes them suitable for the development and preparation of dosage form. There are so many researches available using various natural sources for the extraction and characterization of mucoadhesive components. The well known examples of mucilage extracted from plants are acacia, tragacanth, gum kraya, gum ghati etc. Natural polymers play a key role as pharmaceutical excipients. The wide range of their bio-compatibility with drug and other materials make them important to be used as pharmaceutical excipients. Their easily availability and economic reliability also makes them a choice for pharmaceutical excipient. In the present study, extraction, phytochemical screening and characterization of *Cassia fistula* seed mucilage has been carried out. The extract obtained from the *Cassia fistula* seed has been used as a mucodhesive component in present research [1].

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MATERIALS AND METHODS

The plant seeds were purchased from the local market of Gurgaon (Hariyana), India. Ethyl alcohol was purchased from changshu yangyuan chemical, China. Petroleum ether was purchased from CDH laboratory Reagent Central Drug House P. Ltd. All the chemicals used in the research work were of laboratory grades.

Extraction of Mucilage: The extraction is done from the seeds of *Cassia fistula*. The extraction was done in following two steps [2, 3, 4, 5, 6, 7].

Step 1: *Cassia fistula* seeds were dried in hot air oven at 40°C for two hours. The seeds were then crushed using grinder for obtain coarse powder. The coarse powder was then soaked in cold water for 30 minutes. The soaked seeds was transferred to hot boiling water and boiled for 2 hours to make the solution more concentrated. The mucilage was then filtered with the help of muslin cloth and the material was squeezed to separate mark from the filtrate.

Step 2: In this step the filtrate was centrifuged at 5000 rpm for 20 minutes using centrifugation apparatus (Nitin scientific). The supernatant was collected and kept in refrigerator for 30 minutes. After cooling the product was treated using suitable solvent (*i.e*. ethyl alcohol and petroleum ether) for the precipitation of mucilage and the precipitate was washed so many times using same solvent for removing unwanted impurities. The precipitates was then separated from solvent and kept for drying in hot air oven at 40°C. At last the product was crushed and uniform particle size was obtained by passing the powdered mucilage through sieve #80. The powdered mucilage was packed in suitable container and was stored in desiccators for the further use.

Evaluation of Mucilage: For the evaluation of the mucilage we have determined these parameters such as.

%age yield: The %age yield was determined using following formula [2].

$$\%yeild = \frac{practical\ yield}{theoritical\ yield} \times 100$$

Organoleptic Evaluation: The extracted mucilage was evaluated for organoleptic properties like color, odour, taste, texture and fracture using the standard procedure [8].

Phytochemical Screening of Extracted Mucilage: The aqueous solution of the extracted mucilage was prepared and used for phytochemical screening for indentifying the presence of carbohydrates, tannins, flavanoids, proteins, volatile oil, fats and phenols in accordance to the standard procedure [8].

Determination of Mucilage pH: The pH of mucilage was determined by using 1%w/v solution of extracted mucilage and analysis was carried out using the digital pH meter [8].

Particle Size of Extracted Mucilage: Particle size of extracted mucilage was determined using compound microscope (Hicon) at 10X lens. Magnification value was calculated by calibrating stage micrometer and eye piece. 50 readings of the sample were taken and their mean was calculated [8].

Solubility Study of the Extracted Mucilage: Extracted mucilage was tested for the solubility in different solvents. One part of the powdered mucilage was shaken with the solvent and analysis was carried out using UV spectrophotometer. The solubility determination was carried out using standards specified in Indian Pharmacopoeia [8].

Swelling Index of Extracted Mucilage: Swelling index is defined as the volume in ml absorbed by 1gm of the powder in specified conditions. Swelling index of mucilage was carried out using specific amount of extracted powder mucilage with a standard procedure [8]. The swelling index was calculated using following formula.

Viscosity: The viscosity was determined and calculated for the 1% solution of mucilage using Ostwald viscometer [9].

Surface Tension: The surface tension was determined and calculated for the 1% solution of mucilage by Stalgmometer using drop count method [10].

$$surface\ tension = \frac{no.of\ drops\ of\ water \times density\ of\ test\ solution}{no.of\ drops\ of\ test\ solution \times density\ water} \times 71.8$$

Micromeritic Evaluation of Extracted Mucilage

Bulk Density, Bulkiness and Tap Density: Bulk density of extracted powder mucilage was determined by bulk density apparatus (Nitin scientific). Density can be calculated as weight of the powder divided by the volume

acquired by that weighed powder. The SI unit of density is gm/cm^3. The difference between the bulk density and tapped density is only that, in bulk density we have to use the bulk volume whereas in the tapped density we have to use tapped volume which can be obtained by switching on the equipment for 50 times tapings. The reciprocal of bulk density is called bulkiness [11].

Flow Property of Extracted Mucilage: The flow property of the isolated mucilage powder was determined by calculating the Carr's index, Hausner's Ratio and angle of repose. The observation and calculation was carried out three times to minimize the possible errors and mean was calculated for the discussion of results obtained [11].

Carr's Compressibility Index: The Carr's Compressibility index was determined using following formula [11].

Hausner's Ratio: The Hausner's ratio was calculated using following formula [11].

$$Carr'sCompressibility\ index = \frac{Tapped\ density - Bulk\ density}{Tapped\ density} * 100$$

$$Hausner'sratio = \frac{Tapped\ density}{Bulk\ density}$$

Angle of Repose: The angle of repose was determined using the standard procedure and calculation was carried out using following formula[11]:

$$\theta = \tan^{-1}\frac{h}{r}$$

RESULT AND DISCUSSION

% Age Yield: The extraction of cassia fistula mucilage was carried out and the yield of the extract was found to be 21%w/w.

Phytochemical Screening: The extracted mucilage was evaluated for various phytochemical test and the results obtained are summarized in Table 1. From the results, it was found that the extracted mucilage give the positive test for carbohydrates. Other tests like for tannins, flavanoids, proteins, volatile oil, fats and phenols found negative. When we perform the specific test for distinguishing carbohydrate it was found that the glucose and reducing sugars was absent in extracted mucilage.

Table 1: Phytochemical test of extracted mucilage.

Test	Present/Absent
Carbohydrates	+
Reducing sugar	-
Glucose	-
Tannins	-
Polysaccharides	-
Protiens	-
Volatile oil	-
Fats	-
Phenols	-
Flavanoids	-

+ (Present), - (Negative)

Table 2: Organolapetic properties of extracted mucilage

Color	Odour	Taste	Texture	Fracture
Brown	Odourless	Characteristics	Rough	Irregular

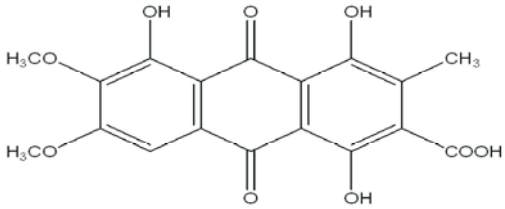
Table 3: pH, particle size, viscosity, surface tension, swelling index of extracted mucilage

Parameters	Observations
pH	6.2
Total Ash (%)	3.56 ±0.115
Particle size (μm)	76.32 ± 1.051
Viscosity (centi poise)	84.04 ± 1.657
Surface Tension (dyne/cm)	47.86 ± 3.613
Swelling Index (%)	72.24 ± 3.395

Organolapetic Properties: The extracted mucilage was evaluated for their organoleptic properties and the results obtained are summarized in Table 2. The organoleptic properties of the extracted mucilage were found to be acceptable after investigation.

pH, Ash Value, Particle Size, Viscosity, Surface Tension, Swelling Index of Extracted Mucilage: The pH, particle size, viscosity, surface tension, swelling index of extracted mucilage were evaluated and the results obtained are summarized in Table 3. The pH of the extracted mucilage was found to be in the range between 6.2. The ash value of the mucilage was found to be 3.56. Particle size of the extracted mucilage was found to be 76.32. The swelling index of the cassia fistula seed mucilage was found to be 72.24%. This shows it has excellent swelling property.

Micromeritics Properties of Extracted Mucilage: The extracted mucilage was evaluated for their micromeritic properties and the results obtained are summarized in Table 4. When we go through the comparison of the results for angle of repose and Carr's index, it was found that the mucilage powder shows excellent flow property as well as good compressibility characteristics.



fistulic acid

Fig. 1: IR spectra of extracted mucilage

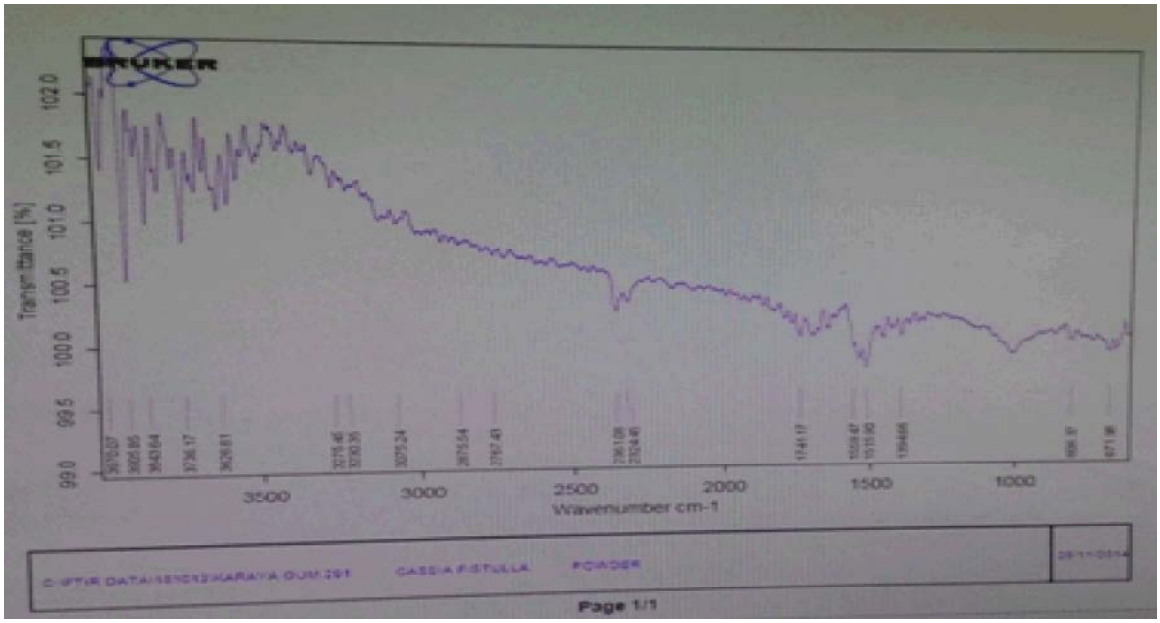


Fig. 2: IR Spectra

Table 4: Micromeritics properties of extracted mucilage

Parameters	Observations
Bulk Density (g/cm^3)	0.57 ± 0.017
Bulkiness (cm^3/g)	1.75 ± 0.051
Tapped Density (g/cm^3)	0.64 ± 0.230
Carr's Index (%)	11.84 ± 0.479
Hausner's Ratio	1.69 ± 0.005
Angle of Repose(°)	24.29 ± 6.007

Table 5: Solubility of extracted mucilage in different solvent

S.No.	Solvent system	Observation
1	Water	Soluble
2	Ethanol	Insoluble
3	Benzene	Insoluble
4	Acetone	Insoluble
5	Petroleum ether	Insoluble

Table 6: Interpretation of the result for IR spectra of extracted mucilage

S.No.	Functional group	Peak (frequency in cm^{-1})
1	OH	3626.81
2	C=O	1741.17
3	N-O	1515.90
4	C-O	1000

Solubility Determination of Extracted Mucilage: The extracted mucilage was evaluated for their solubility using various solvents and the results obtained are summarized in Table 5. When the solubility determination was carried out using water as solvent it was found that the extracted mucilage first swell up in water and then get soluble. The extract was found insoluble in other solvent like Ethanol, Benzene, Ether and petroleum ether. The solubility was also determined using the ash of extracted mucilage and the result was that the ash of the extract was insoluble in the acetone, ethanol, petroleum ether and benzene.

Mucoadhesion Test: This test was performed by using ruthenium red. Positive results are obtained which shows that the extracted mucilage have mucoadhesive property.

Ir Spectroscopy of Extracted Mucilage: Following peaks are obtained in the IR spectroscopy of extracted mucilage from *cassia fistula.* As shown in the Table 6. The main compound present in cassia fistula extract is fistulic acid. 3626.81, 1741.17, 1515.90, 1000, which confirms the presence of C-H, C=O, N-O, C-O, respectively.

CONCLUSIONS

The extraction of *cassia fistula* mucilage was carried out using simple water based extraction and various parameters for characterization of mucilage were done. Form these result, it can be concluded that the extracted mucilage showed all the properties as per the specific requirement for mucilage to be used as the mucoadhesive agent for drug delivery. The physicochemical parameters of extracted mucilage make it suitable and acceptable, which can be used as pharmaceutical adjuvant for drug delivery system.

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