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PHYTOCHEMICAL CHARACTERIZATION AND FUNCTIONAL PROPERTIES OF NATURALLY ISOLATED MUCILAGE FROM *HIBISCUS CANNABINUS* L., AS A POTENTIAL NATURAL PHARMACEUTICAL EXCIPIENT

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ABSTRACT

Hibiscus cannabinus L., fruits are rich in mucilage and have great functional value. This study was performed to examine the *Hibiscus cannabinus* L., mucilage and its functional groups with the help of NMR Spectroscopy as a pharmaceutical excipient for pharmaceutical product development. The plant was collected and authenticated by Dr. A.K.S. Rawat (Scientist & Former Head) Department of Pharmacognosy and Ethnopharmacology Division CSIR-National Botanical Research Institute, Lucknow (no 254060). The mucilage was isolated from the fruits of *Hibiscus cannabinus* L., All the other chemicals used were of analytical grade and distilled water was used throughout the experiments. The mucilage was found to be 9.54% w/w which was off white in colour, tasteless with characteristic odour having neutral pH 7.2±0.2, loss on drying to be 9.34 % w/w and swelling index was revealed to be 9±0.52% w/w. It was found to be soluble in hot water, insoluble in organic solvents & swelled to form a gel in cold water. The FT-IR and NMR spectra confirms the purity of mucilage and presence of polysaccharides. The results of phytochemical and physicochemical tests indicated the suitability of dosage forms due to its: flow ability, neutral pH, swelling potential, and viscous in nature. Therefore, mucilage is a plant-based natural excipient has scope to be utilized in pharmaceutical formulations for product development & it has high scientific relevance particularly as a plant-based natural excipient in pharmaceutical product development.

KEYWORDS: *Hibiscus cannabinus* L., mucilage, NMR Spectroscopy, Natural pharmaceutical excipient.

1. INTRODUCTION

It is a rich and worthy source of mucilage because of easily availability, low cost and non-toxicity as compared to synthetic polymer.[1] Mucilage is a thick, sticky, gluey substance produced by nearly all plants and some microorganisms.[2] It is a metabolised product which is intracellularly formed without injury to the plant and form slimy masses with water.[3] The mucilage acts as a membrane thickener and food reserve in the plants. It is a polysaccharide mixture having high molecular weight (20000 and more) commonly found in various organs of many higher plant species.[4] Mucilage's of different plants are used as natural polymer in various pharmaceutical formulations as a potent candidate for both conventional and novel drug delivery system (NDDS).[5,6] The natural polymers have advantages over synthetic ones, since they are chemically inert, non-toxic, less expensive, biodegradable and extensively available. They hold a variety of pharmaceutical polymer properties, which include binding, disintegrating and suspending properties at a different pharmaceutical dosage form. The synthetic polymer used as excipient suffer from various disadvantages such as high cost, toxicity, non-biodegradability and environmental pollution is caused during their synthesis. So, natural polymers are preferred over semi- synthetic and synthetic polymers due to their valuable characteristics (non – toxic, lower cost, free availability, emollient and – irritating nature).[7,8] Thus, mucilage is a noble candidate to be used as pharmaceutical polymer. Therefore, present study was focused towards the phytochemical characterization, functional properties and evaluation of isolated mucilage of *Hibiscus cannabinus* L., for formulation development as a potential natural polymer for pharmaceutical formulations in pharmaceutical industries and to provide a new way to future technologies.

1.1. Advantage of natural plant-based polymer[9,10]

Environmental friendly processing and bio-acceptable

Biocompatible, biodegradable and non-toxic

Local availability, low cost, and natural in origin

Free from side effects and better patient tolerance as well as public acceptance

1.2. Disadvantage of synthetic polymers used as polymer[9,10]

High cost, toxicity

Non-biocompatible

Non-biodegradability

Environmental pollution caused by their synthesis

2. MATERIAL AND METHODS

2.1 Materials

The plant was collected and authenticated by Dr.A.K.S Rawat (Scientist & Former Head) the Department of Pharmacognosy and Ethnopharmacology Divison CSIR-National Botanical Research Institute, Lucknow (no 254060). All the other chemicals used were of analytical grade and distilled water was used throughout the experiments.

2.2. Isolation of Extraction and Mucilage

100 gm of fruits was collected, soaked in 1 L distilled water for 1-2 hours and then homogenized with mechanical blender after that it was filtered by muslin cloth. It was centrifuged at 1000 rpm for 30 minutes. The supernatant liquid was added with 3 times volume of ethanol and centrifuged for another 10 min, to collect precipitated material and dry in a hot air oven at 40-45 ℃. The mucilage powder thus obtained was passed through sieve # 40 and stored in a desiccator at room temperature.[11]

3. CHARACTERIZATION OF MUCILAGE

Extracted and isolated mucilage was characterized for its various properties by performing the following tests.

3.1. Organoleptic evaluation of isolated mucilage

The isolated mucilage was characterized for organoleptic properties such as colour, taste, odour, and texture, fracture.[12,13]

3.2. Phytochemical characterization of mucilage

Preliminary tests were performed to confirm the nature of isolated mucilage. The chemical tests that were conducted are as follows.

3.3. Qualitative Phytochemical Analysis[13]

3.3.1. Test for Carbohydrates

3.3.1.1. Iodine test[2]

Isolated mucilage was mixed with 2ml of iodine solution. A dark blue or purple coloration showed the presence of the carbohydrates.

3.3.1.2. Molisch's test

Sample (2ml mucilage) was assorted with 2 drop of alcoholic solution of ά - naphthol are added. The combination was shaken well and limited drops of concentrated sulphuric acid was added gradually along the edges of test tube. A violet ring showed the presence of carbohydrates.

3.3.1.3. Benedict's test

Took 0.5ml of mucilage filtrate, 0.5ml of Benedict's reagent and mixed; mixture was heated on a boiling water bath for 2minutes. A specific coloured precipitate showed the occurrence of sugar.

3.3.2. Test for Mucilage's

3.3.2.1. Ruthenium red test

1-2 drops of ruthenium red solution were added to a minor quantity of mucilage put on a glass slide and saw under microscope. Uncertainty mucilage is present spots appear in pink colour.[2,12,13]

The extract (200 mg mucilage) was dissolved in 20ml of distilled water and to this 4ml of absolute alcohol is added with constant stirring. White or cloudy precipitate indicated the presence of mucilage.

3.3.3. Test for Fixed oils and Fats

3.3.3.1. Spot test

Took small amount of mucilage was forced between two filter papers. Oil stain on the paper showed the occurrence of fixed oils.

3.3.4. Test for proteins

3.3.4.1. Millon's test

Took 2ml sample (mucilage) few drops of millon's reagent are added. A white precipitate indicated the presence of proteins.[14,15]

3.4. NMR Spectroscopy Analysis

NMR experiments were done on a Bruker Avance 300MHz instrument with TMS as an internal standard. ESI mass spectra were recorded an Applied Bio system's API-30000 after dissolving the compounds in D_2O elemental analysis was carried out in Heraus CHN analyser.[16]

3.5. Physicochemical Characterization of Isolated Mucilage

3.5.1. Solubility of mucilage

1gm dry mucilage powder was solubilized with polarity gradient solvents and the solubility was determined.[12,13]

3.5.2. pH of mucilage

The mucilage was weighed and dissolved in water separately to obtain 1 % w/v solution. The pH of the solution was determined using a digital pH meter.[12,13]

3.5.3. Loss on drying[17,18]

The moisture content of mucilage can be determined by loss on drying method. Accurately weighed 1g sample is heated at 105 ℃ to get constant weight in a hot air oven and percent loss of moisture on drying is calculated using the following formula:

$$\text{LOD\%} = \frac{(\text{Weight of water in sample})}{(\text{Weight of dry sample})} \times 100$$

3.5.4. Swelling index of isolated mucilage[19,20]

Swelling index of the powdered mucilage was calculated by weighing a butter paper of size 2cm× 2cm, after that the butter paper was dipped in a Petri dish containing water and reweighed the wet butter paper again. After that,10mg of the powdered mucilage was kept on a butter paper placing that on a petri dish containing 15ml of water and the swelling index was calculated at different intervals i.e. 15, 30, 45, 120, 240, 360 min and the final result was calculated using the formula:

$$\text{Swelling index} = \frac{(\text{Final weight of the mucilage} - \text{Initial weight of the mucilage})}{(\text{Initial weight of the mucilage})} \times 100$$

3.5.5. Total ash value

About 2gm of mucilage was accurately weighed and taken in a silica crucible, which is previously ignited and weighed. The mucilage powder was spread as a fine, even layer on the bottom of the crucible. The crucible was incinerated slowly but surely by increasing temperature to make it dull red hot up to free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight. The percentage of total ash is calculated with reference to air dried mucilage sample.

3.5.6. Water-soluble ash value

2gm of ash was boiled in 25ml of water. The insoluble matter was strained and collected on an ash less filter paper washed with hot water and exploded in a tarred crucible at a temperature not beyond 450℃ for 4hrs. The insoluble matter was cooled in desiccators, weighed, and detracted from the total weight of ash. The change in weight denoted weight of water-soluble ash. The percent water – soluble ash was calculated with reference to the air-dried drug by the following formula:

$$\text{Water soluble ash (\%)} = \frac{\text{Weight of total ash} - \text{Weight of water insoluble ash}}{\text{Weight of crude drug taken}} \times 100$$

3.5.7. Acid –insoluble ash value

Acid –insoluble ash value was determined by boiling the 2g of ash for 5min with 25ml of 2M HCl. The insoluble matter was strained and collected on an ash less filter paper washed through the hot water and exploded in a tarred crucible at a temperature not beyond 450℃ for 4hrs. The insoluble matter was cooled in desiccators then weighed and the percentage of acid –insoluble ash was calculated by the reference to the air- dried drug with following formula:

$$\text{Acid} - \text{insoluble ash\%} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of crude drug taken}} \times 100$$

3.6. Flow Properties of Isolated Mucilage

3.6.1. Angle of repose

The angle of repose was determined by following the standard U.S.P 2010 method. For this mucilage powder 10g was accurately weighed and carefully introduced in to a funnel clamped to a stand with its tip 10 cm from a plane paper surface. The mucilage powder was allowed to flow freely onto the paper surface. After complete flow height of the cone, were and the radius of the cone, r and h were measured and used to calculate the angle of repose using the following equation:

$$\text{Angle of repose } \theta = \tan^{-1}(h/r)$$

Where, h= height of the powder cone, r = radius of the powder mucilage

3.6.2. Bulk density

The bulk density of mucilage was measured by putting the accurately weighed (10gm) power into a 100 ml graduated cylinder, and without disturbing the cylinder the volume of powder mucilage was read to give the bulk volume and was calculated using the following formula:

$$\text{Bulk density} = \frac{\text{Weight of powder}}{\text{Volume occupied by powder}} \times 100$$

3.6.3. Tapped density[21]

The tapped density was determined by the tap method. Weighed quantity (10g) of powder mucilage was carefully introduced into a100ml graduated cylinder and dropped on hardwood surface on tiles 500 times from height of 2.5cm. It was calculated using the following formula:

$$\text{Tapped density} = \frac{\text{Weight of powder blend}}{\text{Final volume after tapping}} \times 100$$

3.6.4. Carr's index

It was calculated from the value of bulk density and tapped density. Both these two value used for calculating the compressibility index9 of the powder mucilage.

$$\text{Carr, s index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

3.6.5. Hausner's ratio[21]

It is a measure of flow ability of the mucilage powder and was calculated by the following equation. The low value of hausner's ratio means that the mucilage powder has high flow ability hausner's ratio above 1.25 indicated poor flow:

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \times 100$$

4. RESULT AND DISCUSSION

The percentage yield of isolated mucilage was 9.54% w/w, phytochemical characterization, organoleptic characteristics and physicochemical parameters i.e. colour, odour, taste, texture, pH, solubility, loss on drying, and swelling index were shown in (Table 1). The isolated mucilage showed positive test of carbohydrates in sample and negative test for tannins, alkaloids, and protein confirms the purity of isolated mucilage as shown in (Table 2). The pH measurement reveals that the mucilage at different concentrations was to neutral and non-

irritating to the mucous membrane. The swelling index is very important and most widely accepted general mechanism of action for the disintegration. Thus, it has high swelling properties and this property can be used as a suspending and super disintegrating agent in various pharmaceutical formulations. The weight loss on drying indicated that the mucilage is hygroscopic in nature and some amount of moisture was present in the material which is available to interact with other materials at the time of formulation of dosage forms, then it stores to be an air–tight containers. The chemical profiling of mucilage conforms the presence of four major markers through FT-IR. The functional group at finger print region 2900 cm^{-1} and 2850 cm^{-1} indicates sharp stretching peak of C-H (methyl group), the band between (2920-2830) cm^{-1}C-H stretching, C-H functional group are attributed to neutral polysaccharide components of the mucilage such as arabinose, rhamnose, galactose and xylose.[22,23,24] The characteristic sharp peak at 1620 cm^{-1} indicate COO stretching (Carboxylic group). The characteristic O-H- peak at 1050 cm^{-1} confirms the presence of aliphatic group, the band at 1300-1050 cm-1 indicate the presence of carbohydrate unit.[25] The absorption band between 1185 and 1045 is related to the stretching vibrations of the glycosidic bonds (C-O, ether linkage) and pyranoid and rings (C-C) attributed to polysaccharides that are part of pectin's and mucilage.[38] The O-H stretching band at 3350 cm^{-1} characterize the presence of polysaccharide and the ^{1}H NMR Spectrum of mucilage was found to be in the range of 0.0-8.0 ppm. The NMR spectrum also confirms the presence of polysaccharides, N-acetyl group at 1.90 ppm, aliphatic alkyl groups, uric acid at 2.4 ppm and signals in the regions of 5.18 - 5.11 and 4.66 - 4.58 were attributed to proton of α and β anomeric carbon of hexose or pentose respectively the signals region of 4.18-3.32 ppm were assigned to hydrogen next to the functional -OH group. The regions corresponding to α and β anomeric carbon could be attributed to the presence of sugar residues, composed of arabinose, galactose, rhamanose, xylose as neutral sugars. Presence of acid sugars as D-galacturonic acid agreeing with the uronic acid found in the mucilage[26] were shown in (Figure2) and the mucilage shown that ^{1}H peak at 1.9 and 2.4 ppm region in NMR were directly correlates to wave number of FT-IR at (4000 - 400) cm^{-1}, which is the main mucilage band region in IR spectra. So, FT-IR, and ^{1}H NMR spectroscopy both can be applied the structural analysis of mucilage for confirmatory evaluation of mucilage. The micromeritic properties evaluation of mucilage powder was done with following parameters: angle of repose, bulk density, tapped density, carr's index, and hausner's ratio used for calculating the flow properties of powder mucilage, and all the values were obtained within the range. The angle of repose of the isolated mucilage was found to be 34.81±0.59. The bulk density and

tapped density were found to be 0.74±0.05 and 0.87±0.04 respectively and the bulkiness value indicated that the mucilage powder is 'heavy' in nature. Since this observation, it could be concluded that the mucilage has good flow properties were shown in (Table1).

5. CONCLUSION

The studies conformed that *Hibiscus cannaabinus* L., is an economical important plant and novel source of mucilage with enough mucilage have great functional value. Mucilage has high pharmaceutical significance and can be used as a natural polymer for pharmaceutical formulations in both conventional and novel drug delivery system. *Hibiscus cannabinus* L., mucilage will be a potential candidate for the formulation development in pharmaceutical industries and to provide a new way to future technologies.

Table 1: Organoleptic and Physicochemical Characteristic of *Hibiscus cannabinus* L., Mucilage.

S. No.	Properties of Mucilage	Results
1	Swelling Index	9 ± 0.52% w/w
2	Solubility	Soluble in hot water, Insoluble in organic solvents and in cold water swell to form a gel.
3	Loss on drying	9.34 %w/w
4	pH	7.2 ± 0.2
5	Colour	Off white colour
6	Odor	Odorless
7	Taste	Tasteless
8	Texture	Irregular
9	Fracture	Rough
10	Angle of repose	34.81 ±0.59
11	Bulk density (gm/ml)	0.74±0.05
12	Tapped density (gm/ml)	0.87±0.04
13	Carr's index (%)	14.94±0.25
14	Hausner,s ratio	1.17 ±0.50
15	Flow properties	Good

Table 2: Phytochemical Characterization of Isolated Mucilage of *Hibiscus cannabinus*.

Source	Test of Carbohydrates	Test of Polysaccharides	Test of Monosaccharides	Test of Protein	Test of Fats and Oils	Test of Mucilage
Hibiscus cannabinus	+	+	-	-	-	+

Table 3: Functional Groups and Peak Value of *Hibiscus cannabinus* L., Mucilage by FT- IR Spectroscopy.[36]

S. No	Functional Groups	Range (Wave No) cm^{-1}	Peak Value	Vibration	Intensity
1	Primary Aliphatic alcohol	1050	1050	OH	Strong peak
2	Carbonyl	1750 -1700	1750	C=O Stretch	Strong
3	Aliphatic Hydrocarbon	2962 - 2853	2850, 2800	C-H Stretch	Medium
4	Hydroxyl alcohol	3600 - 2500	3350	OH Stretch	Broad peak

Figure 1: *Hibiscus cannabinus* L., Plant, & *Hibiscus cannabinus* L., fruit mucilage.

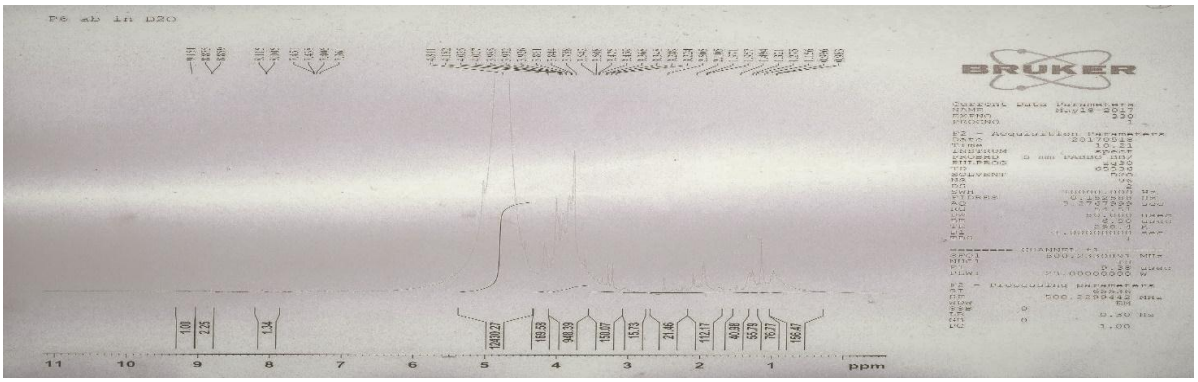

Figure 2: NMR Spectra of C *Hibiscus cannabinus* L., mucilage.

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