RESEARCH PAPER

Extraction and Characterization of Novel Plant Polymer Matrix Formers for Advanced Drug Delivery

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ABSTRACT

The aim of the current investigation is to prepare natural polymers extracted from different plant sources and to characterize them, so that they can be used as drug carriers to improve oral bioavailability and half-life of poorly soluble and permeable drugs to achieve sustained drug release with better therapeutic concentration. In the present study, three different plant sources i.e., *Trigonella foenum-graecum* (Fenugreek) seeds, *Azadirachta indica* (Neem) fruits and *Basella alba* leaves were selected to isolate and purify water soluble fractions of mucilage and then subjected to physical and chemical and structural characterization.

The chemical tests includes preliminary confirmatory tests for mucilage, test for sulphates, chlorides, tannins, phenols, saponins, flavonoids and alkaloids. The physical characterization for the dried mucilage was studied for organoleptic properties such as percentage yield, loss on drying, zeta potential, pH, viscosity, density, solubility, melting point, swelling index, total ash, flow properties and microbiological assays. Additionally, structural elucidation of mucilage was performed using FTIR, Gas chromatography-mass spectrometry (GC-MS) and Differential scanning calorimetry (DSC) studies.

The preliminary biochemical tests confirmed the presence of carbohydrates, mucilage and polysaccharides. All the mucilages were found to be absence of chlorides, sulphates, alkaloids, phenols, saponins, flavonoids and tannins. The isolated mucilage was evaluated for its physical properties and the results were found to be good. In conclusion, the research findings obtained from the studies were found to be satisfactory and therefore the above prepared dried mucilage powders can be used as matrix formers to retard the drug release from the formulation to prolong the therapeutic activity.

Keywords: Natural polymers; Mucilage; *Trigonella foenum-graecum*; *Azadirachta indica*; *Basella alba*; Matrix formers; Sustained release; Characterization

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1. Introduction

The advancement in drug delivery has led to the development of new excipients that are non-toxic and can form matrices that can regulate the rate and extent of drug release from formulation. Many scientists have discovered the importance of plant-based polymers as

pharmaceutical adjuvants. Natural polymers has gained popularity in recent times in novel drug delivery systems due to its biocompatible, biodegradable and low-toxic profile [1]. Natural plant based polymers can be tailored to meet the necessities of various drug delivery systems and therefore they are preferred over synthetic excipients that are available in the market. There are several studies that have proven the efficacy of using plant based polymers in various pharmaceutical dosage forms like transdermal systems, matrix tablets, implants, buccal films, microspheres, microneedle patches, nanoparticles, ophthalmic solutions, and suspensions [2, 3].

Gums and mucilages are natural polysaccharides derived from plants and used as tablet binders, emulsifiers, suspending agents, thickening agents, film forming agents, sustaining agents and coating agents in conventional drug delivery systems [4]. Mucilages are physiological products that are formed during metabolism within the cell and they dissolve in water to form slimy masses as they are made up of hydrophyllic macromolecules, which on hydrolysis yields a mixture of sugars and uronic acids [5].

Mucilages are used in the food industry and are regarded as safe for human consumption. However, there is limited evidence regarding the safety of pharmaceutical excipients derived from natural sources. Therefore, there is a need to screen them as pharmaceutical adjuvants to check their safety, compatibility with other excipients and drug [6].

The physical, chemical, and structural elucidation of plant polymers is essential to identify its composition, functional groups, bonding of atoms, which in turn provides a deep insight regarding its behavioural properties and biological functions [7].

2. Materials and methods

The plant parts of *Trigonella foenum-graecum* L., *Azadirachta indica* A. Juss., *Basella alba* L. were collected from in and around Tirupati, Andhra Pradesh and were authenticated by Professor K. Madhava Chetty, Plant Taxanomist, S.V. University, Tirupati, A. P., India.

2.1 Isolation and purification of water soluble fractions of mucilage

2.1.1. General extraction and purification of *Trigonella foenum-graecum* (Fenugreek) seed mucilage [FG], *Azadirachta indica* fruit mucilage [AI], *Basella alba leaf* mucilage [BA] [8-10]

The high quality fenugreek seeds, fresh *Azadirachta indica* fruits and *Basella alba* leaves were obtained from market locally and cleaned with water to remove the dust and debris. The seeds, fruits and leaves (250g) were soaked separately in a beaker containing 500 ml of double distilled water overnight and then subjected to heat at 50°C for 2 h. The mucilage was released slowly into the water and it was extracted using a multi-layer muslin cloth. To the obtained mucilage, three times the volume of acetone was added and then precipitated mucilage was separated and dried. The sample was collected, size reduced to fine powder using mortar and pestle and passed through a mesh # 80 sieve and stored in desiccator till further use. The powder thus obtained was re-dissolved in water (100ml), filtered and subjected to centrifugation for around 30 min at 3500 rpm. Furthermore, the clear supernatant solution was collected, freeze dried and stored.

a b c

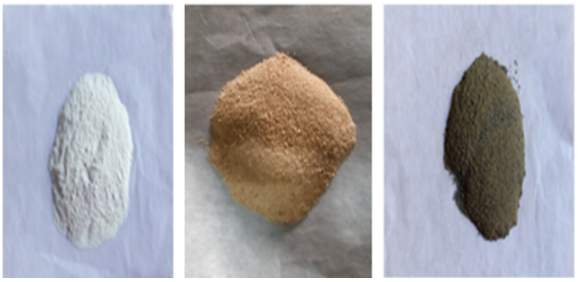

Fig. 1: Plant mucilages isolated from a) FG b) AI c) BA

2.2. Characterization of mucilages

2.2.1. Chemical characterization of mucilages

Preliminary confirmatory test for dried mucilage powder was carried out to confirm the presence of carbohydrates, mucilage, polysachharides, enzymes [11-13].

Table 1: Preliminary confirmatory test mucilages

Test	Observation	Inference
Molisch's test: To the 100mg of dried mucilage powder, Molisch's reagent and concentrated sulphuric acid was added from the sides of a test tube	Violet green colour appears at the junction between two layers	Indicates the Presence of carbohydrates

Ruthenium test: Place 100mg of dried mucilage powder was placed on a slide, to which ruthenium red solution was added and observed under microscope.	Pink colour develops	Presence of mucilage
Iodine test: To 100mg of dried mucilage powder , 1 ml of iodine solution(0.2 N) was added	No colour change	Presence of polysaccharides
Enzyme test: To 100 mg of dried mucilage powder in 20 ml-distilled water; 0.5 ml of benzidine in alcohol (90%) was added. The above solution was shaken and allow to stand for few minutes	No blue colour produced	Absence of enzymes

i) Test for sulphates

To a little amount of the sample, 1ml of 5% barium chloride solution was added, resulting in formation of white precipitate insoluble in hydrochloric acid, which indicates the presence of sulphates.

ii) Test for chlorides

To a few ml of the sample solution prepared in HNO_3, a few drops of $AgNO_3$ solution was added, results in formation of white precipitate insoluble in dilute ammonia solution indicates the presence of chlorides.

iii) Test for tannins

The substance was mixed with basic lead acetate solution. Formation of white precipitate indicates the presence of tannins.

iv) Test for phenols

The substance was mixed with few drops of ferric chloride solution. Formation of red colouration indicates the presence of phenols.

v) Test for saponins

Foam Test: The extract (2g) was taken in a test tube to which 20 ml of water was added and shaken vigorously to observe for persistent foam, which indicates the positive for saponins.

vi) Test for flavonoids

Shinoda Test: To the 2g of dried mucilage, 5 ml of ethanol (95% v/v), 5 drops of hydrochloric acid and 0.5g of magnesium turnings were added. The presence of pink color indicates positive for flavonoids.

vii) Test for alkaloids

To 10 g of dry extract, 20 ml of dilute HCL was added, shaken well and filtered. The following tests were performed using the filtrate. Mayer's Test: To 3 ml of the filtrates, 1ml of Mayer's reagent (potassium mercuric iodide) was added, white precipitate shows the presence of alkaloids.

2.2.2. Physical characterization of gum/mucilages [14-16]

The dried mucilage was studied for different organoleptic properties

i) Percentage yield (%w/w)

The percentage yield of dried mucilage powder was calculated by using the ratio of the amount obtained to the maximum amount that can be obtained theoretically.

ii) Loss on Drying

This parameter was used for determination of moisture content. 100mg of powder was weighed and kept in hot air oven and the weight of the powder was noted every 15 minutes until constant weight was reached. This procedure was repeated for three times and the percentage loss on Drying was calculated using the below formula.

$$LOD\ (\%) = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

iii) Determination of zeta potential, pH, viscosity

1% w/v solution of sample was prepared in distilled water and zeta potential was determined using Nanoparticle analyser SZ-100. The pH and viscosity of same solution was observed using digital pH meter and Brookfield viscometer. All measurements were

performed in triplicates (n=3) and the standard deviation (SD) was recorded.

iv) Density

The density of the dried mucilage was calculated using the following formula

The density of the mucilage solution (ρ solution) = mass of the solution

Volume of the bottle

v) Melting point

Melting point was determined to find out the temperature at which the sample melts and it was used to find out the purity of the sample. The presence of any contamination will indicate difference in the melting point of the sample. It was determined by melting point apparatus.

vi) Determination of % swelling index

One gram of powder was taken in a measuring cylinder and the initial volume of the powder was noted and was made up to 100ml with distilled. Then, the cylinder was gently shaken and kept aside for 24hrs. The volume occupied by the sample sediment was noted after 24 h. The swelling index of the sample was calculated by the formula,

% S.I= {wt-wo/wo}x 100

Where, S.I. = Swelling index

Wt = Final weight of the sample after 24hrs

Wo = Initial height of the powder in graduated cylinder

vii) Total ash

Total ash was determined by placing 3gm of mucilage in a crucible and heated in a muffle furnace at a temperature of 550°C until a constant weight of ash residue was. The crucible was cooled in a desiccator, weighed and the content of total ash in mg/g of air dried material was calculated .

Total Ash (%) = Weight of Ash Residue X 100
Weight of Original Sample

viii) Acid insoluble ash

Acid insoluble ash was determined by boiling the ash with 25 ml of dilute hydrochloric acid for few minutes and then filtered using an ashless filter paper, which was placed in silica crucible and ignited, cooled and the final weight was calculated.

ix) Determination of flow properties

The dried mucilages was tested for the flow properties like angle of repose, bulk densities, compressibility index and Hausner's ratio. All these evaluations were carried out in triplicates as per procedures described in official books.

a) Bulk density and bulkiness

The bulk density was calculated by dividing the total weight of a dry sample by the bulk volume of the sample it occupies in the measuring cylinder. Reciprocal of bulk density gives the bulkiness.

b) Tapped density

Tapped density= weight of dry powder
tapped volume

c) Compressibility index and Hausner ratio

The compressibility index and Hausner ratio were determined by using both bulk density and tapped density of powder.

% Compressibility index = (Tapped density- Bulk density/Tapped density) x 100

Hausner ratio = Tapped density/ Bulk density

d) Angle of repose

The angle of repose (θ) was determined by fixed funnel method. The powder was poured through the funnel on to the flat surface using paper to form a pile. It can be calculated by,

Tan θ = h/r

h = Height of the pile

r = Radius of the pile

x) Microbiological assay

Dried mucilage powders should be free from pathogenic microorganisms. Suitable culture media are selected and prepared as described in Indian Pharmacopoeia 2007.

10 mg of sample was dissolved in 1ml of DMSO, from which 100 µl was taken and spread onto each petri plate containing SS-agar media, Macconkey agar media, cetrimide agar, potato dextrose agar media and incubated over night at 37°C and the plates were observed for the growth of *Salmonella* Typhi, *E.coli, Pseudomonas,* Fungi (*Candida albicans*) respectively

2.2.3. Structural elucidation of mucilages[17,18]

a) Acid-base hydrolysis

The pH of the sample solution (300 mg/ml) was adjusted to 2.0 by adding 0.5M sulphuric acid. The solution was heated to 100ºC for 24 h. After cooling, the pH of solution was adjusted to 10 by the addition of 0.5M NaOH and heated to 100ºC for 24 h. After cooling the solution was neutralized with barium carbonate and filtered. The solution was then dialyzed for 24 h against de-ionized water and freeze dried. The obtained hydrolysed dry samples were subjected for FTIR and Gas chromatography-mass spectrometry (GC-MS) studies and X-Ray Diffraction (X studies.

b) FTIR, GC-MS analysis of hydrolysed fraction and XRD

The FT-IR spectrum of mucilages was recorded on FT-IR spectrophotometer. The sample were mixed with KBr in ratio of (1:4) and pressed into pellets under mechanical pressure using hydraulic press. The scans were obtained with a spectral resolution of 2 cm^{-1} from wave number 4000 to 200 cm^{-1}. The GC-MS analysis was carried out using HP-5 conventional capillary column (30m x 0.25 mm with

internal diameter of 0.25 µm) coupled to ion trap mass spectrometry functioned at 70ev. The columns were automated from 50 to 250°C at 50°C/min. X-ray diffraction (XRD) patterns of mucilages were carried out using Shimadzu, XRD 6000 equipment, with nickel filtered tube CuKα1 at a voltage of 45 kV and current of 45 mA, The scanned angle was set at 2θ from 5º to 90º and scanned rate was 1º/min.

3) Results and Discussion

3.1. Characterisation of mucilages

3.1.1. Chemical properties of the mucilages

Table 2: Chemical properties of mucilages

S.No.	Test	Observation			
		GK	FG	AI	BA
1.	Molisch's test (for carbohydrates)	+	+	+	+
2.	Mucilage mounted with ruthenium red	+	+	+	+
3.	Mucilage mounted with iodine solution	+	+	+	+
4.	Mucilage mounted with benzidine in alcohol (Enzyme test)	-	-	-	-
5.	Silver Nitrate test (for chlorides)	-	-	-	-
6.	Barium Chloride test (for sulphates)	-	-	-	-
7.	Lead Acetate test (for tannins)	-	-	-	-
8.	Ferric chloride test (for phenols)	-	-	-	-
9.	Foam test (for saponins)	-	-	-	-
10.	Shinoda test (for flavanoids)	-	-	-	-
11.	Mayer's Test (for alkaloids)	-	-	-	-

Note: (+) Presence, (-) absence.

The chemical tests confirmed that the isolated compound was mucilages, Molisch's test confirmed the presence of carbohydrates and with the test for chlorides, sulphates, tannins, phenols, saponins, flavonoids and alkaloids resulted negative (Table 2).

3.1.2. Physical properties of the mucilages

Table 3: Physical properties of mucilages

S. No	Description	FG	AI	BA
1.	Appearance	White	Light brown	Greenish
2.	Yield (% w/w)	46.8	43.56	41.2
3.	Zeta potential (mV)	-38.3±0.9	-57.5±1.3	-32.4±1.5
4.	Viscosity (Poise)	1.521±0.12	1.625±0.12	1.325±0.22
5.	Loss on drying (w/w)	2.81±0.22	1.71±0.32	2.11±0.12
6.	Swelling Index (%)	83.26±0.23	86.72±0.14	81.34±0.29
7.	% Total ash (w/w)	5.6±0.26	4.2 ± 0.27	5.2±0.23
8.	% Acid insoluble ash (w/w)	1.2±0.13	1.0±0.21	1.4±0.22
9.	pH	6.2±0.1	6.5±0.22	6.7±0.03
10.	Melting point (ºC)	79.8	96.2	105.6
11.	Density (g/ml)	1.023±0.01	1.061±0.01	1.064±0.01

12.	Bulk Density (g/cm^3)	0.624± 0.02	0.721±0 .01	0.716±0 .01
13.	Tapped Density (g/cm^3)	0.736± 0.03	0.826±0 .010	0.852±0 .010
14.	Compressi bility Index (%)	15.2±0. 01	12.7±0. 03	15.9±0. 02
15.	Hausner's Ratio	1.17±0. 04	1.14±0. 05	1.18±0. 01
16.	Angle of Repose (θ°)	36.52± 0.4	34.74±0 .2	38.24±0 .5
17.	*Pseudomo nas, Salmonell a E.coli* and *candida albicans*	Negativ e	Negativ e	Negativ e

Values are reported as mean ± Standard Deviation, n=3

All the physical properties of the mucilages were found to be within limits. The zeta potential measurement of mucilages was found to anionic in nature. The high percentage of swelling index of mucilages indicate they are better carriers as sustained releases agents in drug delivery as it absorbs a large amount of water and form a slimy mass, which acts as a barrier to slow down the rate of drug diffusion. The flow property of the mucilages was found to be good. The mucilages were found to be free from pathogenic organisms (Table 3).

3.1.3. Structural characterization of mucilages by FT-IR, GC-MS and XRD

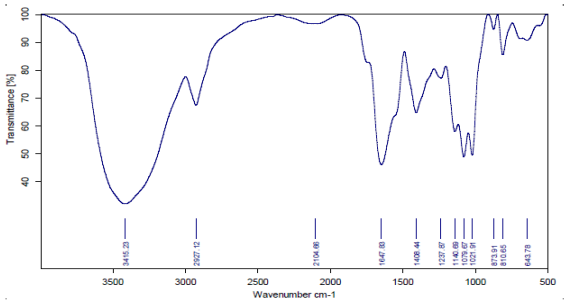

Fig. 2: FT-IR spectra of FG at a resolution of 2 cm^{-1} from 4000 to 500 cm^{-1}

The major peaks present in the FT-IR of FG at 3415.23 cm^{-1} indicates stretching vibrations of free –OH groups and N-H groups, characteristic peak at 2927.12 cm^{-1} corresponds to C-H stretching vibrations of carboxyl group, absorption band at 2104.66 cm^{-1} represents C≡C stretching band, the peak at 1647.83 cm^{-1} represents stretching mode of keto groups, the band at 1408.44 cm^{-1} represents C-H bending vibrations, the peak at 1237.87 cm^{-1} indicates O-H bending mode, the peaks at 1079.67 cm^{-1} and 1021.91 cm^{-1} corresponds to stretching vibrations due to C-O, C-N, C-C groups, the bands at 873.91 cm^{-1} represents N-H, C-H rocking mode. The frequency at 643.78 cm^{-1} indicates rocking vibrations of C-H group (Fig. 2).

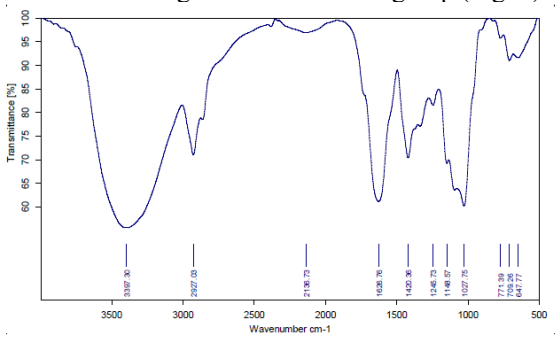

Fig. 3: FT-IR spectra of AI at a resolution of 2 cm^{-1} from 4000 to 500 cm^{-1}

The major characteristic peaks present in the FT-IR spectra of AI at 3397.30 cm^{-1} indicates stretching vibrations of N-H and O-H groups, the characteristic peak at 2927.03 cm^{-1} corresponds to C-H stretching band, the absorption band at 2136.73 cm^{-1} corresponds to C≡C stretching band, the peak at 1626.76 cm^{-1} represents stretching mode3f carboxylate groups, the peak at 1245.73 cm^{-1} indicate bending vibrations of –OH groups, the band at 1420.36 cm^{-1} represents C-H bending mode, the peak at 1027.75 cm^{-1} suggest the presence of stretching vibrations of C-OH group, the frequency at 771.39 cm^{-1} suggest the presence of N-H rocking and the peak at 647.77 cm^{-1} indicates rocking vibrations of C-H group (Fig. 3).

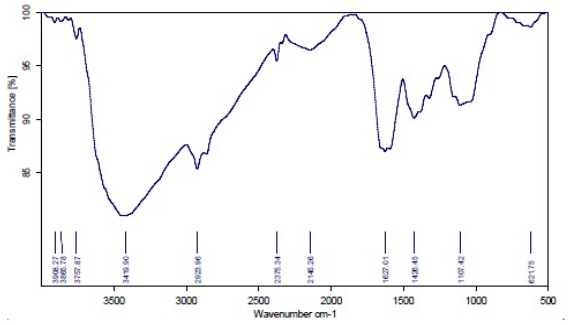

Fig. 4: FT-IR spectra of BA at a resolution of 2 cm^{-1} from 4000 to 500 cm^{-1}

From the FT-IR spectra of BA, the peak at 3419.90 cm^{-1} indicates OH stretching of hydroxyl group, the characteristic peak at 2923.96 cm^{-1} corresponds to C-H stretching band of carboxyl group, the absorption band at 2145.26 cm^{-1} corresponds to C≡C stretching band, the peak at 1627.01 cm^{-1} represents stretching mode of keto groups, the band at 1426.45 cm^{-1} represents C-H bending mode, the peak at 1107.42 cm^{-1} suggest the presence of stretching vibrations of C-OH group. The frequency at 621.77 cm^{-1} indicates rocking vibrations of C-H group (Fig. 4).

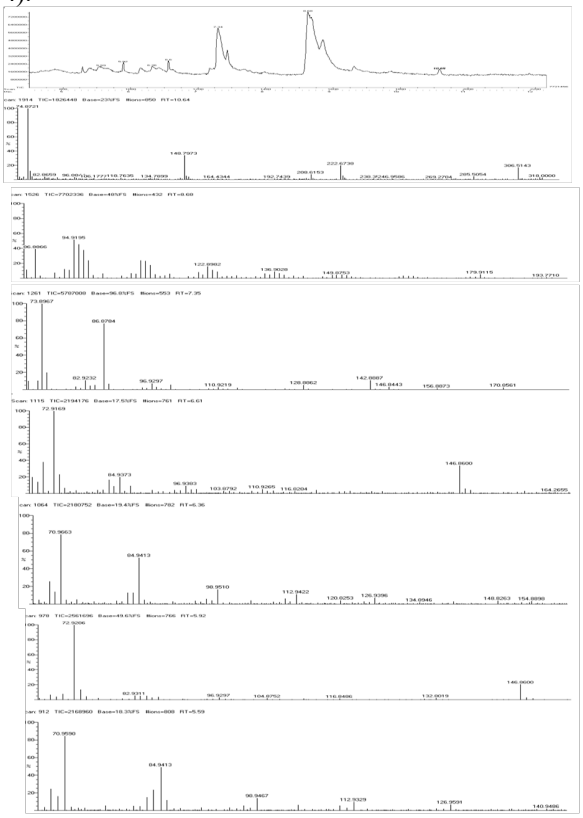

Fig. 5: GC-MS analysis of acid base hydrolysed fraction of FG

The GC-MS of hydrolysed fraction of FG showed seven peaks at retention time (T) 10.64 min, 8.68 min, 7.35 min, 6.6 min, 6.36 min, 5.92 min and 5.59 min, with molecular ion peaks of mass/charge (m/z) ratio 318, 193.77, 170.85, 164.26, 154.77, 146.86 and 140.94 respectively, confirmed the presence of major neutral sugars such as rhamnose, D-galactose, D-arabinose and uronic acid such as D-galacturonic acid (Fig. 5).

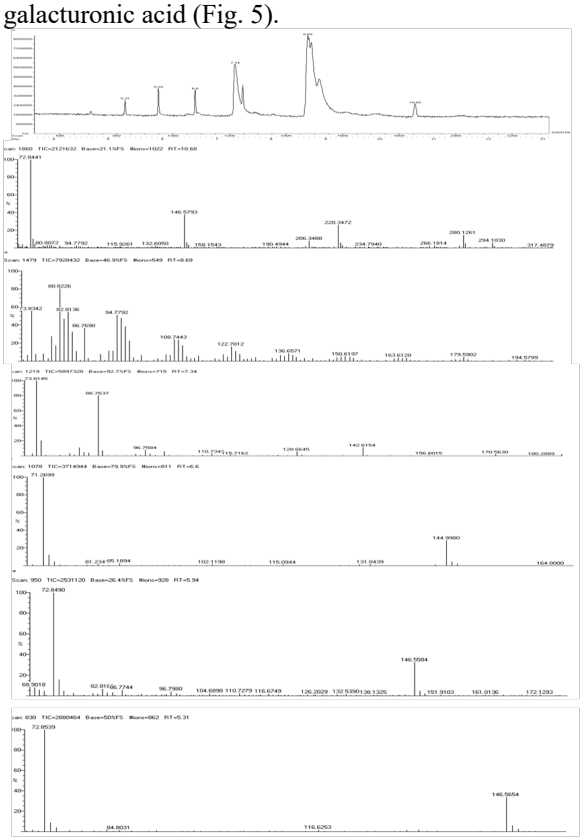

Fig. 6: GC-MS analysis of acid base hydrolysed fraction of AI

The GC-MS of hydrolysed fraction of AI showed six peaks at retention time (RT) 10.65 min, 8.69 min, 7.34 min, 6.6 min, 5.94 min and 5.31 min, with molecular ion peaks of mass/charge (m/z) ratio 317.48, 194.57, 180.28, 164, 172.12 and 146.54 respectively, confirmed the presence of major neutral sugars such as rhamnose, mannose, D-arabinose and uronic acid such as D-galacturonic acid (Fig. 6).

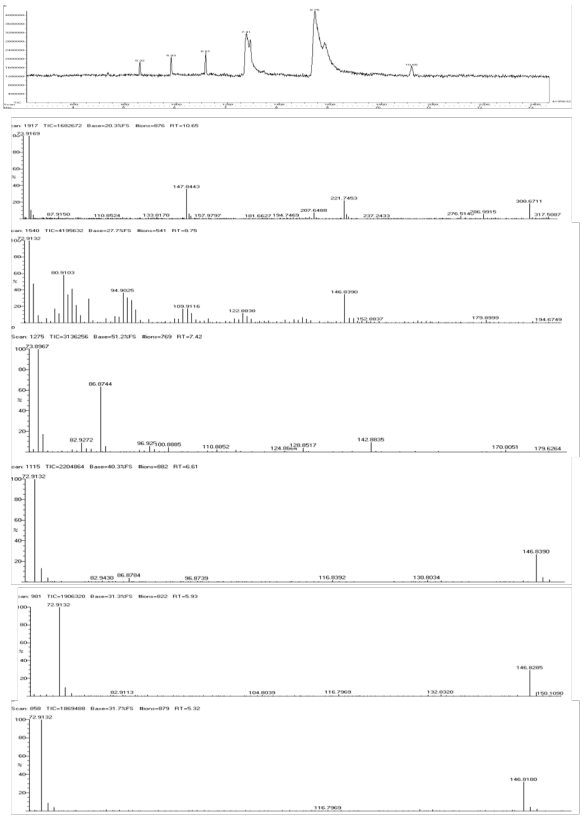

Fig. 7: GC-MS analysis of acid base hydrolysed fraction of BA

The GC-MS analysis of hydrolysed fraction of BA showed six peaks at retention time (RT) of 10.65 min, 8.75 min, 7.42 min, 6.61 min, 5.93 min and 5.32 min with molecular ion peaks of m/z ratio, 317.5, 194.67, 179.62, 146.83, 150.10 and 146.81 respectively, indicating the presence of D-arabinose, D-galacturonic acid, xylose and rhamnose (Fig. 7)

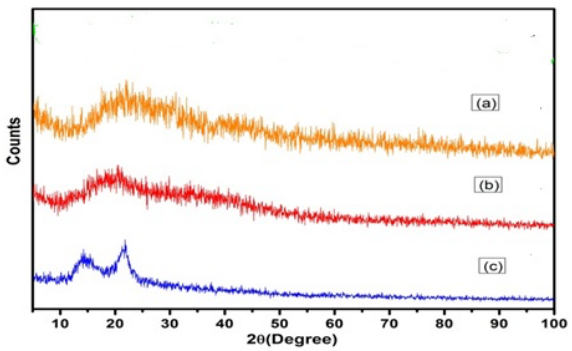

Fig. 8: XRD pattern mucilages a) FG b) AI c) BA

Powder XRD analysis of mucilages depicted in Fig. 8. No characteristic peaks in the spectrum were observed, indicating that the native mucilages were completely amorphous in nature. The amorphous nature suggests that the molecular structure is not in orderly manner, irregular with repeating lattice units as in the crystal.

4) Conclusion

The research demonstrated the successful isolation and evaluation of mucilage extracted from three different plant sources i.e., *Trigonella foenum-graecum* (Fenugreek) seeds, *Azadirachta indica* (Neem) fruits and *Basella alba* leaves. The preliminary chemical test confirms the presence of mucilage. The physical tests were found to be within the limits and high % swelling index confirmed that the isolated mucilages are better candidates as matrix formers in drug delivery. The structural elucidation by FT-IR, GC-MS and XRD were used to identify the major functional groups, compounds and crystallinity present in the mucilage, which are essential for understanding the interaction within the molecular groups and chemical nature of the mucilage.

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