EXTRACTION, ISOLATION AND CHARACTERIZATION OF ABELMOSCHUS ESCULENTUS MUCILAGE

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ABSTRACT:

In Recent days, natural polymers have played an important role in the development of Novel formulations because of their many advantages over synthetic polymers cost-effective, Non-toxic, abundant available, biodegradable, biocompatible, Eco eco-friendly. So, it's necessary to explore the knowledge of natural polymers to meet the industrial demand. So, in this current work, an attempt was made to extract, isolate, and characterize the mucilage extracted from Abelmoschus Esculentus (Okra). the isolated mucilage was assessed for different parameters like Organoleptic, Physical, and Chemical properties. Further isolated mucilage was identified by FTIR studies. Based on the organoleptic properties evaluation the isolated mucilage was a brownish characteristic odour with a mucilaginous taste. All the results obtained from physical properties & chemical evaluation found that the isolated mucilage meets standard limits of the mucilage. For the detection of mucilage characters polymer test was performed and the result obtained was found to be positive. So, in this current work, we successfully isolated mucilage from Okra which can be used extensively in the design of Novel drug delivery systems.

Key words: Abelmoschus esculentus, Acetone, mucilage, Natural excipient

Introduction:

Abelmoschus esculentus L. is popularly known as okra or lady's finger. It belongs to the Malvaceae family. [1] Okra (Abelmoschus esculentus (L) Moench) or bhendi also known as ladies' finger is an important vegetable crop being native to tropical Africa. [2] The okra mucilage (OM) is composed of natural polysaccharides containing D-galactose, L-rhamnose, and galacturonic acid monosaccharides and is abundant in proteins, vitamins, and minerals. [3] The okra fruit/pod is a greenish capsule with a length of 10–30 cm long and a diameter of 1–4 cm, it is slightly curved, tapers to a blunt point, a six-chambered pod of fibrous texture, and contains numerous seeds [4]) Okra is a popular health food due to its high fibre, vitamin C, and folate content. It is also a good source of calcium and potassium. [5] Anatomically, the fruits, stems, and leaves of okra are covered with minute soft, hairy structures. Although the flowering of the okra plant is perennial, it is highly dependent on various biotic and abiotic factors. The leaves of okra are polymorphous, characterized by hairy upper and lower surfaces, whereas the petioles are around 15 cm long. The flowers of okra can be easily recognized due to their slight yellowish color with a crimson center. The edible part of okra or its capsule (pod) measures approximately 15–20 cm in length and has a pyramidal-oblong, pentagonal, hispid appearance. Historically, okra pods were utilized for various purposes, such as in food, appetite boosters, astringents, and as an aphrodisiac. Furthermore, okra pods have also been recommended to cure dysentery, gonorrhea, and urinary complications. [6] Okra pods especially when heated produce more sticky mucus. This mucilage has a good potential for pharmaceutical applications such as diluents, binders, pharmaceutical excipients over synthetic excipients, disintegrants in tablets, thickeners in oral liquids, protective colloids in suspensions, film forming agents in transdermal and periodontal films and gelling agents in a gel base for topical application due to ease of application for better percutaneous absorption. [7]) Recently, the mucilage obtained from Abelmoschus esculentus was reported to have a sustained release property in tablet formulations [8].

Materials and Methods:

Okra fruits were purchased from local market, Anantapur. Distilled water was procured from the METRO products. Acetone, Sulphuric acid, Ammonia solution, silver nitrate, Lead acetate Glacial acetic acid was obtained from S D Fine Chem Limited. Ethanol was

purchased from Changshu Hong Sheng Fine Chemical Co.Ltd. Acetic anhydride was obtained from Reechem Private Limited.

1. Collection and drying of okra fruits:

Okra (*Abelmoschus esculentus*) was obtained from the local market. [9] It was washed thoroughly with running tap water for 1Hr. Immature pods were selected because they contain more mucilage content than matured fruits [10]. Collected okra was dried under shade for 24 h [9].

2. Extraction of mucilage:

Method I: Cut the pods longitudinally into extremely thin slices, remove the seeds, and soak them for 24 hours in distilled water [11]. a white muslin cloth was used to extract the viscous gum by squeezing the swollen slices. [12]

Method-II: Nicely cleaned and sliced okra were dried under shade for 12 – 24h, further dried in a hot air oven at 40° C for 5 – 6 h to obtain constant weight. Dried okra was blended into powder, and then passed through a sieve. The okra powder is transferred to a beaker containing water; then soaked for 12 – 24 h and filtered through muslin cloth and the extract [13].

3. Isolation of mucilage:

Obtained filtrate was treated with twice volume of acetone to obtained Okra polysaccharide. Polysaccharide dried in hot air oven below the temperature of 50°C. [14] Hard mucilage cake was ground into a fine powder using motor and pestle. [15] Dried polymer was sieved through #100.

4. Storage of Dried mucilage:

The powdered mucilage was stored in an airtight container in a desiccator for further studies ([14].

CHARACTERIZATION OF OKRA MUCILAGE:

I. Organoleptic Evaluation:

Isolated mucilage was characterized by organoleptic properties such as color, odour, taste and texture [9].

II. Physical characterization:

1. Percentage yield of isolated mucilage:

Based on the quantity of fresh okra and dry mucilage obtained by solvents, the percentage yield for extracted mucilage was calculated and expressed as mucilage percentage (%). The weights of the dried mucilage obtained and the weight of the fresh material are used to calculate the percentage yield ratio. Using the formula, the yield of mucilage was determined by weighing the fresh material and dried, isolated mucilage [16].

Percentage yield = Weight of dried mucilage obtained/weight of fresh okra used × 100

2. Swelling index:

The swelling index of the mucilage was determined by accurately weighing 1g of mucilage, which was further introduced into a 25ml glass-Stoppard measuring cylinder. 25ml of water was added and the mixture was shaken thoroughly every 10 min for 1 h. It was then allowed to stand for 3h at room temperature. Then the volume occupied by mucilage was measured. The same procedure was repeated thrice and the mean value was calculated. [17]

3.Solubility:

Powdered OFG (2 g) was added to 200 ml of distilled water and left undisturbed (10-12 hrs), allowing it to swell totally. After stirring at room temperature (25-30°C) and elevated temperature (55-65°C) for approximately 50 minutes, the solution was cooled and centrifuged at 5000 ×*g* for 25 minutes to remove the insoluble material. The settled portion was then moved into a Petri dish and dried at 110°C in an oven till stable weight was obtained [2]. The following equation was used to determine solubility:

$$\text{Solubility S (\%)} = (S2\text{-}S1/S2) \times 100$$

Where S1 is the sediment fraction (mg) while S2 is the initial concentration of the solution (mg). The solubility of OFG was also investigated in other solvents such as ethanol, acetone, and chloroform.

4. Water sorption time (WST):

The OFG (50 mg) was soaked in distilled water, HCl (0.1 N), or phosphate buffer pH 1.2, 6.8, or 7.4, respectively (100 cc) for 24 hrs. The time taken by the liquid to reach to top of the powder bed was estimated as WST. [18]

5. Loss on drying (LOD%):

A 1.0 g quantity of the sample was transferred into a Petri dish and then dried in an oven at 105°C until a constant weight was obtained. The moisture content was then determined as the ratio of the weight of moisture loss to the weight of the sample expressed as a percentage. [8]

The moisture content was determined using the following equation:

$$LOD\% = (Wf - Wi/W_i) \times 100$$

Where Wi is the initial weight of the sample and

Wf is the final weight after drying. [18]

6.pH determination:

The pH of the okra mucilage was measured using a digital pH meter by dispersing the okra mucilage 25ml of distilled water. [19]

7. Surface tension:

It was determined by the drop count method, using a stalagmometer. Surface tension was calculated as per the equation:

$$\sigma solution = \sigma water \times m\ (solution)/m\ (water).$$

Where, m (solution)=Surface tension of solution,

m (water)=Surface tension of water

σsolution=Weight of solution, and

σwater=Weight of water.

8.Viscosity:

It was determined using Oswald's viscometer using the equation:

$$S = w \times ts\rho s/tw\rho s$$

where, s=Viscosity of solution,

w=Viscosity of water,

t=Time, and

ρ=Density [12].

9. Melting Range:

The melting range of a substance is defined as those points at which, the substance begins to coalesce and is completely melted. The melting point of Okra polysaccharide was carried out in the Thieles tube apparatus. [14]

10. Particle Size Analysis:

The particle size was determined by microscope [9].

11. Total ash content:

The gum sample (1.0 g) was weighed into a preignited and preweighed crucible, and a furnace at an ignition temperature of 550°C for 24 hrs. The recovered ash was transported into a desiccator for equilibration to room temperature before weighing. The resultant ash from the above was mixed with distilled water, boiled, filtered, and the filter was rinsed. Both filter paper and residue were transferred into the crucible and ignited for 24 hrs until a constant weight was reached. Thereafter, cooling was carried out in a desiccator and the product was weighed. The percent total ash was calculated from the formula:

%Total ash= (Ash weight/Original sample weight) ×100

12. **Acid insoluble ash:**

Acid-insoluble ash, which is a part of total ash insoluble in dilute hydrochloric acid is required to be determined for certain drugs. Adhering dirt and sand may be determined by acid-insoluble ash.

Method: Above obtained ash washed with 25 ml of dil. HCl; the ash was washed from the crucible used for total ash into a 100 ml beaker. The residue obtained after filtration was washed twice with hot water crucible was ignited in the flame, cool and weighed. Crucible was further heated until vapours cease to be evolved. Cool it. Calculate the percentage of acid insoluble ash with reference to the air-dried substance [14].

III. Flow Properties:

1.Angle of repose:

The fixed funnel and free-standing cone method were employed in which a funnel is secured with its tip at a given height, H, above graph paper that is placed on a flat horizontal surface. OFG powder was precisely weighed and vigilantly introduced into the funnel until the peak of the conical stack just touches the funnel tip. Angle of repose was calculated using the following equation:

$$\text{Tan} = H/R$$

Where, H=Height of the pile

R is the radius of the base of the conical stack.

2.Bulk density:

A known amount of the powdered OFG sample (M) was placed in a measuring cylinder (100 mL) and the volume (V0) occupied by the OFG sample was noted as bulk volume. [20]

$$\text{Bulk density (B)} = M/Vo$$

3.Tapped density:

The tapped density is obtained mechanically by tapping a graduated measuring cylinder or vessel containing the powder sample. [21] The volume occupied after 100 taps was noted as tapped volume (Vt). The tapped density was calculated using the equation:

$$\text{Tapped density (T)} = M/Vt$$

4.Hausner's ratio (H):

H was calculated as the ratio of tap density to bulk density of the sample.

$$H = T/B$$

5.Compressibility index (C %):

C % was calculated using the following equation: [20]

$$C\% = (T - B/T) \times 100s$$

iv. chemical characterization:

1.Limit test:

(i). Heavy metals: Test was designed to determine the content of metallic impurities that are coloured by sulphide ion under specific conditions and determined by visual comparison of the colour produced by the substance with that of control (standard lead solution). When a solution of hydrogen sulphide is added to concentrated solution of lead salts, a black precipitate of lead sulphide is obtained. With much diluted solution, a brown colour is produced. The intensity of which depends on the quantity of lead present.

Standard solution: Into a 50 ml Nessler cylinder 1 ml of lead standard solution (20 ppm pb) and dilute with water to 25 ml. Adjust with dilute acetic acid or dilute ammonia solution to a pH between 3 and 4 dilute with water to about 35 ml and mix.

Test solution: Weigh Okra powder, add sufficient sulphuric acid to wet the sample, ignite carefully at a low temperature until thoroughly charred. Add to the charred mass 2 ml of nitric acid and 5 drops of sulphuric acid and heat cautiously until thoroughly charred, heat cautiously until white fumes are no longer evolved. Ignite, preferably in a muffle furnace at 500o to 600oC, until the carbon is completely burnt off. Cool it, add 4 ml of hydrochloric acid, cover, digest on a water bath for 15 min. uncover and slowly evaporate to dryness on a water bath, Moisten the residue with 1 drop of hydrochloric acid and add 10 ml of hot water and digest for 2 min. Add ammonia solution drop wise until the solution is alkaline to litmus paper. Dilute to 25 ml with water add adjust with dilute acetic acid to a pH between 3 and 4. Filter, if necessary, rinse the crucible and the filter with 10 ml of water, combine the filtrate and washing in a 50 ml Nessler cylinder, dilute with water to about 35 ml and mix.

Procedure:

To each of the cylinders containing the standard and test solution add 10 ml of freshly prepared hydrogen sulphide solution, mix, dilute to 50 ml with water, allow to standard for 5 min and view downwards over a white surface, the colour produced with the test is not more intense than standard solution.

(ii). For chlorides:

Dissolve the specified quantity of the substance being examined in water. Add 10 ml of dilute nitric acid, dilute to 50 ml with water and add 1 ml of 0.1 M silver nitrate. Stir immediately

with a glass rod and allow to stand for 5 min protected from light. When viewed transversely against a black background any opalescence produced is not more intense than that obtained by treating of 10 ml of chloride standard solution (25 ppm Cl) and 5 ml of water in same manner [14].

2.Test for tannins:

This was done by boiling 1g of each of the dried powdered samples (separately) in 40 ml of water in a test tube and then filtered. A brownish green or a blue-black coloration was observed after addition of a few drops of 0.1% ferric chloride.

3. Test for steroids:

This was carried out by addition of 4 ml of acetic anhydride to 1 g of each of the crude extract (separately) with further addition of H2S04(2ml). The presence of steroids was indicated by change of colour from violet to blue or green.

4. Test for flavonoids:

This was determined through heating of 0.5g of the powdered okra mucilage extract sample (separately) with ethyl acetate (10 ml) over a steam water bath for 3 min. To 1 ml of dilute ammonia solution, 4ml of filtrate from the filtered mixture was added and shook. Appearance of yellow coloration is an indication of presence of flavonoids.

5. Test for Saponin:

To 10 ml of distilled water, 1 g of the powdered okra mucilage sample (separately) was added and boiled in a water bath. The mixture was then filtered and to resultant 5ml of filtrate, 2-3 ml of distilled water was added and shook vigorously for attainment of a stable persistent froth. Then, followed by mixture of frothing with 1-2 drops of olive oil and shook vigorously, then observed in the formation of emulsions.

6. Test for alkaloids:

5 ml of the crude mucilage extracts were added to 8 ml of 1% HCl mixed, warmed and later filtered. Meyer's and Dragendorff's reagents were added to the 2 ml of the filtrate, then alkaloids absence or presence were determined based on the turbidity or precipitate development.

7. Test for Anthraquinone:

5 ml of each of the crude mucilage extracts (separately) was boiled with 10 ml of sulfuric acid (H2SO4) and was filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes.

8. Test for Phenols:

To 2ml of various crude mucilage extracts of sample, 4ml of distilled water, followed by a few drops of 10% aqueous ferric chloride solution was added. Development of blue or green colour indicated the presence of phenols.

9. Test for Resin:

2 ml of crude mucilage extracts was added to a few drops of acetic anhydride solution, followed by addition of concentrated Sulphuric acid (2ml). Presence of resins was determined by change in colour from orange to yellow.

10. Test for Terpenoids:

This was carried out by Salkowski's test described by Parekh and Chands (2008), To 4ml of chloroform, 10ml of the crude extract was added, followed by the careful further addition of 5ml concentrated (H2SO4). Formation of the reddish-brown coloration at the interface is an indication of a positive result for the presence of terpenoids.

11. Test for Cardiac Glycosides

The Keller-Kilani test method described by Parekh and Chands [15] was used for Cardiac Glycosides determination. To 2 ml of glacial acetic acid containing one drop of ferric chloride (FeCl3) solution, 5 ml of the extract was added, this was followed by addition of 1 ml concentrated Sulfuric acid. Brown ring was formed at the interface which indicated the presence of deoxy sugar of cardenolides. A violet ring may appear below the brown ring, though in the acetic acid layer, a greenish ring may also form just progressively throughout the layer.

12. Test for Phlobatannins:

An aqueous extract of the dry okra mucilage sample was boiled with 1% aqueous hydrochloric acid. The appearance of red precipitate indicates the presence of Phlobatannins [22].

V. Detection of mucilage:

1. Ruthenium red:

A few drops of ruthenium red were added to the powdered medication, which was then placed on a watch glass to produce a red tint**.**

2.Polymer Test:

The Powdered drug was swells in water or aqueous KOH.

VI. Instrumental analysis:

1. FTIR Spectroscopy:

The chemical structure of the components of okra fibres was analysed using FTIR (Bruker). The main absorbance peaks of interest in this study have been identified. The FTIR spectrum of the okra shows absorption bands of chemical groups characteristic of lingo cellulosic fibre compounds: cellulose, hemicellulose, and lignin. Such components are mainly composed of alkenes and aromatic groups and various oxygen-containing functional groups (ester, ketone, and alcohol). The distinctive functional groups in the selected extracts were located using an FTIR (Fourier transform infrared) spectrophotometer. To make thin, translucent sample discs, potassium bromide (KBr) was fully mixed with the AE and ME (5 mg), respectively, and then pressed at a pressure of 6 bars within 2 minutes. The Perkin Elmer 2000 spectrophotometer equipment with a scan range of 400 to 4000 cm1 was used to generate the FT-IR spectra, which were then analysed using Bruker OPUS software [16].

2. UV Analysis:

Ultraviolet and visible absorption spectrophotometry is the measurement of the absorption of monochromatic radiation by solutions of chemical substances, in the range of 185 nm to 380 nm, and 380 nm to 780 nm of the spectrum, respectively. The UV analysis of Okra polysaccharide powder was carried out using the Shimadzu apparatus [14].

RESULTS AND DISCUSSION

Okra Mucilage was isolated from okra fruit and evaluated for various tests

1. **Collection and drying of okra fruits:**



Fig 1: Collected *okra* fruits

2. **Extraction of mucilage:**







Fig.2: Removal of *Okra* Seeds Fig.3: Soaking of *okra* in Distilled water Fig.4: Extraction of mucilage

3. Isolation of mucilage:



Fig.5: Filtering the mucilage

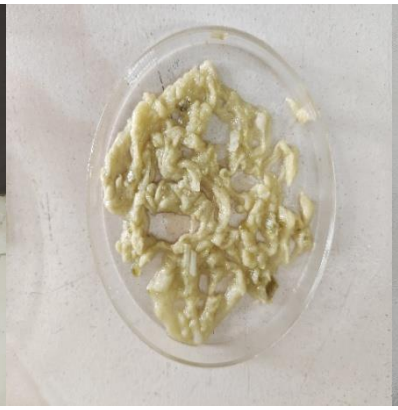


Fig.6: Isolated mucilage



Fig.7: Dried mucilage

4. Storage of Dried mucilage:

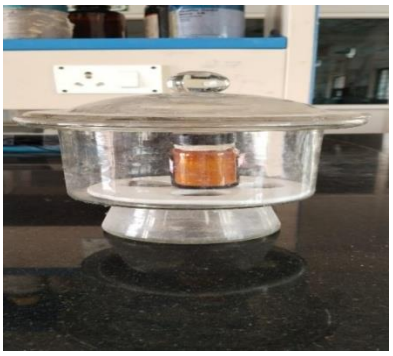


Fig.8: Storing of dried mucilage

S. No	Parameter	Observation
I. ORGANOLEPTIC CHARACTERIZATION		
1.	colour	Brownish
2	Odor	Characteristic
3	Shape	Irregular
4	Taste	Mucilaginous
II. PHYSICAL CHARACTERIZATION		
1.	Percentage yield	8.5%
2.	Swelling index	10 ml/g
3.	Solubility in water, ethanol, acetone, and chloroform	Soluble in water, Insoluble in ethanol, acetone, and chloroform.
4.	Water sorption time	7.21 minutes
5.	Loss on Drying	10.9%

6.	pH	6.2
7.	Surface tension	73 dynes/cm
8.	Viscosity	387 cps
9.	Melting point	162° c
10.	Particle Size	53.5µm
11.	Total Ash content	0.64%
12.	Acid insoluble ash	0.03%
III. FLOW PROPERTIES		
1.	Angle of repose	53.1°
2.	Bulk density	0.583
3.	Tapped density	0.875
4.	Hausner's ratio	1.5
5.	Compressibility	33.3%

IV. CHEMICAL CHARACTERIZATION

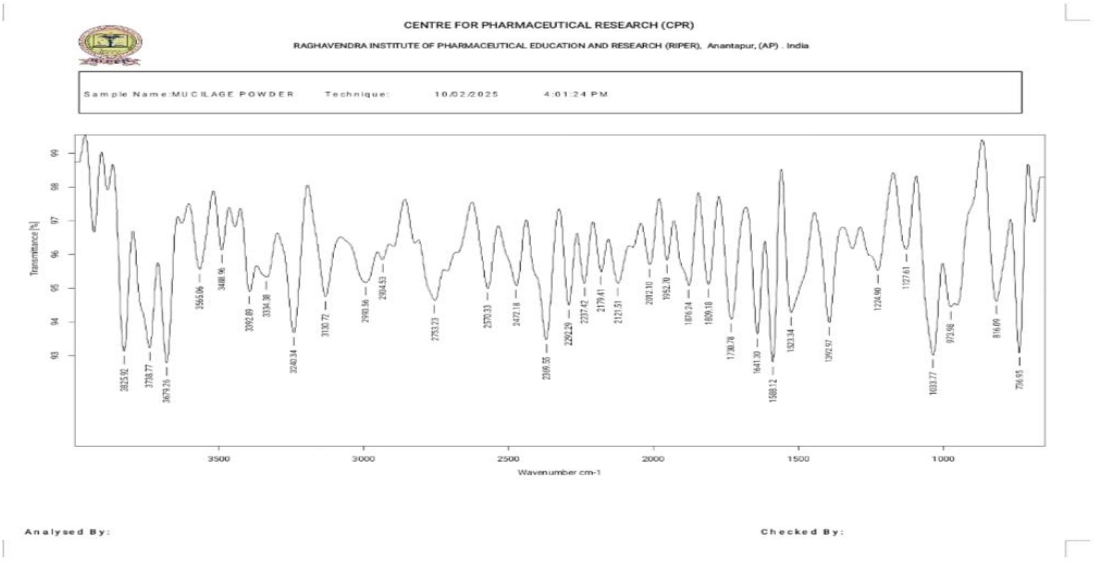
S. No	Chemical test	Observations	Inference
1.	Limit test for heavy metals	No dark colour formation	Passes
	Limit test for chlorides	No visible turbidity	Passes
2.	Test for Tannins	Brownish-green colour	Present
3.	Test for Steroids	Violet to blue	Present
4.	Test for flavonoids	Yellow colour	Present
5.	Test for Saponins	Persistent froth formation	Present
6.	Test for Alkaloids	Cream precipitate	Present
7.	Test for Anthraquinone	No colour change	Absent
8.	Test for phenols	Blue-green color	Present
9.	Test for Resin	No colour change	Absent
10.	Test for Terpenoids	Reddish brown interface	Present
11.	Test for Cardiac glycosides	No interface formed	Absent
12.	Test for Phlobatannins	No formation of red precipitate	Absent

V. DETECTION OF MUCILAGE			
1.	Ruthenium red Test	Light pink	+ve
2.	Polymer Test	Gelatinous mass	+ ve

VI. Instrumental Analysis:

1. FTIR Spectroscopy:

The obtained FTIR spectrum of the mucilage powder shows characteristic peaks corresponding to polysaccharides, indicating that the sample is rich in carbohydrates. A broad peak around 3500–3200 cm^{-1} suggests the presence of hydroxyl (-OH) groups. This is indicative of polysaccharides, which are major components of mucilage. Peaks in this range confirm the presence of C-H stretching vibrations, characteristic of aliphatic structures found in carbohydrates. A strong peak near 1700 cm^{-1} suggests the presence of carboxyl (-COOH) or ester groups, often seen in pectin and uronic acids in mucilage. Peaks in this range of 1624 cm^{-1} indicate C=C stretching, often found in ring structures of polysaccharides. The peak near 1234 cm^{-1} confirms glycosidic linkages, essential in the polysaccharide backbone structure. These peak near 816-726cm^{-1} indicates glycosidic linkages, essential in the polysaccharide backbone structure.



Characteristic peaks of Okra polysaccharide powder:

Functional group	Characteristic peak (cm^{-1})	Obtained peak (cm-1)	Interpretation
O-H stretch	3600-3200	3506-3099	Broad peak due to polysaccharide hydroxyl groups
C-H stretch	2950-2850	2926-2854	Indicates CH_2, CH_3 Stretching vibrations
C=O stretch	1740-1650	1698	Carbonyl presence, likely from uronic acids or protein impurities
C=C Stretch	1650-1450	1624-1514	Corresponding to polysaccharide backbone structures
C-O-C Stretch	1250-1000	1234	Strong peaks confirming polysaccharide either linkage
C-H Bending	900-750	816-726	Typical fingerprint region for mucilage-based polysaccharides.

2. UV Analysis

Okra fruit polysaccharide powder solution (in water) showed no absorption in the UV-visible range (i.e. 185-780 nm). Ultraviolet and visible absorption spectrophotometry is the measurement of the absorption of monochromatic radiation by solutions of chemical substances, in the range of (185-380) nm and (380-780) nm of the spectrum, respectively. Okra powder solution (in water) showed no absorption in the UV range (i.e. 185-7

CONCLUSION:

The study found that mucilage derived from Abelmoschus esculentus is a promising natural excipient with outstanding bioadhesive, emulsifying, and stabilizing capabilities. Its solubility in water and polar solvents makes it useful in pharmaceutical applications, especially medication formulations. The swelling capacity contributes to its role in moisture retention and controlled drug release, while its viscosity makes it useful as a thickening and stabilizing ingredient in emulsions and suspensions. FTIR research confirmed its polymeric composition and excipient characteristics. Okra mucilage is a biodegradable, non-toxic, and inexpensive alternative to synthetic excipients. Its uses include pharmaceuticals, food, and cosmetics,

providing a sustainable and environmentally favourable choice for formulation development. Given its good qualities, more research into formulation stability and large-scale production is required to increase its commercial viability. With further research, okra mucilage could serve as a valuable natural excipient in a variety of businesses.

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