

Physicochemical Standardization of Mucilage Obtained from *Althaea officinalis* Linn – Root

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ABSTRACT

Introduction: Plant derived mucilage has been explored as a drug, pharmaceutical excipient, and in cosmetics. Several mucilage and mucilage-containing drugs are being utilized in Unani medicine. These are to be standardized for authentications owing to immense utilization. A mucilage-containing drug obtained from root of *Althaea officinalis* L. (AO) – family Malvaceae, an important Unani drug, has been subjected to physicochemical studies for standardization.

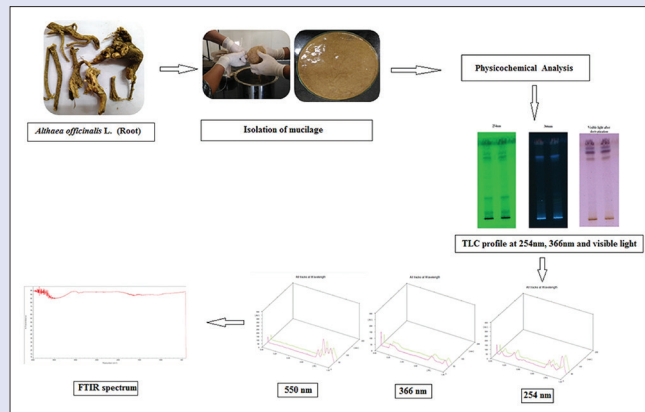
Materials and Methods: Mucilage of roots of the drug was isolated by classical and reference method. The physicochemical method included determination of ash values, moisture content, viscosity, swelling index (SI), and pH value. Powder characterization study included bulk density, tapped density, Hausner's ratio, and angle of repose. For preliminary phytochemical analysis, qualitative tests for organic constituents and test for mucilage were carried out. Analytical methods, namely Fourier transform infra-red (FTIR) and high-performance thin-layer chromatography (HPTLC) were also applied. **Results:** The yield percentage taken by acetone method was 36.80 ± 1.25 whereas that of classical method was 42.93 ± 1.35 . Values of pH, loss on drying, viscosity, and SI were 4.08 ± 0.032 , 14.46 ± 0.13 , 34.40 ± 0.61 , and 334.36 ± 23.77 , respectively. Data for ash value and powder characterization (Micromeritic Properties) were set in. Preliminary confirmative test confirmed that the isolated polysaccharide is mucilage. HPTLC fingerprinting of aqueous extract gave 6 and 4 peak at 254 nm, 4 and 5 peak at 366 nm and 5 and 6 peaks at 550 nm in mobile phase chloroform (90): methanol (10): acetic acid (2). FTIR data for the mucilage were also set in. **Conclusion:** Physicochemical standardization data/monograph for AO root mucilage were developed.

Key words: *Althaea officinalis*, mucilage, physicochemical characterization, root, Unani medicine

SUMMARY

- The present study included preliminary phytochemical studies and literature review for *Althaea officinalis* L root mucilage. The mucilage is indicated as therapeutic constituent utilized in various formulations as an ingredient, pharmaceutical excipients, and as a corrective for adverse effect of several drugs. Isolation Method mentioned in Unani medicine gave little higher percentage yield. Physicochemical parameters for standardization of acetone method of isolation were set in. Data obtained can act as a monograph/

reference and may guide the users of the mucilage for pharmacological activities and pharmaceutical utility.



Abbreviations used: HPTLC: High-performance thin-layer chromatography; FTIR: Fourier transform infrared; AO: *Althaea officinalis* L.; FRLHT: Foundation for revitalization of local health traditions; SI: Swelling index; Pet: Petroleum; IR: Infrared; KBr: Potassium bromide; TLC: Thin-layer chromatography; IR: Infrared; KBr: Potassium bromide; UV: Ultraviolet; DNA: Deoxyribonucleic acid; μ l: Microliter; %: Percentage; pH: Potential of hydrogen; rpm: Revolution per minute; SEM: Standard error of mean.

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INTRODUCTION

Mucilage is translucent, amorphous substance, and polymer of monosaccharides or mixed monosaccharides combined with uronic acid forming slimy mass in water which is typically heterogeneous in composition.^[1] In recent years, plant-derived dietary fibers (mucilage) are being studied for their use in food, cosmetics, and pharmaceutical industries.^[2] Mucilage is obtained mainly from seeds and sometimes other parts of plants. Some mucilage is also obtained from marine algae and selected microorganisms. Plant-derived mucilage has been widely explored as pharmaceutical excipients. They are widely used in industry as thickeners, emulsion stabilizers, water-retention, gelling, and suspending agents, binders,

and sustained release agents. Apart, it has been known for its medicinal uses since long times.^[1]

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Many mucilage (called *Lu'ab* in Unani medicine) or mucilaginous drugs are widely used in Unani medicine as therapeutic agent, correctives and pharmaceutical aid. *Lu'abe Behidana* (*Cydonia vulgaris* Mill), *Lu'abe Hulba* (*Trigonella foenum graecum* L.), *Lu'abe Aspaghol* (*Plantago ovata* Forsk), *Lu'abe Alsi* (*Linum Usitatissimum* L.), *Lu'abe Samaghe Arabi* (*Acacia Arabica* Willd.), *Lu'abe Gheekwar* (*Aloe barbadensis* L.), etc., are some best examples.^[3,4]

The genus *Althaea* belongs to the family Malvaceae. *Althaea officinalis* L. (AO) is an important Unani medicinal plant known as *Khatmi*. Almost all parts of the plant contain mucilage and possess activities such as emollient, expectorant, demulcent, soothing, cleansing, laxative, analgesic, astringent, hemostatic, concoctive, diuretic, and emmenagogue,^[5,6] used in various ailments such as metritis, enteritis, mastitis, and arthritis in Unani medicine.^[7,8] It is also used traditionally for the treatment of irritation of oral and pharyngeal mucosa, dry cough, mild gastritis, skin burns, insect bites, catarrh, gastrointestinal tract and urinary tract complaints, inflammation, ulcers, abscesses, burns, constipation, and diarrhea.^[9,10]

The root of AO contains relatively large amount of mucilage, which contains glucose, xylose, uronic acid, methyl pentose, and hexose. Studies have reported that the crude mucilage of the root contained a glucan, an arabinogalactan, and an acidic polysaccharide. A neutral fraction composed of 21% of glucose, 52% galactose, and 27% of arabinose, acidic polysaccharide composed of 58% galacturonic acid, 39% rhamnose, and 3% of galactose and trace amount of glucose.^[11] The present study aimed at standardizing the mucilage because in spite of its regular use in Unani medicine, it has not been standardized by parameters used so far.

MATERIALS AND METHODS

Materials

The drug was procured from crude drug market of Bangalore and was identified by Dr. S. Noorunnisa Begum, Senior Assistant Professor, Pharmacognosy foundation for revitalization of local health traditions, Bangalore vide authentication certificate no. 4537. The chemicals and reagents used in this study were of analytical grade.

Methods

Various pharmacognostic methods such as preliminary phytochemical/physicochemical studies were performed including determination of ash values, moisture content, viscosity, swelling index (SI), pH value, etc. Powder characterization study includes bulk density, tapped density, Hausner's ratio and angle of repose, qualitative tests for organic constituents, and test for mucilage were also carried out. Two analytical studies, namely Fourier transform infra-red (FTIR) and high-performance thin layer chromatography (HPTLC) were also applied for setting appropriate standards.

Isolation of mucilage

For isolation of mucilage, fresh plant material was washed with distilled water to remove dirt and debris; dried material was powdered with the help of grinder, of which 100 g was soaked in 800 ml distilled water for 5–6 h, boiled for 30 min, and allowed to stand for 1 h. The material was then squeezed through muslin cloth to remove the marc from the solution. Three times volume of acetone was added to the squeezed material to precipitate the mucilage which was separated, dried in an oven at a temperature

not more than 50°C for 4–5 h, collected and the dried material was passed through a sieve no. 80 and stored carefully in desiccators until required.^[12,13] Method of isolation of mucilage was adopted as per Ameena *et al.* and Kulkarni *et al.*^[12,13] with slight modification in respect to drug: water ratio.

Percentage yield

The percentage yield of extracted mucilage was calculated based on the amount of drugs used for the extraction process and the amount of dry mucilage obtained individually depending on solvents used. The percentage yield was calculated from the ratio between weight of dried mucilage obtained and weight of fresh material as:^[14]

Percentage yield = Weight of dried mucilage obtained/weight of drug used × 100.

Classical method of isolation

The drug (100 g) was soaked in 800 ml of purified water for 24 h. Then, the content was boiled for 10–15 min to separate the mucilage from the drug and then filtered and squeezed through a muslin cloth to get the mucilage. This method was as per Unani Pharmacopoeia with slight modification in respect of drug: water ratio.^[15]

Physicochemical characterization of mucilage obtained from acetone method

The isolated mucilage was subjected to preliminary identification tests, *viz.* ruthenium red and Molisch's test, organoleptic evaluation and other preliminary physicochemical tests which were carried out by standard test procedures.^[16–18]

The observations of the entire test were made in triplicate and represented as average of successive measurements. Other tests applied in the study were as follows:

pH of solution

pH of percentage solution was determined using pH meter.^[16–18]

Loss on drying

Loss on drying was determined for an appropriate quantity of dried mucilage at 105°C for 2 h in a hot-air oven.^[16–19]

Determination of ash

Ash values were calculated by the method described in Indian Pharmacopoeia.^[20]

Swelling index

SI was determined by the method described by WHO, expressed as a percentage and was calculated using formula $SI (\%) = (V_{\text{final}} - V_{\text{initial}}) / V_{\text{initial}} \times 100$.^[21,22]

Viscosity of mucilage powder

The viscosity was determined using 1% and 2% solution of mucilage prepared with distilled water carried out using Brook field viscometer using spindle no. 2. The viscosity was measured at 100 rpm at 25°C using spindle No. 2. The study was done in triplicate to ensure the accuracy.^[23]

Solubility

Solubility of the mucilage powder was determined in different solvents

such as water, chloroform, ethanol, acetone, petroleum ether and benzene.^[14]

Powder characterization

Bulk density

Bulk density was determined by measuring the volume of known weighed quantity of mucilage powder using bulk density apparatus.^[14,24,25]

Bulk density = Mass/bulk volume.

Tapped density

Tapped density was calculated by measuring the volume of known weighed quantity of granules using bulk density apparatus and using the formula:^[14,24,25]

Tapped density = Mass/tapped volume.

Compressibility index

The compressibility index of mucilage powder was determined by Carr's Index and was calculated using the formula:^[25]

Carr's index (%) = (tapped density – bulk density/tapped density) × 100.

Hausner's ratio

The Hausner's index is calculated by dividing the tapped density by the bulk density of the granules.^[25]

Hausner's ratio = Tapped density/bulk density.

Angle of repose

Flow characteristics of isolated mucilage powder were measured by angle of repose by fixed funnel method. Angle of repose was calculated by using formula:^[25]

$\tan \theta = 2 h/D$

h = Height of powder (from graph paper to tip of funnel), D = Mean diameter of the powder.

High-performance thin-layer chromatography

HPTLC fingerprinting was carried out as per the following chromatographic condition: Stationary phase, Plate size (X × Y): 20.0 cm × 10.0 cm, Material: HPTLC plates silica gel 60 F₂₅₄ (Manufacturer: E. MERCK KGaA), Calibration parameters, Calibration mode: Single-level statistics mode: CV, Evaluation mode: Peak height; Linomat 5 application parameters, Spray gas: Inert gas, Sample solvent type: Methanol, Dosage speed: 150 nl/s, Predosage volume: 0.2 ul; Sequence, Syringe size: 100 µl, Number of tracks: 2, Application position Y: 12.0 mm, Bandlength: 10.0 mm; Instrument: CAMAG thin layer chromatography (TLC) Scanner 3 "Scanner3_140525" S/N 140525 (1.14.28), Number of tracks: 2, Position of first track X: 35.0 mm, Distance between tracks: 20.0 mm, Scan start pos. Y: 12.0 mm, Scan end pos. Y: 90.0 mm, Slit dimensions: 10.00 mm × 0.20 mm, macro, optimize optical system: Light, scanning speed: 20 mm/s, Data resolution: 100 µm/step; Measurement table, Wavelength: 254/366/550, Lamp: D2 and W, Measurement Type: Remission, measurement mode: Absorption, optical filter: second order, Detector mode: Automatic, PM high voltage: 382 V; Detector properties, Y-position for 0 adjust: 12.0 mm, Track # for 0 adjust: 0, Analog Offset: 10%, Sensitivity: Automatic (19); Integration Properties, Data filtering: Savitzky–Golay 7, Baseline correction: Lowest Slope, Peak threshold min. Slope: 5, Peak threshold min. Height: 10 AU, Peak threshold min. Area: 50, Peak threshold max. Height: 990 AU, Track start position: 12.0 mm, Track end position:

90.0 mm, Display scaling: Automatic, mobile phase selected for study is chloroform (90):methanol (10):acetic acid (2), two track (track 1 and track 2) for each wavelength was studied for checking reproducible data 2.6.

Fourier transform infrared

The FT-IR spectrum of the sample was recorded in an IR spectrometer (Nicolet 6700 FT-IR) using potassium bromide (KBr) discs prepared from powdered samples of mucilage of AO mixed with dry KBr. Instrument: Thermofisher Scientific, Model: Nicolet 6700 FT-IR, Source: IR, Beam splitter: XT-KBr, Detector: DTGS KBr, Scan range: 4000 cm⁻¹ to 400 cm⁻¹, No of scans: 64, Resolution: 4, Sample gain: 8, Optical velocity: 0.4747, Aperture: 80, and Sample preparation: KBr pellet method.

RESULTS

Percent yield

The yield percentage of test drug taken by acetone method was 36.80 ± 1.25. The percentage yield value by classical method was estimated as 42.93 ± 1.35, respectively.

Physicochemical standardization/characterization of mucilage isolated with acetone method

Organoleptic properties

The isolated mucilage was evaluated for their organoleptic properties; results are summarized in Table 1.

pH, ash value, loss on drying, viscosity, swelling index of isolated mucilage

The pH, ash value, loss on drying, viscosity, and SI of isolated mucilage were evaluated, and the results are summarized in Table 2.

Table 1: Organoleptic characteristic of *Althaea officinalis* L. root mucilage

Parameters	Observation
Appearance	Amorphous
Color	Yellowish brown
Odor	Characteristic
Taste	Mucilaginous
Texture	Rough

Table 2: Physicochemical characterization of *Althaea officinalis* L. root mucilage

Parameters	Value (mean±SEM)
Loss on drying (%)	14.46±0.13
pH (1%)	4.08±0.032
Total ash (%)	11.67±0.17
Acid-insoluble ash value (%)	1.17±0.17
Water-soluble ash value (%)	9.67±0.17
Swelling index %	334.36±23.77
Viscosity (1 w/v %) cP	34.40±0.61

SEM: Standard error of mean

Table 3: Micromeritic characteristics of powder of *Althaea officinalis* L. root mucilage

Parameters	Value
Bulk density (g/ml)	0.49±0.0040
Tapped density (g/ml)	0.58±0.0056
Hausner's ratio	1.17±0.0016
Compressibility (%)	14.75±0.12
Angle of repose (θ)	36.91±0.21

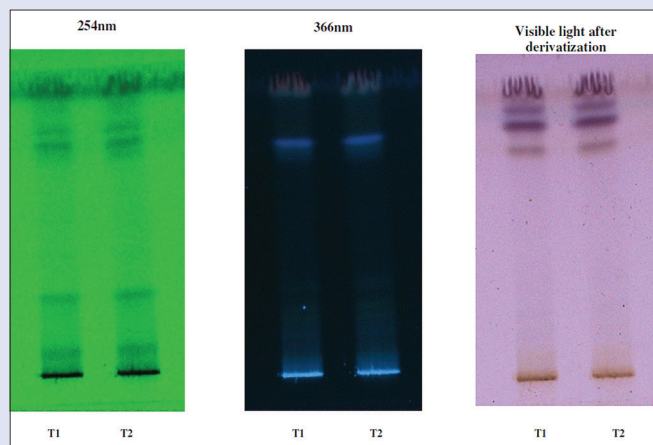


Figure 1: Thin-layer chromatography profile of mucilage of aqueous *Althaea officinalis* L. track 1 and 2 at 254 nm, 366 nm, and visible light

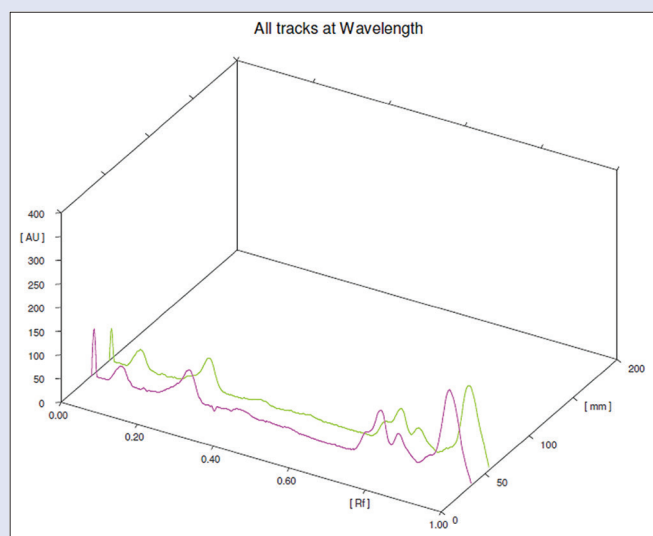


Figure 2: High-performance thin-layer chromatography fingerprinting overlay of aqueous extract of mucilage of *Althaea officinalis* L. root at 254 nm track 1 and 2

Solubility

Mucilage was insoluble in most organic solvents except water in which it was sparingly soluble.

Powder characterization (micromeritic properties) of isolated mucilage

The isolated mucilage was evaluated for their micromeritic properties; the results are summarized in Table 3.

Phytochemical screening

The identification of isolated mucilage was confirmed by color reaction with ruthenium red and Molisch's reagent. Phytochemical investigation showed the presence of carbohydrate. This entire preliminary confirmative test confirms that the isolated polysaccharide is mucilage [Table 4].

High-performance thin-layer chromatography

Chromatogram (fingerprinting) of aqueous extract of AO gave 6 peak in track one at 254 nm, 5 peak in track two at 366 nm, and 5 and 6 peak in track one at 550 nm wavelength with mobile phase as chloroform (90):methanol (10):acetic acid (2). Visible light derivatization was done with spray of anisaldehyde sulfuric acid, TLC picture is depicted in Figure 1, and fingerprint/peaks of the same is depicted in Figures 2-7.

Fourier transform infrared

FTIR spectroscopic analysis of drug data was set in, FTIR spectrum of mucilage of AO is depicted in Figure 8, and FTIR was used in the

Table 4: Confirmatory phytochemical test of *Althaea officinalis* root mucilage

Chemical properties	Test	Observations
Mucilage	Ruthenium red	Positive
Alkaloids	Mayer's test	Negative
Carbohydrates	Molisch's test	Positive
Glycosides	Borntreger's test	Negative
Terpenes/phytosterols	Salkowski's test	Positive
Phenols	Ferric chloride test	Negative
Fixed oils	Filter paper test	Positive
Flavonoids	Ammonia test	Positive
Tannins	Ferric chloride test	Positive
Diterpenes	Copper acetate test	Positive
Saponins	Froth test	Negative
Proteins and amino acids	Ninhydrin test	Negative

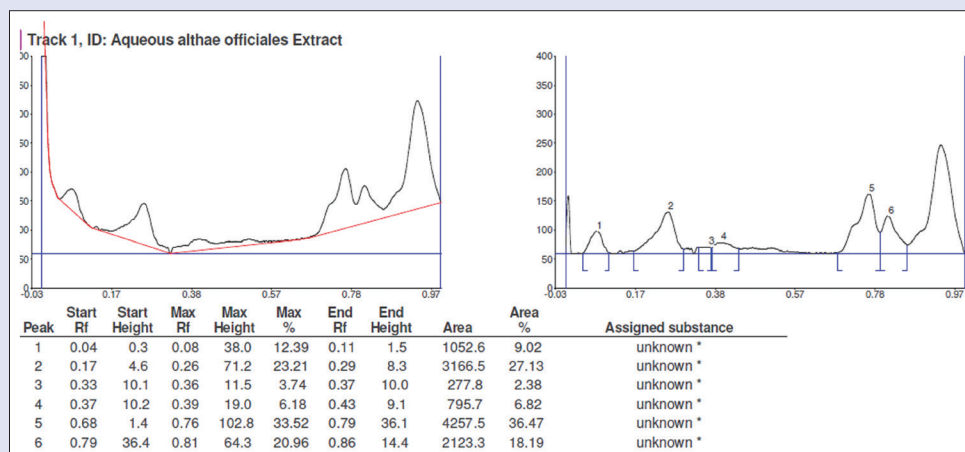


Figure 3: High-performance thin-layer chromatography densitogram and peaks of aqueous extract of mucilage of *Althaea officinalis* L. at 254 nm (T1)

identification of vibrational frequency of the molecules. The list of frequency and corresponding intensity is recorded.

DISCUSSION

Traditional systems of medicine are often being held responsible for use of substandard drugs. With increasing demand and resurgence of traditional medicines, regulatory authorities frame guidelines for making these drugs safe for the consumers.^[26] Now, the world is much more concerned over the safety of herbal drugs. Therefore, herbal drugs have also been brought under the scanner of pharmacovigilance for improving quality, safety, and efficacy.^[26,27]

Many mucilage and gums have significant anticoagulant, hypoglycemic, anticancer, anti-inflammatory, and wound healing activities which make them very exciting constituent, and it can become an important thrust area in this regard owing to its new applications such as in microencapsulation, coating agents, gelling agents, transdermal film-forming agent, and excipients.^[28] Any substance including mucilage and gum intended to be used as drug or excipients must be pure, safe,

and effective. Purity, safety, and efficacy are three important parameters for deciding good quality of any drug, especially that of herbal drugs and their components.

Organoleptic characters of crude drugs give useful information. Color, odor, appearance, taste, and texture of the mucilage were recorded. Estimation of percentage yield in case of mucilage containing drugs may be an important parameter. It can give an idea about the mixing of other mucilage with the original drug.

The yield percentage of mucilage by acetone method was 36.80 ± 1.25 , and by classical method, it was estimated as 42.93 ± 1.35 . The mean percentage yield of mucilage of the test drugs differed widely in classical and acetone method. Since mucilage is water soluble, the findings displayed higher yield in classical method.^[14,15] Difference of percentages taken by two methods indicates that the method applied has an effect on the yield. The two methods are not superseding over each other; therefore, either method can be applied depending on the individual need and to get maximum yield.

The mean value taken by loss on drying method is $14.46\% \pm 0.13\%$. It is also a method by which moisture content of drugs can be estimated. The high percentage of moisture may be due to more water content. Moisture content of the drugs if exceeded beyond limit can deteriorate the drug. Findings of loss on drying suggest further stress stability study for assessing the effect of moisture content in the drug.^[29]

The mean (%) of total ash value, acid-insoluble ash value, and water-soluble ash value were $11.67\% \pm 0.17\%$, $1.17\% \pm 0.17\%$, and $9.67\% \pm 0.17\%$, respectively. Ash value is an important parameter for detection of adulteration and impurities.^[29] Low ash value of the mucilage indicates its purity.

pH of drug in 1% solution was 4.08. The mildly acidic pH can contribute to its emulsifying capacity. Acidic pH may also be due to uronic acid in its structure.^[30]

Mucilage powder was characterized for its flow property. The values were 1.17° , 14.75° , and 36.90° , respectively, for Hausner's ratio, Compressibility index and angle of repose [Table 3]. Findings establish its flow and Micromeritic property, it displayed adequate flow property.

The viscosity of mucilage solution was 34.40 ± 0.61 cP. Less viscous suspension tends to pour more easily than the more viscous ones and hence study of rheology or viscosity is critical to understand the stability of suspensions.^[31] Mucilage isolated was found to be water soluble which is its characteristic.

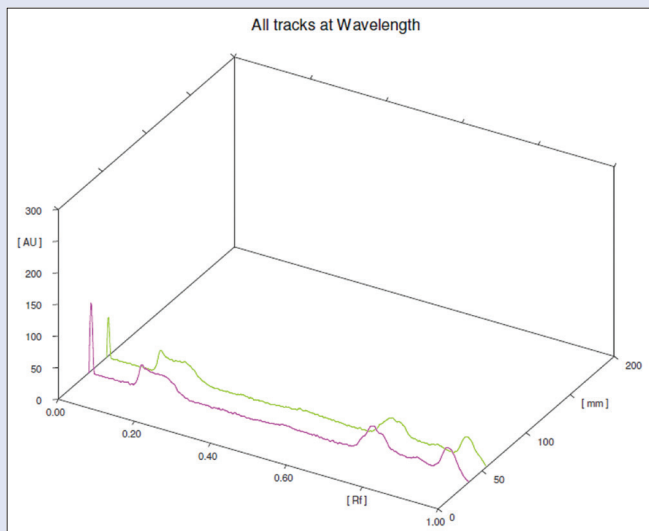


Figure 4: High-performance thin-layer chromatography overlay of aqueous extract of mucilage of *Althaea officinalis* at 366 nm track 1 and 2

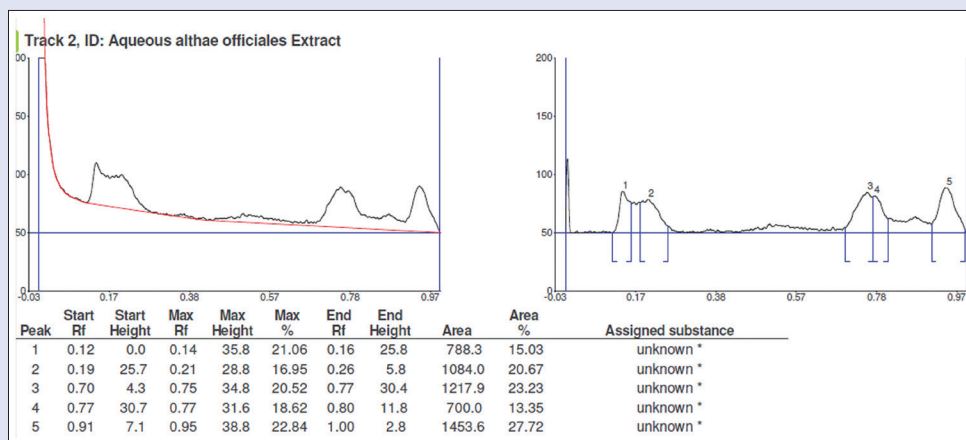


Figure 5: High-performance thin-layer chromatography densitogram and peaks of aqueous extract of mucilage of *Althaea officinalis* L. at 366 nm (T2)

The SI of mucilage of AO was $334.36\% \pm 23.77\%$. Determination of swelling factor of root mucilage is of prime importance which is evaluated on the basis of their SI. The reported data set the SI and viscosity of the sample in respect of the method used. Generated data of swelling factor and viscosity can be used as reference value for identification and to check the adulteration and can also be useful in formulations or its combinations with other drugs. Swelling factor can also reveal the chemical composition of different mucilage. It is essential to know its therapeutic importance as well as its pharmaceutical applications as excipients in design for dosage forms.^[32]

Qualitative phytochemical screening for its functional group displayed the presence of carbohydrate; ruthenium red test for the presence of mucilage was also found positive.^[16,18] Apart from this, it also displayed the presence of terpenes, fixed oil, flavonoids, tannins, and diterpenes.

HPTLC fingerprinting was performed on the mucilage, aqueous

extract of the mucilage gave 6, 5, and 6 peak at 254 nm, 366 nm, and 550 nm wavelength, respectively, with the selected mobile phase. Visible light data was also generated. Samples were run in two tracks to check the reproducibility. Identical graph was found in both the track [Figures 1-8], obtain fingerprinting is for isolated crude mucilage of AO root, further study with reference marker for identification and quantification of the specific phytochemical is needed. FTIR spectrum of aqueous extract of mucilage was set in for future reference [Figure 8]. FTIR is used in the identification of vibrational frequency of the molecules; the list of frequency and corresponding intensity are recorded in the work.

Curnow and Owen studied root phytochemicals derived from AO as potential natural components of ultraviolet (UV) protecting dermatological formulations and find out that AO root extract significantly reduce UVA-induced DNA damage in cultured human lung and skin fibroblasts, indicating that it can be predominantly protected against indirect UVA-induced oxidative stress and can be utilized in dermatological formulations.^[33] Masashi *et al.* isolated and characterized mucous polysaccharide, "Althaea-Mucilage O" from the roots of *A. officinalis*.^[11] Géciová and Babor characterized starch from AO root and suggested the presence of Alpha-D-glucans by confirming the presence of reserve starch besides the moderately branched (1→6)-alpha-D-glucan resembling microbial dextrans.^[34] Peterc *et al.* isolated acidic heteropolysaccharide from the mucilage of the roots of the AO; its insoluble barium salt contained D-galactose, L-rhamnose, n-glucuronic acid, and n-galacturonic acid.^[35] Similarly, several reported activities on AO mucilage are immunomodulatory, demulcent, soothing, antitussive, hypoglycemic effect, etc.^[36]

Further work with sophisticated technique is needed for comment on compatibility and stability issues of the isolated AO root mucilage. Concept of use of *Luab* (Mucilage) of several drugs is mentioned specifically in Unani medicine and is indicated for the treatment of several disease conditions. The mucilage is indicated as therapeutic constituent utilized in various formulations as an ingredient, pharmaceutical excipients, and as a corrective for adverse effect of several drugs. Physicochemical standardization data for AO mucilage indicated for various ailments in Unani medicine can act as a monograph and reference.

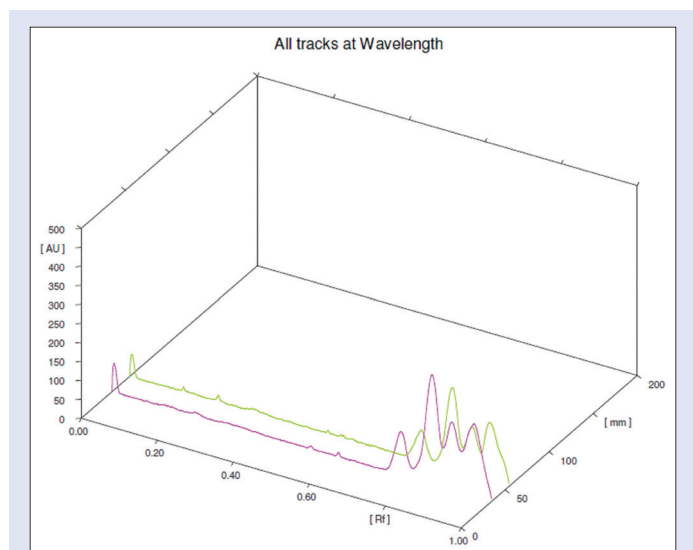


Figure 6: High-performance thin-layer chromatography overlay of aqueous extract of mucilage of *Althaea officinalis* L. at 550 nm track 1 and 2

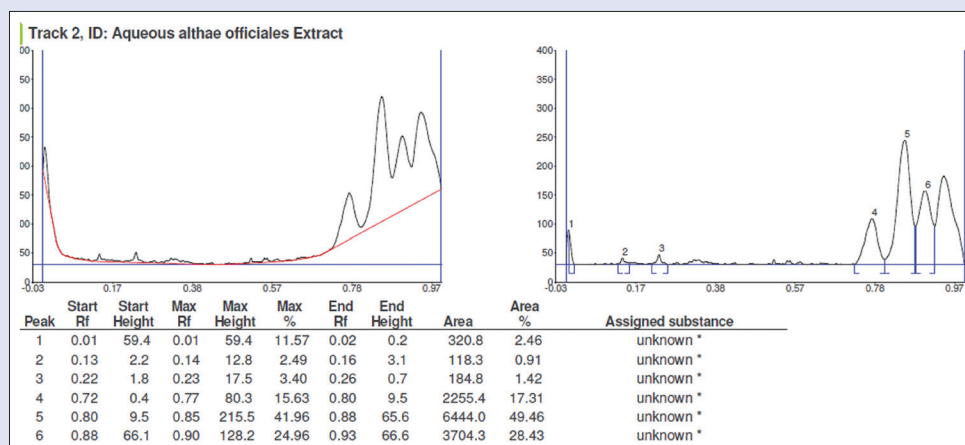


Figure 7: High-performance thin-layer chromatography densitogram and peaks of aqueous extract of mucilage of *Althaea officinalis* L. at 550 nm (T2)

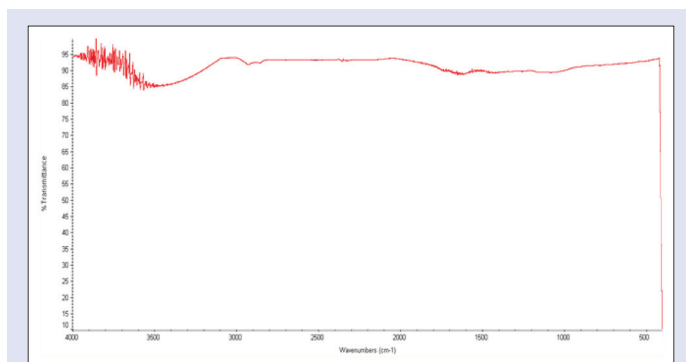


Figure 8: Fourier transform infrared spectrum of aqueous extract of mucilage of *Althaea officinalis* L

CONCLUSION

Classical method mentioned in Unani medicine text gave little higher percentage yield of the mucilage than acetone method. Physicochemical parameters for standardization of acetone method were set in. The data obtained may guide the users of the mucilage for pharmacological activities and pharmaceutical utility.

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Conflicts of interest

There are no conflicts of interest.

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