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PHARMACOGNOSTICAL, PHYSICOCHEMICAL AND PHYTOCHEMICAL INVESTIGATION OF *ALTHAEA ROSEA* LINN.

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ABSTRACT
Althaea rosea belongs to the family Malvaceae. It is an erect, simple or sparingly branched, stellately hairy, annual or biennial herb and 0.5-2.0 m in height. The seeds of this plant having anti-pyretic, diuretic, anti-inflammatory, demulcent and analgesic properties. In present era, the adulteration has become a major problem due to unavailability of standards relating to genuineness of herbal drug. Hence, efforts have been made to identify the pharmacognostical characters and phytochemical analysis of Althaea rosea seed. HPTLC fingerprint profiles of methanolic extract and its chloroform fraction of the seed were also developed. This will serve as a standard reference for identification, authentication and distinguishing the plants from its adulterants.

Keywords *Althaea rosea, HPTLC, pharmacognostical, phytochemical*

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INTRODUCTION

Althaea rosea belongs to the family Malvaceae. It is an erect, simple or sparingly branched, stellately hairy, annual or biennial herb and 0.5-2.0 m in height. It is a stately ornamental plant, producing large single, semi-double, double or frilled flowers of many colours[1-2]. This is distributed from the east Mediterranean region to Central Asia. Native to China and Greece, very commonly cultivated in Indian gardens. Sometimes found as an escape in waste places and along roadsides[1-4]. All parts of the plants contain mucilage and are used in medicine[1]. The seeds of this plant are having anti-pyretic, diuretic, anti-inflammatory, demulcent and analgesic properties[2-5]. It has also been used in traditional medicines for ailments like chest complains, boils and abscesses, constipation, peptic ulceration, renal calculi, burning micturition, gastritis, cough, etc.,[2-6]. Since, adulteration and substitution have become a major problem due to

the absence of standards relating to genuineness of herbal drug. The WHO has emphasized the need to ensure quality control of medicinal plant products by using modern techniques. Keeping in view the importance, the seed of *Althaea rosea* Linn. was standardized according to WHO and Pharmacopoeial guidelines available for herbal drugs.

MATRIAL AND METHODS

Collection and authentication of drug

The seeds of *Althaea rosea* Linn. were purchased from Khari Baoli, local market of Delhi and authenticated by Dr. H. B. Singh, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. A voucher specimen No. NISCAIR/RHMD/Consult/-2010-11/1657/255 is submitted in the herbarium of NISCAIR, New Delhi.

Macroscopical and microscopical study

Macroscopical and microscopical characters of the drugs were studied according to the WHO and pharmacopoeial guidelines[7-8].

Physico-chemical studies

Different physicochemical values such as extractive values (cold and hot extracts), ash values (total ash, acid-insoluble ash & water soluble ash), loss on drying, swelling index and pH of 1% and 10% solution of *Althaea rosea* seed were determined according to the standard methods[7].

Preliminary phytochemical analysis

The preliminary phytochemical screening was carried out using the extracts for different types of chemical constituents as per method described by Trease and Evans[9].

Fluorescence analysis

Chemical tests of powder drug with different reagents were performed to observe the colour reactions according to the reported method[10].

Effect of different chemical reagent on crude drug powder

Chemical tests of powder drug with different reagents were performed according to method described by Sama et al.,[10]. Most of these tests were based on colour indication of powder drug with specific substance.

Determination of total phenolic contents by UV spectrophotometer

The phenol was determined in powdered crude drugs, extracts and beverages by Folin Ciocalteu method[11]. Standard stock solution was prepared by dissolving 25 mg of catechin standard in 100 ml distilled water. Different concentrations of the standard solutions were prepared for standard calibration curve starting from 4 to 24 µg/ml in water. The commercial Folin Ciocalteu reagent was diluted (1: 10) with distilled water on the day of use. 1M sodium acetate was prepared by dissolving 82 g of sodium acetate in 1000 ml distilled water

Sample preparation - 500 mg of the samples were taken in 50 ml volumetric flasks and added around 25 ml of distilled water and sonicated for 10 minutes then made up the volume with water.

Procedure - Take 3ml of each standard and sample solution in a 10 ml test tube and to this add 3 ml of FC reagent and 3 ml of sodium carbonate solution. A blank solution was prepared by adding 3 ml each of distilled water, sodium carbonate solution, and FC reagent in test tube. Keep the solution in dark for 30 minutes for colour development. Absorbance was taken at 765nm against blank solution. After taking the absorbance of standard dilutions the calibration curve was plotted (Fig. 1). Phenolic contents in drug were calculated by using standard calibration curve.

Determination of total flavonoid contents by UV spectrophotometer

The flavonoid content was determined in powdered crude drugs according to method described by Pourmorad et al.[12]. $AlCl_3$ (0.1 g/ml) and CH_3COONa (1M) were prepared, Prepared dilutions for Rutin (standard) from 10 µg /ml to 100 µg/ml.

Samples Preparation - 500 mg of the samples were taken in 50 ml volumetric flasks and added around 25 ml of methanol and sonicated for 30 minutes then made the volume with methanol.

Procedure - 0.5ml of each standard and sample solution was taken in a 10 ml test tube and added

1.5 ml methanol. To this added 0.1ml of $AlCl_3$ and 0.1 ml of CH_3COONa reagents and added 2.8 ml Distilled water and kept for 30 minutes. A blank solution was prepared by adding 2 ml of methanol, 0.1ml of $AlCl_3$, 0.1ml of CH_3COONa reagents and then added 2.8 ml Distilled water. Absorbance was taken at 415 nm against blank solution. After taking the absorbance of standard dilutions the calibration curve was plotted (Fig. 2). Flavanoid contents in drug were calculated by using standard calibration curve.

Development of chromatographic HPTLC fingerprint profile of different extracts

The plant material was coarsely powered and extracted in Soxhlet apparatus for 6-24 h using methanol. The extract was evaporated to dryness in a rota-vapour and the solvents were recovered. The methanolic extract of drugs were treated with boiling chloroform for one hour and filtered. The process was repeated two times. All the chloroform soluble fractions were combined together. Removal of chloroform by distillation method under reduced pressure gives chloroform soluble fraction of *Althaea rosea* seed. Gummy residues so obtained, were stored in deep freezer at -20 °C till further application. TLC and HPTLC samples were prepared by dissolving each extract in their respective solvent to get the concentration; 40 µg/ml. These solutions were further passed through syringe filter to remove any impurities and applied on TLC plate for finger printing analysis. The extract was applied on TLC aluminum sheets silica gel 60 F 254 (Merck) 10 µl each with band length 6 mm using Linomat 5 sample applicator set at a speed of 100 nl/sec CAMAG, Switzerland.

Different solvent systems were used for separation of constituents of extract and its fraction. The chromatograms were developed in twin trough chamber for 20 min up to the distance of 80 mm and the spots were visible at 254, 366 nm and after spraying 10 % H_2SO_4 450 nm wavelengths.

RESULT AND DISCUSSION

Standardization expression is used to describe all measures, which are taken during the manufacturing process and quality control leading to a reproducible quality. It also encompasses the entire field of study from birth of a plant to its clinical application[13]. The process of standardization can be achieved in a stepwise manner by using standard pharmacognostical methods. Medicinal plant materials are categorized according to sensory, macroscopic and microscopic characteristics. An examination to determine these characteristics is the first step towards establishing the identity and degree of purity of such materials [7].

Althaea rosea seeds are small to moderate size, approximately 6 mm, usually brownish black, reniform, rugose, hairy at margin; become mucilaginous when soaked in water. Microscopic study of T.S. of seed shows testa thinly pulpy; an outer multicellular layer comprising of outer most thick walled epidermis; this is followed by several layers of parenchymatous cells; the inner epidermis of testa also thick. Tegmen is two layered; outer tegmen 4-5 cells deep and inner tegmen shows the row of palisade like malphighian cells followed by a slightly thick walled, nonlignified two layered hypodermis of cells. Endosperm cells filled with starch grains which are polygonal to round, 5 to 20 µm in size.

Powder study of *Althaea rosea* seeds showed brownish black in colour, odorless, mucilaginous and sweetish taste; shows patches of parenchyma with mucilage and starch grains polygonal to round. A small amount of powder when treated with 1 % ruthenium red, powder becomes pink in colour showing the presence of mucilage.

Physicochemical values such as extractive values, ash values, loss on drying, pH value, and swelling index of *Althaea rosea* seed were determined. The extractive value is used to determine the amount of active constituents and the ash values are used to determine the extraneous matter like sand and soil adhering to the plant surface. Swelling index is an important parameter as many herbal materials

of specific therapeutic or pharmaceutical utility because of their swelling properties[7]. Using these standards, the seed can be differentiated from other related species of seeds. The results of physicochemical parameters are summarized in table 1.

Table 1: Physicochemical results of seeds of *Althaea rosea*

	Physicochemical parameters	Value
A.	Individual extractive values cold extract	% Extractable matter
1.	Petroleum ether extract	5.2 ± 0.49 %
2.	Chloroform cold extract	6.2 ± 0.15 %
3.	Methanol cold extract	5.8 ± 0.46 %
4.	Aqueous extract	5.6 ± 0.26 %
B.	Individual extractive values hot extract	% Extractable matter
1.	Petroleum ether extract	8.42 ± 0.63 %
2.	Chloroform extract	8.08 ± 0.30 %
3.	Methanolic extract	9.83 ± 0.23 %
4.	Aqueous extract	16.0 ± 0.95 %
C.	Successive extraction	% Extractable matter
1.	Petroleum ether extract	8.18 ± 0.85 %
2.	Chloroform extract	2.76 ± 0.12 %
3.	Methanol extract	3.63 ± 0.32 %
4.	Aqueous extract	11.24 ± 0.14 %
D.	Ash value	% Ash
1.	Total ash	7.3 ± 0.32 %
2.	Acid insoluble ash	1.48 ± 0.16 %
3.	Water soluble ash	3.33 ± 0.24 %
E.	Loss on drying in crude drug (%)	8.2 ± 0.38 %
F.	pH of the drug 1%	7.18± 0.001
G.	pH of the drug 10%	7.13 ± 0.001
H.	Swelling index	5.3 ± 0.16 ml

The preliminary phytochemical screening was carried out using the extracts to determine the presence of different types of chemical constituents, which are responsible for various therapeutic effects. Therefore, the extracts were analyzed for detection of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, proteins and amino acids. Presence and absence of different phytoconstituents are presented in table 2.

Table 2: Phytochemical screening of individual extracts of *Althaea rosea* seed

Constituents	**Extracts**			
	Petroleum ether	**Chloroform**	**Methanolic**	**Aqueous**
Alkaloids	-	-	+	+
Carbohydrates	-	+	+	+
Phenolic compounds	-	-	+	+
Flavonoids	-	-	+	+
Proteins and amino- acids	-	-	+	-
Glycosides	-	-	+	-

Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material which is an important parameter of pharmacognostical evaluation[14]. Results of fluorescence analysis of powdered drug with distilled water, Dil.HNO_3, Dil. H_2SO_4, Dil. HCl, ethyl acetate, 5% ferric chloride, ammonia, methanol, chloroform, petroleum ether, 10% aq. NaOH and glacial acetic acid gave different characteristic colour under ordinary light and under ultraviolet (254 and 366 nm) are summarized in table 3.

Table 3: Fluorescence behavior of crude drug powder with different chemical reagents

S. No.	Treatment	Day light	UV light 254 nm	UV light 366 nm
1.	Powder + distilled water	Dark brown	Light yellow	Greenish yellow
2.	Powder + Dil.HNO_3	Yellowish green	Brown	Green
3.	Powder + Dil. H_2SO_4	Brown	Dark brown	Black
4.	Powder + Dil. HCl	Brown	Dark brown	Brownish green
5.	Powder + Ethyl acetate	Turbid	White	Transparent
6.	Powder + 5% Ferric chloride	Brown	Blackish brown	Light green
7.	Powder + Ammonia	Greenish brown	Light yellow	Light brown
8.	Powder + Methanol	Transparent	White	Transparent
9.	Powder + chloroform	Turbid	Brown	Black
10.	Powder + petroleum either	Turbid	White	Transparent
11.	Powder + 10% Aq. NaOH	Dark brown	Brownish yellow	Greenish yellow
12.	Powder + Glacial acetic acid	Light brown	Cream	grey

Effect of crude powder of *Althaea rosea* seed with different chemical reagents such as 10% Aq. NaOH, Con.HNO_3, Con. H_2SO_4, Con. HCl, Iodine, glacial acetic acid and picric acid (saturated) exhibited blackish brown, orange, reddish brown, dark brown, dark maroon, light brown and yellowish green respectively (Table 4).

Table 4: Powdered drug reaction with different reagents

S. No.	Chemical treatment	Observation
1.	Powder as such	Dark brown
2.	Powder + 10% Aq. NaOH	Blackish brown
3.	Powder + Con.HNO3	Orange
4.	Powder + Con. H2SO4	Reddish brown
5.	Powder + Con. HCl	Dark brown
6.	Powder + Iodine	Dark maroon
7.	Powder + Glacial acetic acid	Light brown
8.	Powder + Picric acid (saturated)	Yellowish green

Many of the compounds like phenolic and flavonoid serve as antioxidants or play other important roles in maintaining the health. Besides this, some other important roles of these compounds are anti-allergic, anti-inflammatory, antimicrobial, anticancer activities, etc.[15]. Therefore, quantification of these compounds is important in determination of quality of drug. Thus, quantitative analysis of phenolic and flavonoid content of *Althaea rosea* seed were

carried out by UV spectroscopic method. The amount of phenolic content of 10 mg/ml of crude powder of *Althaea rosea* seed was calculated with the help of standard calibration curve (fig. 1) and found to be 0.5 % w/w. The amount of flavonoid content of 10 mg/ml of crude powder of *Althaea rosea* seed was calculated with the help of standard calibration curve (fig. 2) and found to be 0.24 % w/w.

Fig. 1: Calibration curve of standard catechin for total phenolic contents

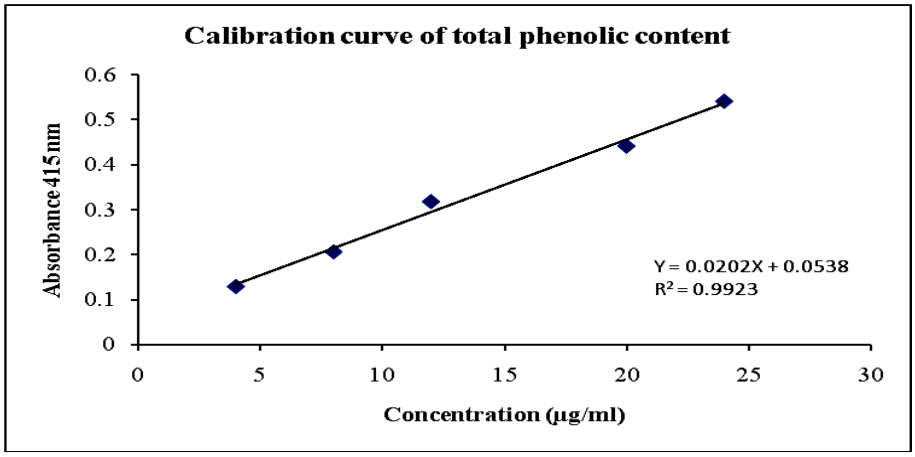
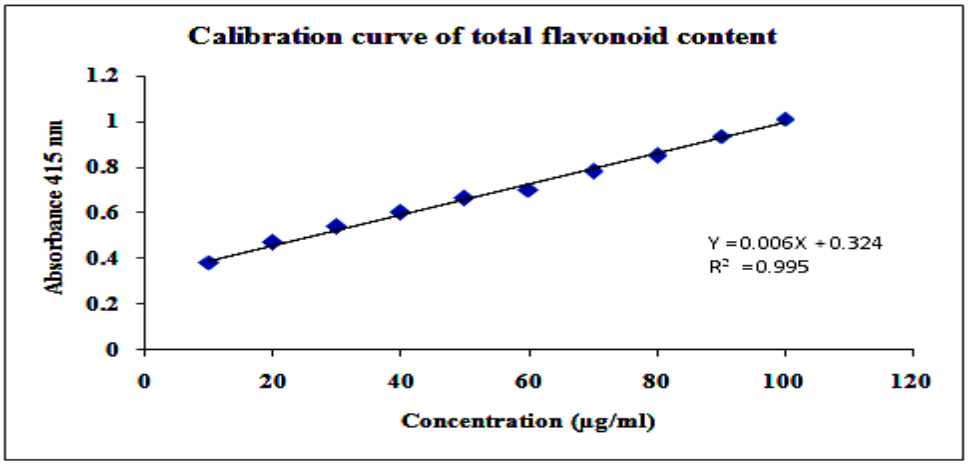


Fig. 2: Calibration curve of standard rutin for total flavonoid content



HPTLC is one of the most widely used modern, sophisticated separation technique to establish reference fingerprints of herbs, against which raw materials can be evaluated and finished products can be assayed. HPTLC results indicate the number of constituents and further facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds. HPTLC finger printing analysis of methanolic extract of *Althaea rosea* and its chloroform fraction was carried out at 254 nm, 366 nm and after spraying 10% H_2SO_4 at 450 nm using different solvent systems as given in table 5 and 6.

Table 5: Results of HPTLC fingerprint analysis of methanolic extract of *Althaea rosea* at 254, 366 and 450 nm.

Solvent system	**No. of peak observed (Rf values)**		
	254 nm	**366 nm**	**450 nm after spraying 10 % H_2SO_4**
Tolune : Ethyl acetate (9 : 1)	0.11, 0.15, 0.19, 0.31, 0.35, 0.53, 0.57, 0.72	0.11, 0.17, 0.33, 0.46, 0.56, 0.74	0.11, 0.15, 0.18, 0.24, 0.32, 0.36, 0.43, 0.52, 0.57, 0.59,0.61, 0.79, 0.81, 0.84

Table 6: HPTLC fingerprint of chloroform fraction of methanolic extract of *Althaea rosea* at 254, 366 and 450 nm.

Solvent system	**No. of peak observed (Rf values)**		
	254 nm	**366 nm**	**450 nm after spraying of 10 % H_2SO_4**
Tolune : Ethyl acetate: Formic acid (8.5 : 1.5: 2 drops)	0.11, 0.16, 0.20, 0.22, 0.29, 0.35, 0.52, 0.59, 0.64, 0.69, 0.73, 0.93	0.11, 0.15, 0.21, 0.61, 0.84, 0.93	0.10, 0.18, 0.22, 0.31, 0.34, 0.39, 0.44, 0.60, 0.75, 0.82, 0.90, 0.94

HPTLC fingerprint of methanolic extract of *Althaea rosea* showed 8 spots in Toluene: Ethyl acetate (9: 1) at 254 nm. At 366 nm and after spraying 10% H_2SO_4 at 450 nm it showed 6 and 14 spots respectively. HPTLC fingerprint of chloroform fraction of methanolic extract of *Althaea rosea* showed 12 spots in Tolune: Ethyl acetate: Formic acid (8.5 : 1.5: 2 drops) at 254 nm. At 366 nm and after spraying 10% H_2SO_4 at 450 nm it showed 6 and 12 spots respectively. Fig. 7 shows developed chromatogram and fig. 8-10 show the 3D view of methanolic extract of *Althaea rosea* at 254, 366 and 450 nm. Fig. 11shows developed chromatogram and fig. 12-14 show the 3D view of chloroform fraction of methanolic extract of *Althaea rosea* at 254, 366 and 450 nm.

Fig. 3:T. S. of seed of *Althaea rosea*

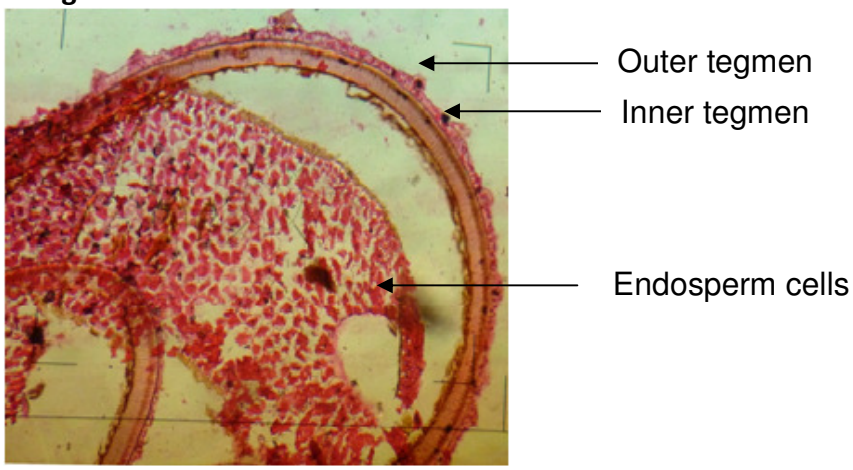


Fig. 4:T. S. of tegmen of seed of *Althaea rosea*

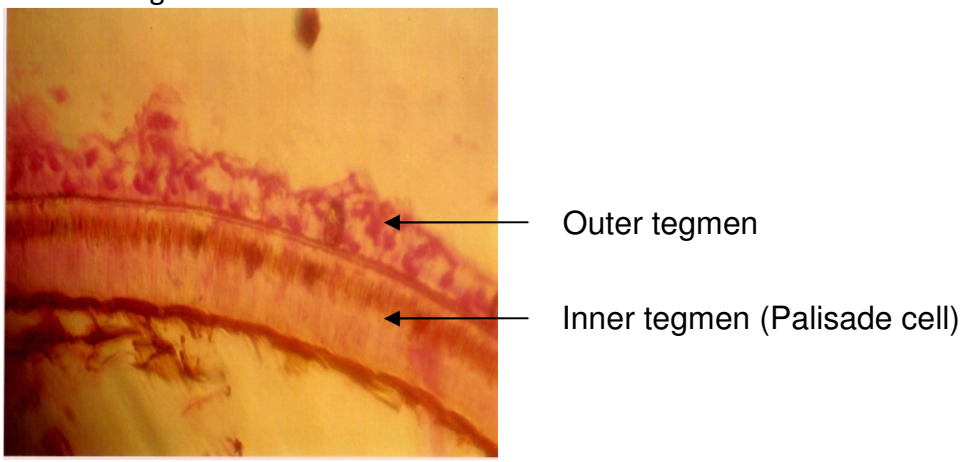


Fig. 5: T. S. of testa of seed of *Althaea rosea* seed

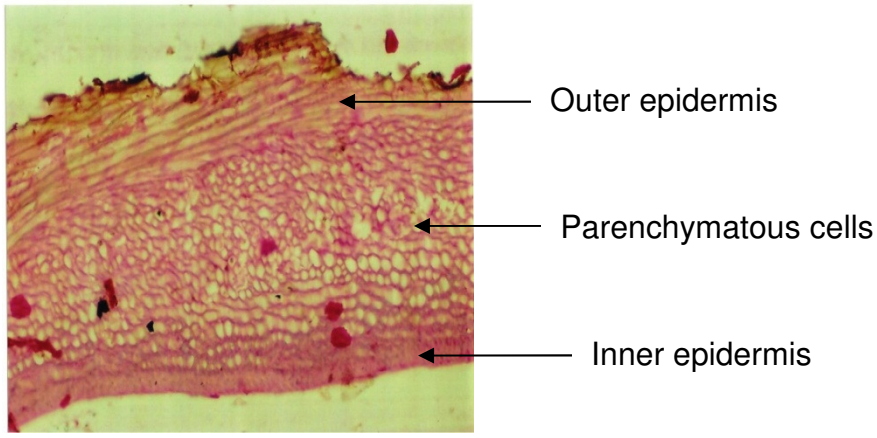


Fig. 6: T. S. of endosperm cells

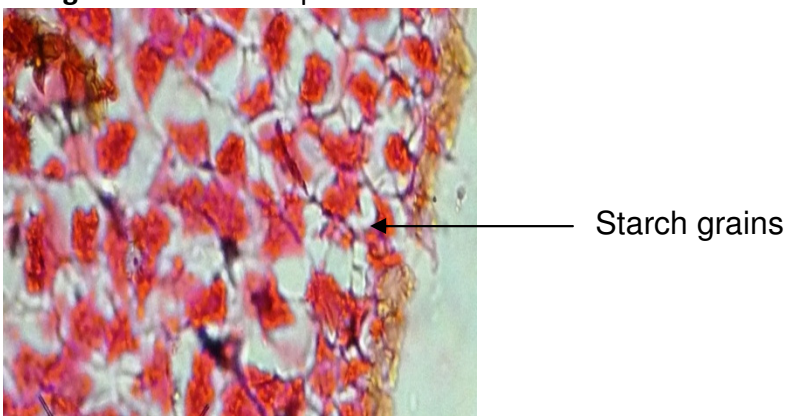


Fig. 7: Chromatogram of methanolic extract of *Althaea rosea* at 254 (A), 366 (B) and 450 (C) nm.

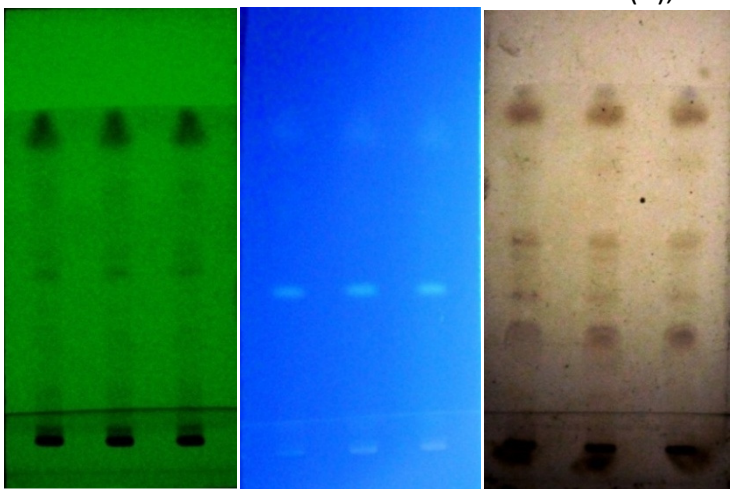


Fig. 8: 3D view of methanolic extract of *Althaea rosea* at 254 nm

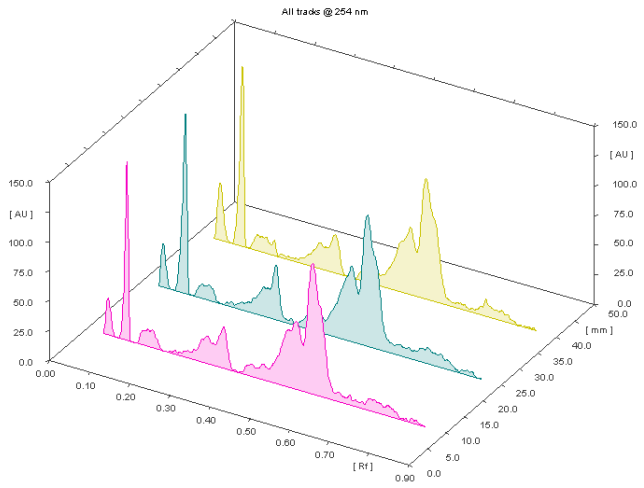


Fig. 9: 3D view of methanolic extract of *Althaea rosea* at 366 nm

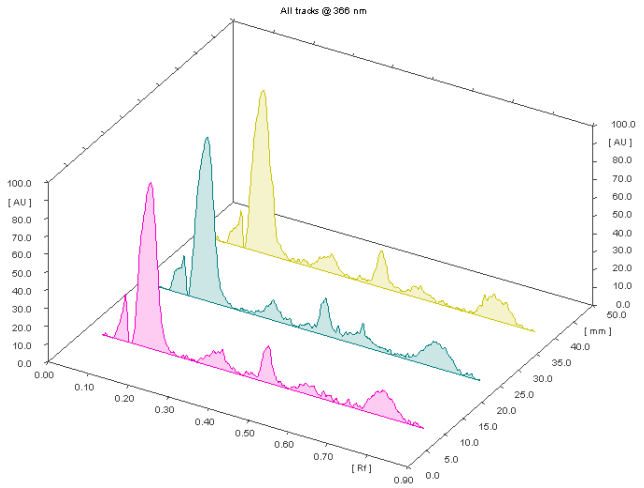


Fig. 10: 3D view of methanolic extract of *Althaea rosea* at 450 nm after spraying 10 % H_2SO_4.

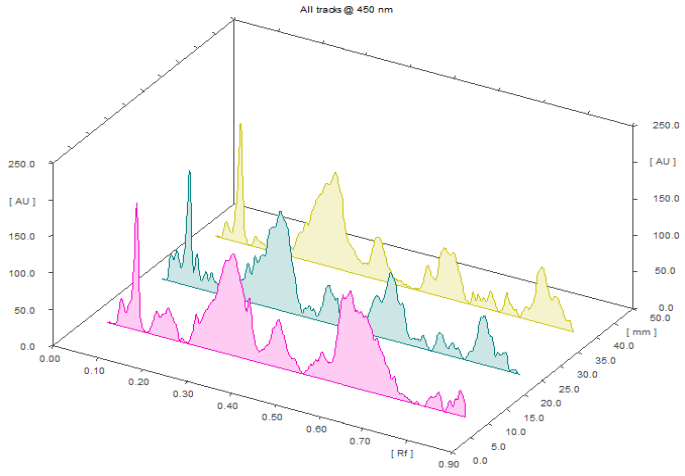
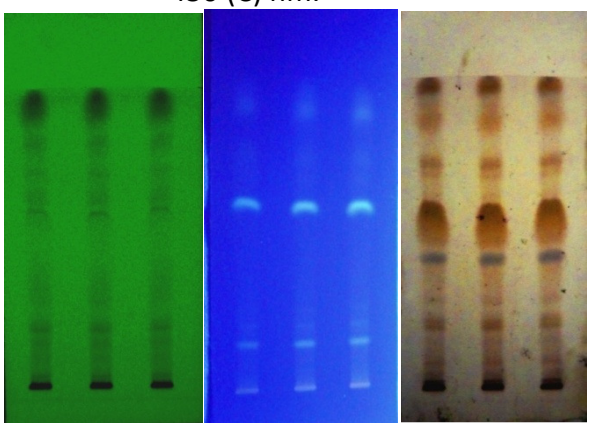


Fig. 11: Chromatograms of chloroform fraction of methanolic extract of *Althaea rosea* at 254 (A), 366 (B) and 450 (C) nm.



(A) **(B)** **(C)**

Fig. 12: 3D view of chloroform fraction of methanolic extract of *Althaea rosea* at 254 nm.

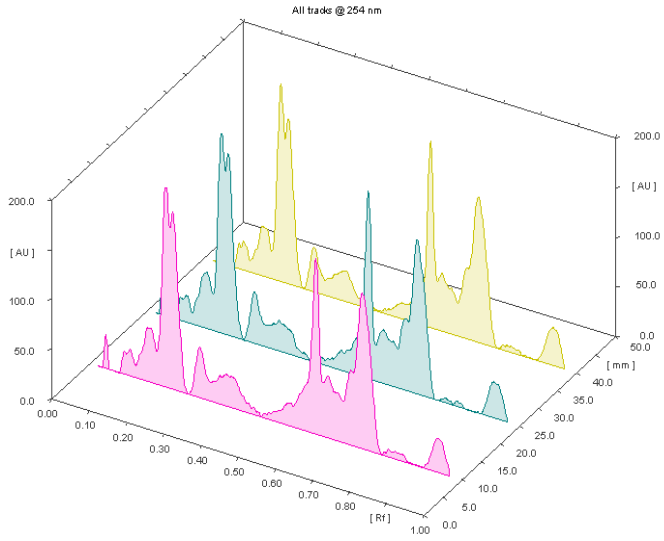


Fig. 13: 3D view of chloroform fraction of methanolic extract of *Althaea rosea* at 366 nm.

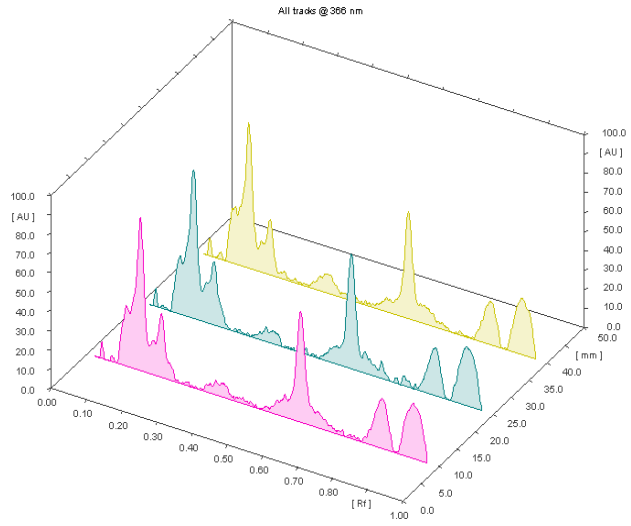
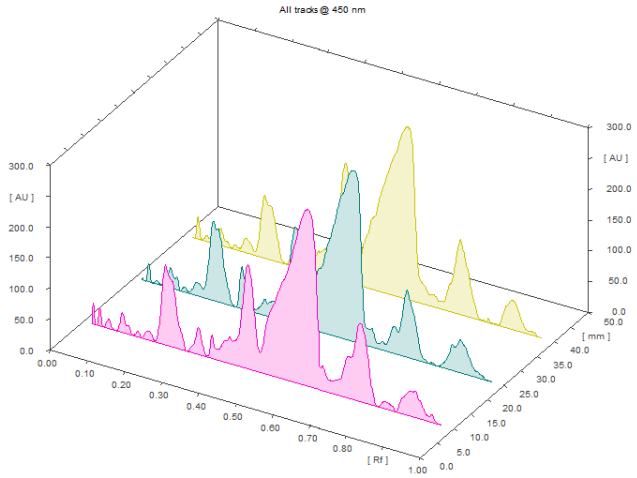


Fig. 14: 3D view of chloroform fraction of methanolic extract of *Althaea rosea* at 450 nm after spraying 10 % H_2SO_4.



CONCLUSION

In present context, traditional / herbal remedies are having a vital role in health care systems, because these drugs are easily available at low cost, safe and people have faith in them. As the usage of these herbal medicines has increased, issues regarding their quality, safety, and efficacy have raised up. The purpose of standardization of medicinal plant products is obviously to ensure therapeutic efficacy. Therefore, maintaining the quality of these plant products is an essential factor.

Althaea rosea seed is an important drug with various biological properties. Hence, efforts have been made to provide scientific data on standerdisation of *Althaea rosea* seed. The morphological, microscopic, physicochemical and chromatographic studies of this seed would help to identify and determination of its quality and purity and detection of its nature of adulteration, further these data could be utilized to standardize the plant material for further studies.

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