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Pharmacognostic Evaluation of the Flower of *Alcea rosea* L.*

Shanta Mehrotra*,†, A. K. S. Rawat and Usha Shome

Pharmacognosy Section, National Botanical Research Institute, Lucknow-226 001, India

Abstract – The flowers of *Alcea rosea* L., Malvaceae, sold in the Indian market under the trade name 'Gulkhairo', are well known for their expectorant, cooling and diuretic properties and used in many indigenous cough mixtures in India. The present paper deals with the detailed pharmacognosy of the floral parts including morphological, anatomical, phytochemical and fluorescence characters. Some of the diagnostic features of the drug are : pedicel characterized by multicellular appendages, stellate hairs, rosette crystals of Ca-oxalate, starch sheath and large sized mucilage canals; sepals having distinctive multicellular appendages arranged in a semilunar fashion present adaxially at their base; monadelphous stamens, pollen grains pentaporate provided with dimorphic spines; placentation axile, ovules campylotropous; dark green fluorescence of the powder with nitrocellulose in amyl acetate and yellow fluorescence of trichomes under Fluorescence microscope.
Key words – Pharmacognostic studies, *Alcea rosea*, Flower

Introduction

Alcea rosea L. Syn. *Althaea rosea* (Linn.) Cav., belonging to family Malvaceae is known as 'Gulkhairo', 'Khaira', 'Khitmigajhar' in Hindi; 'Simaitut' in Tamil; 'Hatmi' in 'Turkish', 'Shü kuf hüa' in Chinese and 'Hollyhock' in English. Though originally a native of China (Clapham *et al.*, 1962), it is being cultivated as an ornamental in the Indian gardens since long and forms an ingredient of various cough mixtures, pectoral syrups. Mixed with sesamum oil, the leaves and flowers are applied as poultice and for fomentation (Nadkarni, 1954). In Punjab the flowers are used in rheumatism (Kirtikar and Basu, 1933). In its native place the decoction of the flowers is used to improve blood circulation, for constipation, dysmenorrhoea, haematuria, haemorrhage and malaria (James & Ayensu, 1985). In England also the flowers are used internally or externally as emmolient & decongestant (Collins, 1979). Similarly, in America, an infusion and decoction of the flowers is used to soothe and relieve coughing, and as a refreshing drink in cases of gastritis, enteritis and cystitis (Dejey, 1977).

In view of its medicinal importance the chemical screening of the flowers has been carried out by several workers. Obara (1964) isolated a flavonoid altheanin (aromadendrin 3-glucoside) from the flowers. Meanwhile, Nair *et al.*, (1964) studied the flavonoids from major purple portions of the petals and minor yellow portions separately and identified quercetin, kaempferol, isoquercitin and kaempferol 3-glucoside pigments from yellow portion, while the purple portion contained cyanidin, glucose and rhamnose. The total anthocyanidin content of flowers was found to be 13.5% (dry basis). Subsequently, Parthasarthy and Seshadri (1965) isolated a new flavonol glycoside herbacin, from the yellow colored flowers from Srinagar, which on hydrolysis gives herbacetin (see Anon., 1985). In addition to these the flowers also yield a red dye, which may be used as an indicator in acidimetry and alkalimetry (Anon., 1948). Together with this a fair amount of work on the constituents of leaves, fruits and seeds has also been carried out (Anon., 1985) and some work on the mucilage of this plant is on record (Turowska *et al.*, 1966; Tomoda *et al.*, 1983 and 1986). However, no detailed work on the pharmacognosy is on record, hence, the present study has been carried out.

Material and Methods

The present study is based on the material collected from Banthra Research Station, NBRI, Lucknow, India in the month of March and preserved in 70%

*Author for correspondence
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alcohol for section cutting. Hand sections were cut and stained with safranin, phloroglucinol followed by a drop of c-HCl, iodine, ferric chloride and a solution of ruthenium red in 10% lead acetate to detect lignin, starch, tannin and mucilage respectively. Phytochemical studies were performed with the shade dried powdered material.

Observations

Brief Taxonomic Description of the plant – *Alcea rosea* L. is erect, sparingly branched, stellately hairy, annual or biennial herb, 0.5-2.0 m in height, commonly cultivated in the gardens. Leaves alternate, lower ones long petioled, cordate or reniform, margin irregularly crenate, upper ones short petioled, smaller; flowers born in terminal racemes.

Macroscopic Characters of the Flower and Fruit

The flowers are large, white, pink or purple, short pedicelled, 7-10 cms in diameter, and in long terminal racemes (Fig. 1). Involucre cupshaped consisting of 6-9 acute segments (0.2×1.3-0.5×1.7 cm), united at the base; calyx 5-6 large, cupshaped with acute lobes (0.7×2.0-0.8×2.3 cm); corolla-5, free, broad, waxy, obcordate, white, pink or purple, finely veined (3.5×4-5.7×5.7 cm); androecium indefinite, monadelphous, staminal tube short (0.9-1.2 cm), filaments branched bearing reniform antherlobes; carpels numerous, syncarpous, ovary many celled, style as many as the ovary cells, united at the base, separate and filiform above, stigma recurved; fruit schizocarpic, schizocarps densely hairy, 2.0-2.5 cm in diameter, mericarps 30-34 per schizocarp, 6.0-7.5 mm long and 5.0-7.5 mm wide, indehiscent, black when mature, having a deep groove in its posterior side. The weight per 100 mericarps is 1.4500-1.4556 g.

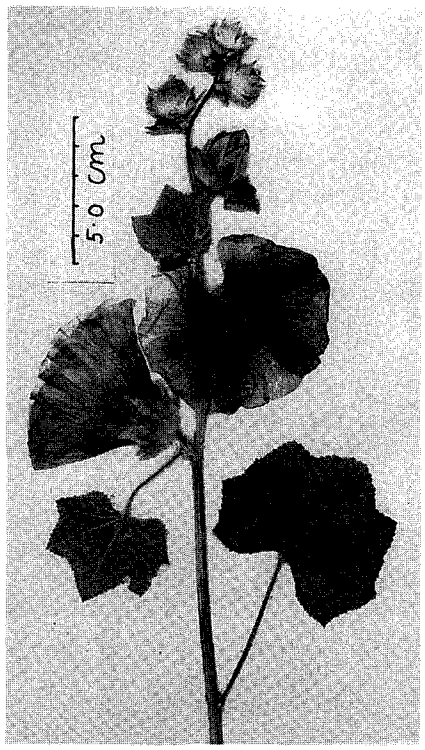

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Fig. 1. A photograph of flower showing macroscopical characters.

Microscopic Characters

Pedicel – (Figs. 2A-2E) A transection of the pedicel is almost circular in outline (Fig. 2A). The epidermis is cuticularized and formed of isodiametric cells. It is covered with multicellular appendages (31.36×50.96-27.44×78.40 μm) and stellate hairs of various sizes (111-1554 μm; Figs. 2A, 2B). Each arm of these hairs consists of two cells. The upper one being longer and free, while the lower one is short and united with the basal cells of the other arms (Fig. 2C). All these type of hairs and multicellular appendages (Fig. 2D) when studied under Fluorescence microscope, fluoresced yellow. Occasionally the stomata are observed on the surface of the pedicel (Fig. 2E).

The cortex is formed of isodiametric, collenchymatous cells having very prominent cellulosic thickenings in the outer 4 or 5 layers (Fig. 2B-a). The cells of innermost three or four layers contain starch grains forming the starch sheath, which delimits stellar tissue from the cortex (Fig. 2B-b).

The phloem is very well developed and present in the form of a concentric cylinder. The xylem is centrifugal and formed of vessels, tracheids and parenchyma. In between the two, there is a mild suggestion of vascular cambium (Fig. 2B-c). The well developed pith consists of large isodiametric collenchymatous cells containing large sized mucilage canals (54.88×54.88-98.00×113.68) (Fig. 2B-d). The rosette crystals of calcium oxalate (19.60×19.60-27.44 ×27.44 μm) are scattered in almost all the tissues of the pedicel (Figs. 2A and 2B).

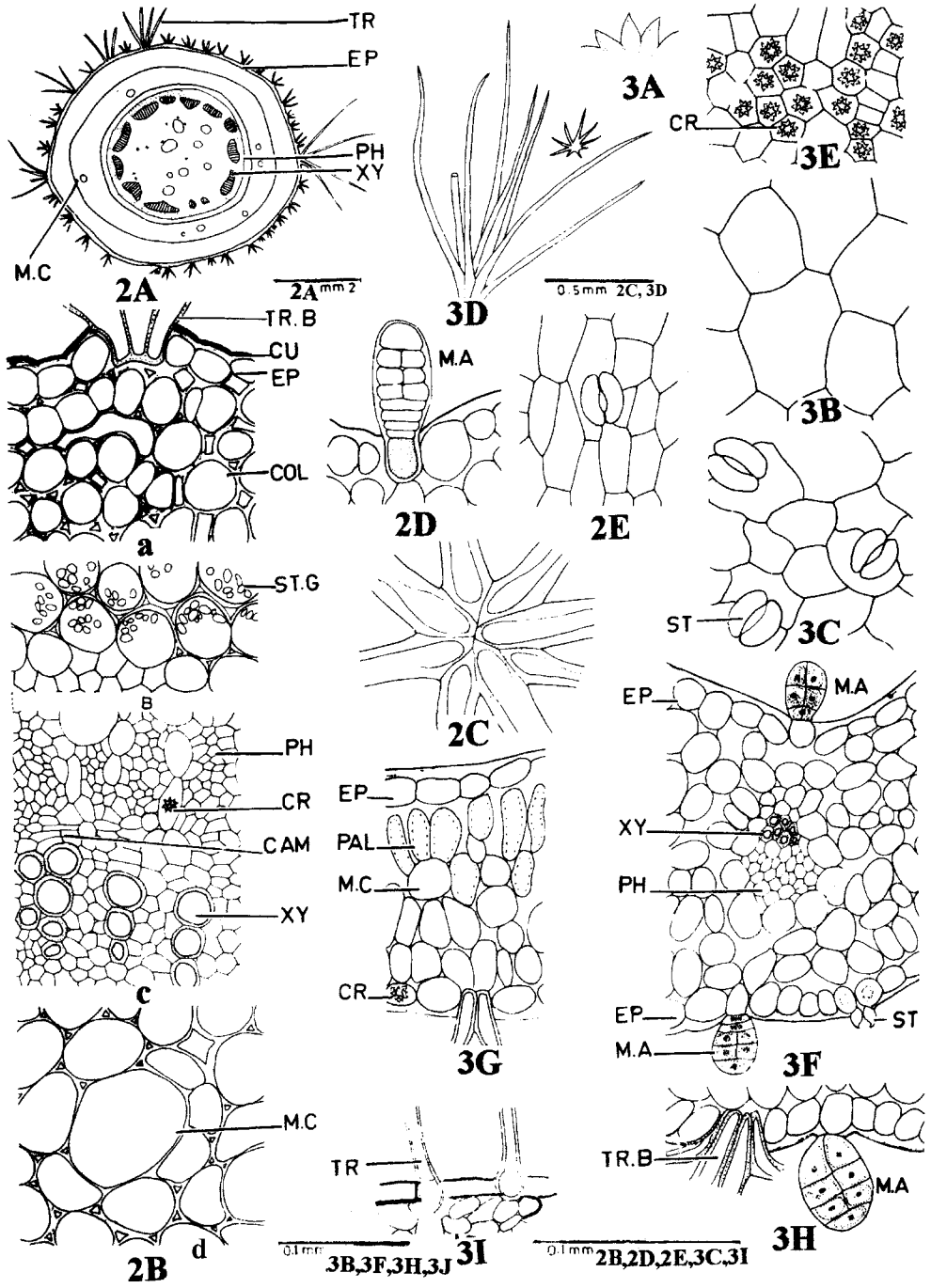

Fig. 2A. T. S. Pedicel (Diagrammatic). Fig. 2B. A portion of transverse section through the pedicel showing details. Fig. 2C. Basal portion of the hair showing the union of basal cells with each other. Fig. 2D. A portion of t. s. Pedicel showing a multicellular appendage. Fig. 2E. Epidermal cells of the pedicel showing a stomata.

Fig. 3A. Epicalyx lobes. Fig. 3B. upper epidermal cells of the epicalyx. Fig. 3C. Lower epidermal cells of the epicalyx showing diacytic and anisocytic stomata. Fig. 3D. Small and large stellate hairs from epicalyx. Fig. 3E. Hypodermal cells from epicalyx showing rosettes of Ca-oxalate crystals. Fig. 3F-3I. T. S. Epicalyx showing details.

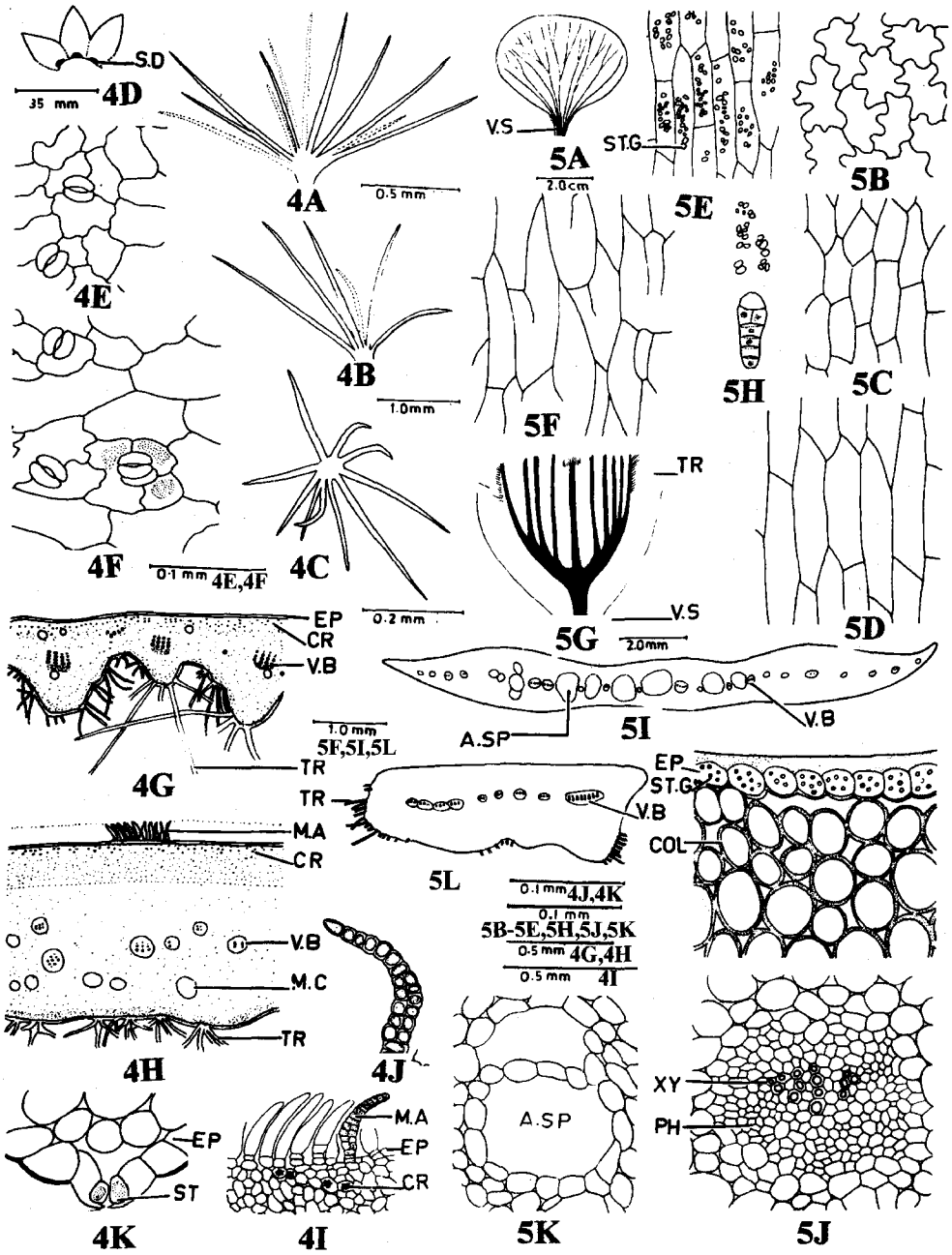

Fig. 4A-4C. Stellate hairs of various sizes from sepal. Fig. 4D. Calyx lobes showing semilunar discs. Fig. 4E.and 4F. Lower and upper epidermal cells of the sepal respectively. Fig. 4H.and 4G. Transverse sections of the sepal from its base and middle respectively (Diagrammatic). Fig. 4I. A portion of the figure 4H magnified showing multicellular appendages. Fig. 4J. A multicellular appendage. Fig. 4K. A portion from transection of calyx (abaxial side) magnified showing a stoma.

Fig. 5A. A petal. Fig. 5B-5D. Lower epidermal cells of a petal from its apical, middle and basal regions respectively. Fig. 5E. Upper epidermal cells of a petal from its middle showing starch grains. Fig. 5F. A portion of the petal magnified showing its vasculature. Fig. 5G. Base of the petal showing its vascular supply. Fig. 5H. A multicellular appendage from the petal. Fig. 5I. T. S. Petal from its middle (Diagrammatic). Fig. 5J. A portion from figure 5I magnified. Fig. 5K. A portion from figure 5I showing air spaces highly magnified. Fig. 5L. T. S. Petal from its base.

Epicalyx – (Figs. 3A-3I) The epicalyx is formed of 6-9 large, acute lobes, which are free above and united below (Fig. 3A). In a surface view the cells of the upper epidermis of epicalyx are large, penta to hexagonal (Fig. 3B) while those of lower epidermis comparatively smaller (Fig. 3C). The trichomes are of two types, uniseriate and stellate, the former being present only on the adaxial side and the latter-small as well as the larger ones-are confined to adaxial surface forming a thick mat (Figs. 3D, 3G, 3I). The stomata which are present only on the lower surface are usually anisocytic, (19.60×23.58-23.58×37.24 μm) and abundant and can be seen only after scrapping the stellate hairs (Fig. 3C).

A transection through epicalyx from its central portion shows that the multicellular appendages (Figs. 3F-3H) are present on both the surfaces. The mesophyll consists of loosely arranged parenchymatous cells (Fig. 3F). However, on the margins it is differentiated into well defined palisade present on the adaxial side and loosely arranged parenchymatous cells on the abaxial side (Fig. 3G). The vascular bundles are collateral (Fig. 3F) and are present in the middle. Rosette crystals of calcium oxalate (11.76 ×11.76-27.44×27.44 μm) are present in the hypodermal and epidermal layers (Figs. 3E, 3G).

Sepals – (Figs. 4A-4K) The sepals, which are almost identical to epicalyx lobes, except the size, are covered with trichomes of two types (i) large, uniseriate and cylindric present only on the adaxial surface in the upper half and (ii) stellate hairs of various sizes present only on the abaxial side with 5-12 arms radiating out in various directions (Figs. 4A-4C). In addition to these a very characteristic type of multicellular appendages (31.36×94.08-30.09×138.16 μm) arranged in semilunar fashion are present adaxially at the base of each sepal (Fig. 4D).

Cells of the upper epidermis are twice as large as those of the lower surface. The stomata, present on both the surfaces are anisocytic-diacytic (Figs. 4E and 4F).

The transection of a sepal in its basal region shows the multicellular appendages (Figs. 4I and 4J) arranged in a row adaxially and stellate hairs all along the lower epidermis. The ground tissue is differentiated into a much denser zone just below the multicellular appendages and is full of starch grains. The rest of the ground tissue consists of loosely arranged parenchymatous cells having mucilage canals. The vascular bundles are numerous and arranged in the middle of the ground tissue. The rosette crystals of calcium oxalate are present in abundance (15.68×15.68-23.58×23.58 μm) (Figs. 4H and 4I).

The transection passing through the middle region of a sepal shows ridges and furrows abaxially, while the adaxial side is smooth (Fig. 4G). The upper epidermis is highly cuticularized and formed of rectangular cells. The hypodermal layer is characterized by the presence of Ca-oxalate rosettes almost in each cell. The ground tissue consists of aerenchymatous tissue in the upper half and compactly arranged parenchymatous tissue on the lower side, which is again covered by stellate hairs. The vascular bundles are present in a median row. Slightly raised stomata can also be seen on adaxial side (Fig. 4K).

In the apical region grooves and ridges are not very conspicuous. The upper epidermal layer is thinly cuticularized and interrupted at several places by the foot cells of long uniseriate trichomes. The ground tissue is compact in this region. The vascular bundles are represented by a few longitudinally or transeverely cut tracheids.

Petals – (Figs. 5A-5L) The petals are broad, reticulately and finely veined, obcordate, (Figs. 5A and 5F). Cells of the upper as well as lower epidermis are alike. These are elongated in the basal region and penta or haxagonal a little higher up (Figs. 5C, 5D). However, the cells in the upper half of the petal are almost as long as broad with their anticlinal walls undulated (Fig. 5B). Simple starch grains are present in the basal region of the petal (Fig. 5E).

Long unicellular trichomes are arranged on either side of the claw and in its middle (Fig. 5G). Multi--cellular appendages (19.60×64.68-27.44×74.4 μm), which fluoresce yellow are present sparsely on both the surfaces of the petal (Fig. 5H).

In the basal region just above the claw the petal in transection shows vascular bundles arranged in a median row (Figs. 5I and 5L) usually alternating with the large air spaces (Figs. 5I and 5K). On either side of the vascular bundles the ground tissue is differentiated into an outer collenchyma and an inner parenchyma (Fig. 5J). The vascular bundles are represented by a few xylem elements which consist of mainly annular or spiral elements. Even scalariform tracheids have been observed.

Androecium – (Figs. 6A-6D) The stamens are monadelphous. The bases of filaments are united to form a staminal sheath, although the apices are

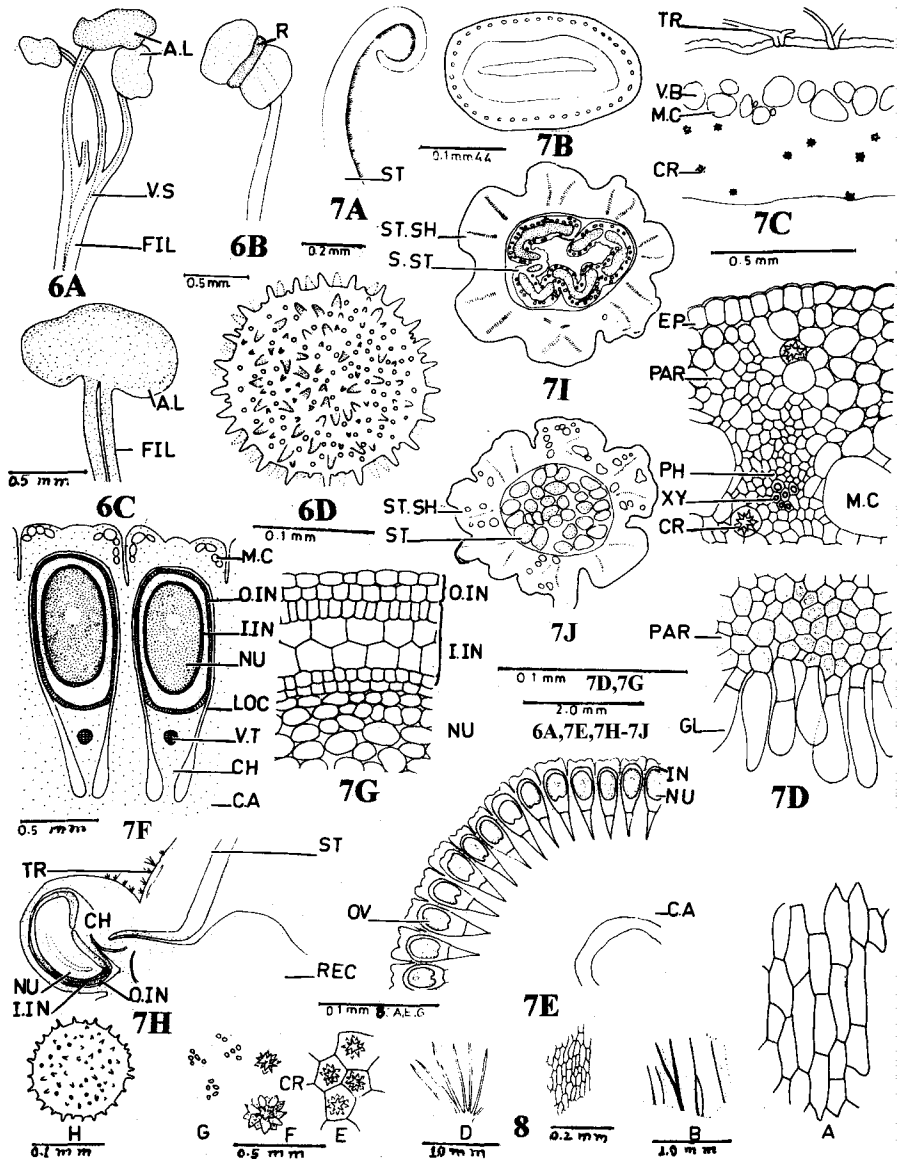

Fig. 6A. Branched filament showing anther lobes. Fig. 6B. A dehisced anther lobe showing the ridge. Fig. 6C. A typical reniform anther lobe. Fig. 6D. A pollen grain.

Fig. 7A. Curved tip of the style. Fig. 7B. Transverse section of the style at its base. Fig. 7C. An outer portion of figure 7B slightly magnified. Fig. 7D. A portion of figure 7B highly magnified. Fig. 7E. Transverse section of the ovary showing ovules. Fig. 7F. A portion from figure 7E magnified showing the details of the ovule. Fig. 7G. A portion of ovular wall highly magnified. Fig. 7H. Longitudinal section of the ovary showing the ovule. Fig. 7I.and 7J. Transverse sections of staminal sheath from its middle and apex showing separating & separated styles respectively.

Fig. 8A-8H. Different tissues from the drug powder.

branched (Fig. 6A). The antherlobes (370×740-481×1591 μm) are reniform and dehisce by slit running across the top and dividing the anther lobes into two halves (Figs. 6B and 6C). The pollen grains are large, pentaporate provided with dimorphic spines, and measuring 150.87×151.99 μm (124.92×124.92-166.56×166.56 μm) (Fig. 6D).

Gynoecium – (Figs. 7A-7J) The carpels are numerous, syncarpous, arranged in a whorl around the central axis. The styles are free above with their apices

curved and united at the base (Figs. 7A and 7B). The transections of the style in its basal portion, where it is united, shows two clearly demarcated tissues. The outer one having a ring of mucilage canals and vascular bundles embedded in the ground tissue consisting of loosely arranged parenchymatous cells (Figs. 7B-7D). The inner one is formed of hexagonal cells having nuclei full of contents (Fig. 7D). The inner most cells of the tissue are drawn out into glandular cells as is also reported in several numbers of Cactaceae (Maheshwari, 1950). The transections from still higher levels show that the styles are getting gradually separated (Figs. 7I and 7J).

Each carpel is one ovuled and is marked by a fissured contour on the dorsal wall in transection (Figs. 7E and 7F). On the angles of the fissure are found mucilage canals. The ovules are campylotropous (Fig. 7H). The vascular supply can be seen in the chalazal region (Fig. 7F).

The ovule or young seed has two integuments i. e. outer consisting of two layers forming the testa and inner 4 to 5 layered forming the tegmen in the seed. The median layers of the inner integument are multiplicative in the seed (Fig. 7G).

Study of Powder – (Figs. 8A-8G) The powder of the whole flower is yellowish brown in color with no specific odor and taste. The powder was sieved through No. 40 mesh, cleared in chloral hydrate and mounted in glycerine. A microscopic examination revealed the following elements (i) a piece of tissue from the basal portion of the petal (Figs. 8A and 8C) (ii) a tissue again from the petal showing the vasculature in low magnification (Fig. 8B) (iii) stellate hair (Fig. 8D) (iv) rosettes of Ca-oxalate crystal (Figs. 8E and 8F) (v) starch grains (Fig. 8G) and (vi) pollen grains (Fig. 8H).

The behavior of the powdered drug with different chemical reagents was also studied as per methods described by Chase & Pratt (1949) and Kokoski *et al.*, (1958) and the results presented in the Table 1.

Phytochemical Studies

Air dried material was used for quantitative determination of ash value, sugar, tannin and successive extractive percentages. The recommended procedures were followed for calculating total ash percentage (Anon, 1966), whereas tannins (calculated as gallotannins), total sugars were calculated as per procedures prescribed by AOAC (Anon, 1965). The values obtained are recorded in Table 2.

For phytochemical studies a known quantity of dried powder was extracted in Soxhlet with hexane, chloroform, alcohol and water successively and tested for different constituents (Peach and Tracy, 1955) viz. steroids and triterpenoids (LB test), flavonoids (Shinodas test), alkaloids (Mayer's reagent) tannins (ferric chloride test) and sugars (Fehling solution test). The study revealed that the alkaloids are present in chloroform and alcoholic extractives and flavonoids and tannins in ethanol extractive only. On the contrary resins and steroids are indicated in the hexane extractive.

Thin layer chromatography of different extracts was also performed to characterize the drug and hRf values were calculated and recorded in Table 3.

Table 1. *Alcea rosea* Fluorescence analysis of the powder

Sl. No.	Treatment	Color under day light	Fluorescence under U.V. Light (365 nm.)
1.	Drug powder as such	Light brown	Dark green
2.	Powder + nitrocellulose in amyl acetate	Dark brown	Dark green
3.	Powder + 1N NaOH (Methanolic)	Brown	Dark green
4.	Powder + 1N NaOH (Methanolic)+nitrocellulose in amylacetate	Yellowish brown	Greenish brown
5.	Powder + 1N NaOH (Aq)	Brown with yellowish tinge	Purple with greenish tinge
6.	Powder + 1N NaOH (Aq)+nitrocellulose in amylacetate	Light brown	Green
7.	Powder + 1N HCl	Yellowish brown	Light bottle green
8.	Powder + 1N HCl + nitrocellulose in amylacetate	Yellowish brown	Light green with purple tinge
9.	Powder + 50% HNO_3	Brown	Dark bottle green
10.	Powder + 50% H_2SO_4	Brown	Dark green

Table 2. *Alcea rosea* Physico-chemical values

Sl.No.	Values	Range	Average
1.	Loss in drying at 105°C	77.72-79.34	78.53%
2.	Total ash	10.84-11.94	11.39%
3.	Total phenolics	1.52-1.56	1.54%
4.	Sugar by Shaffer's Somogyi method	0.580-0.587	0.583%
5.	Hexane extract	0.848-1.00	0.924%
6.	Chloroform extract	0.42-0.868	0.644%
7.	Alcohol extract	2.95-3.136	3.043%
8.	Water extract	16.808-19.87	18.339%

Table 3. *Alcea rosea* L.-hRf values of the different extracts

Extractive	Solvent system	hRf values
Hexane extract	hexane: chloroform: acetic acid 60 : 140 : 0.3	2.1, 8.5, 4.9, 6.3, 8.3, 13, 15, 20.4, 57, 66, 74, 75, 79, 83, 88, 91.
Chloroform extract	chloroform : methanol : acetone 170 : 20 : 10	4, 15, 18, 21, 38, 47, 55, 69, 73
Chloroform extract	chloroform : methanol : acetone : formic acid 50 : 3.5 : 2.5 : 0.2	5, 20, 25, 55, 61, 77
Alcohol extract	ethylacetate : formic acid : glacial acetic acid : water 100 : 11 : 11 : 27	9, 23, 33, 35, 38, 44, 62

Hexane extract – The hexane extractive was first run on silica gel GF-254 precoated plates using hexane, chloroform and acetic acid (60 : 140 : 0.3) as a solvents system for 6 cms. The plate was air dried and again run in the same solvents but in different proportions (72 : 128 : 0.3) for 14 cms. Fourteen spots, of varying colors were obtained. Out of these 8 spots were present near the point of origin, one in the middle and 5 near the solvent front (Fig. 9A).

Chloroform extract – The chloroform extractive was eluted in two different solvent systems (viz. chloroform: methanol: acetone: formic acid -50 : 3.5 : 2.5 : 0.2 and chloroform: methanol: acetone 170 : 20 : 10) resulting in 6 and 9 distinct spots, respectively (Figs. 9B and 9C).

Alcohol extract – Seven distinct spots of brown, yellow and purple colors were obtained when the alcohol extractive was eluted with ethylacetate: formic acid: glacial acetic acid and water (100 : 1 : 11 : 27) as the solvent system (Fig. 9D).

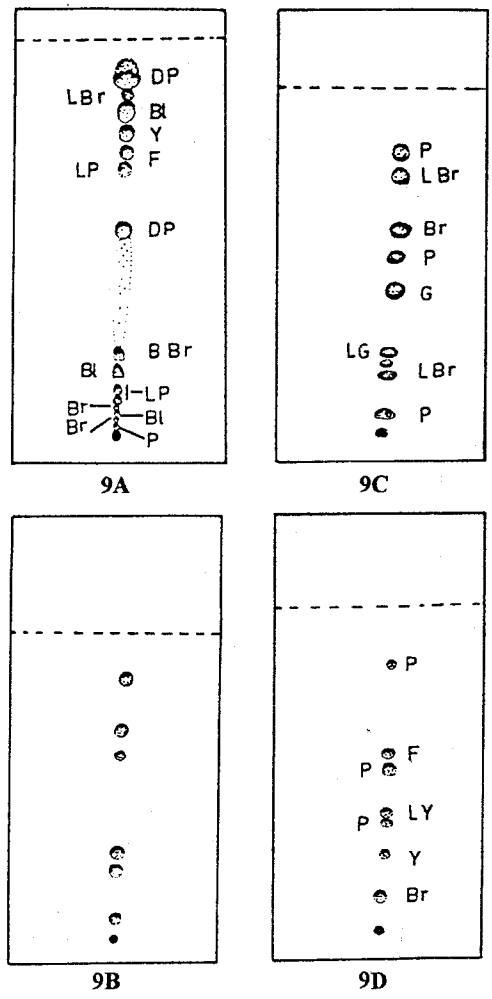

Fig. 9A, hexane extract; Figs. 9B and 9C, chloroform extract; 9D, alchohol extract.

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