



Pharmacognostical and Phytochemical Study of Kokilaksh (*Astercantha longifolia*)

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Pharmacognosy is the study of naturally occurring biological substances, principally those derived from plants, that find use in medicine. The word 'pharmacognosy' is derived from the Greek 'pharmacon', 'a drug' and gignosco, 'to acquire knowledge of'. It is closely related to both botany and plant chemistry and, both originated from the earlier scientific studies on medicinal plants. As late as the beginning of the 20th century, the subject had developed mainly on the botanical side, being particularly concerned with the description and identification of drugs, both in the whole state and in powder and with their history, commerce, collection, preparation and storage. Such branches of pharmacognosy are still of fundamental importances, but rapid development in other areas, particularly phytochemistry and pharmacology, have enormously expanded the subject. As a result it is now possible to approach the study of medicinal plants from the botanical, phytochemical and pharmacological view points (Trease and Evans, 14th ed., 1997). Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value

which have yet to be discovered; large numbers of plants are constantly being screened for their possible pharmacological value.

The plant selected for the present study – *Astercantha longifolia*, of the family Acanthaceae. Here an attempt is made to study the plant pharmacognostically in this chapter. The whole plant has taken for study.

Kokilaksh consists of dried whole plant of *Asteracantha longifolia* Nees. Syn. *Hygrophila spinosa* T.Anders (Fam.Acanthaceae); a spiny, stout, annual herb, common in water logged places throughout the country.

Chief Characters: 1. Annual thorny plant. 2. Dagger shaped simple leaf. 3. Light blue coloured flowers. 4. Fruit- Capsule.

Vernacular names

Sanskrit : Kokilaksh, Ikshura, Ikshuraka

Hindi : Talmakhana, Kantakaliya

English : Long leaved barleria

Assamese : Kulekhara

Bengali : Kuliya khara, Kulekhade

Gujrati : Ekharo

Kannada : Kolavali, Kolarind, Kolavankal

Malayalam : Culli, Nirchulli, Vayalculli
 Marathi : Talikhana, Kalsunda
 Tamil : Golmidi, Kettu, Nirguvireru, Nerugobbi
 Telugu : Kokilaksamu, Nirguvi veru.
 Urdu : Talmakhana
 Traditional : Gokhula

Distribution

Common weed growing in marshy and water logged areas. Found throughout India, in ditches from Himalaya to southern states.

Parts Used-

Whole plant, root, seed, leaf

Properties and action:

Rasa : Madhura, Amla, Tikta

Guna : Picchila, Snigdha

Virya : Shita

Vipaka : Madhura

Doskaghanta : Vatapittashamaka

Rogaghanta : Vata-Rakta, Vatavyadhi, Nadidaurbalya, Kamla, Kasa, Jalodara, Yakridudara, Anaha, Udarroga, Pittashmari, Shotha, Shukradaurbalya, Kailbya, Ashmari, Mutrakrichchhra, Bastishotha, Daurbalya.

Karma : Shothahar, Mutral, Anuloman, Nadibalya, Santarpana, Ykriduttejaka, Ruchya, Stanyajanana, Vrishya, Vajikara, Shukrashodhan, Brihana, Balya

Doses : 3 -6 g of the drug in Powder form, Ash 1-3gm, Decoction-50-100 ml

Botanical Description

a) Macroscopic

Root - Mostly adventitious, whitish to brown; no characteristic odour and taste.

Stem- Usually unbranched, fasciculate, sub-quadrangular, swollen at nodes, covered with long hairs which are numerous at the nodes, externally grayish-brown, creamish brown in cut surfaces; no characteristic odour and taste.

Leaf- Greenish-brown, 1-7 cm long, 0.5-1 cm wide, sub sessile, lanceolate, acute, entire and hairy.

Flower- Purple blue, usually occurring in apparent whorls of eight (in 4 pairs) at each node; bracts about 2.5 cm long, with long white hairs; **calyx 4-** partite, upper sepal 1.6-2 cm long, broader than the other three, which are 1.3 cm long, all linear-lanceolate, coarsely hairy on the back and with hyaline ciliate margins; corolla 3.2 cm long, widely 2 lipped, tube 1.6 cm long, abruptly swollen at top; stamens 4, didynamous, second pair larger; filament quite glabrous; anthers two celled, sub equal, glabrous; ovary two celled with 4 ovules in each cell; style filiform, pubescent; stigma simple, involutes with a fissure on upper side.

Fruit- Two celled, linear-oblong, compressed, capsule about 0.8 cm long, pointed, 4-seeded.

Seed - Ovate, flat or compressed, truncate at the base, 0.2-0.25 cm long and 0.1 - 0.15 cm wide, hairy but appearing smooth; when soaked in water immediately get coated with mucilage, light brown; taste slightly bitter and odour not distinct.



Fig. of Kokilaksh

Fig. of Kokilaksh Churna

b) Microscopic**Root**

Root shows a single layered epidermis of thin-walled, rectangular to cubical, parenchymatous cells having unicellular hairs; secondary cortex composed of round to oval or oblong, thin-walled cells having large intercellular spaces; most of these cells divided longitudinally and transversely with walls forming 4-6 or more chambers; size of these cells and intercellular spaces gradually reduce towards the inner region, where these cells are mostly radially elongated, arranged in radial rows, a few thick-walled cells found scattered singly throughout secondary cortex; secondary phloem narrow consisting of small, thin-walled, polygonal cells; phloem fibres thick-walled, occur in groups of 2-6 or singles, scattered throughout the phloem region; secondary xylem forms continuous ring; vessels angular, broader towards centre, arranged radially having spiral thickenings, surrounded by thick-walled parenchyma and xylem fibres; fibre walls uniformly thickened; multi and uniseriate medullary rays occur from primary xylem region upto secondary cortex; ray cells thin walled, radially elongated in xylem region, circular to transversely elongated in phloem region.

Stem

Stem shows somewhat sub-quadrangular outline; cork consists of 5-10 rows of rectangular, radially arranged, moderately thick-walled, brownish cells; collenchyma 4-8 layered consisting of isodiametric cells; a few thick-walled, isolated cells found scattered in this zone; cortical cells thin-walled, round, oblong, variable in size, with a number of large air cavities; a special feature of these cells is the formation of tangential and radial walls within the cell dividing it into 4-5 or more parts; most of cells contain numerous acicular crystals of calcium oxalate; endodermis single layered, composed of transversely elongate, thin-walled cells; phloem narrow, consisting of round to polygonal cells, peripheral ones larger, inner cells smaller; fibres thick-walled, single or in groups of 2-3, some cells contain calcium oxalate

crystals similar to those found in cortical cells; xylem present in a ring; vessels with spiral thickenings, arranged radially; fibres elongated with wide lumen and pointed tips, medullary rays uni to multi seriate extend upto secondary cortex; ray cells thin-walled, radially elongated in secondary xylem, transversely elongated in secondary phloem; pith large, composed of polygonal, thin-walled parenchymatous cells, having small intercellular spaces; a few cells contain calcium oxalate crystals similar to those found in secondary cortex.

Leaf

Midrib- Shows concavo-convex outline; epidermis on either surface covered with thick cuticle; collenchyma 2-5 layered; stele composed of small strands of xylem and phloem having some groups of fibre; rest of tissues composed of thin-walled, parenchymatous cells, a few of them containing acicular crystals of calcium oxalate ; cystolith present beneath upper and above the lower epidermal cells. *Lamina* - Shows epidermis single layered on either surface, composed of thin-walled, parenchymatous, tangentially elongated cells, covered with thick cuticle; stomata diacytic, 1-5 celled hairs present on both surfaces; palisade 1-2 layered; spongy parenchyma composed of 3-5 layered, loosely arranged cells traversed by a number of veins; palisade ratio 6.25-15.75; stomatal index 17.24-30.78; vein islet number 17-42.

Fruit- Shows single layered epidermis covered with striated cuticle followed by 5-10 layered, thick-walled and oval to hexagonal, lignified, sclerenchymatous cells.

Seed- Shows hairy testa composed of thin-walled, tangentially elongated cells covered with pigmented cuticle; embryo composed of oval to polygonal, thin-walled, parenchymatous cells containing oil globules.

Powder- Light brown; shows aseptate, elongated fibres; vessels with simple pits and spiral thickening; palisade, acicular crystals of calcium oxalate, unicellular hairs and globules.

Physical constants: Whole plants- Foreign matter- not more than 2%, Total ash- not more than 9%, Acid- insoluble ash-not more than 1%, Alcohol- soluble extractive- not less than 4%, water – soluble extractive- not less than 20%.

Chemical constituents: Apigenin-7-0-glucuronide and 7-0glucoside (flowers), histidine, lysine, phenylalanine, linoleic, oleic, palmitic and stearic acids, xylose, uronic acid, polysaccharides, xylan, lipase, protease, saponin, sterols, asterol I,II,III, IV, astercanthine, asteracanthicine (seeds), lupeol, betulin, phytosterol, essential oil (root),lupeol, ascorbic acid, nicotinic acid(leaves), betulin, lupeol, stigmasterol, n-hydrocarbons (c27-35) {plant}, 3-methylnonacosane, 23- ethylcholesta-11 (12), 23 (24)-dien-3 beta-ol, lupeol, 25-oxohentriacontanyl acetate and methyl acetate and methyl 8- n- hexyltetracosanoate (arial parts).

Pharmacological Activities- Anti-convulsant, antineoplastic, hepatoprotective, antifungal, antispasmodic, respiratory stimulant, antibacterial, anti-inflammatory, diuretic, moderate antipyretic, hypotensive, vasodilator, anabolic cum androgen like activity, bronchodilatory, antitumour promoting activity against chemically induced hepato carcinogenesis in Wister rats. The plant is an important medicinal herb, widely distributed in India and is used by local population for different medicinal purposes. It is also used commercially as an ingredient of some Over-The-Counter (OTC) formulations used in liver disorder and those prescribed as general tonic. The herb has been reported to contain chemical constituents such as β -sitosterol, Lupeol (Saleem *et al.*, 2005; Mazumdar and Sengupta, 1978; Tiwari *et al.*, 1967), linoleic acid and oleic acid as main constituents along with other fatty acids, polysaccharides, histidine and phenyl alanine (Haq and Nabi, 1978). Lupeol and β -sitosterol both have been reported to have antipyretic (Ali, 1967), hepatoprotective (Shailajan *et al.*, 2005; Singh and Handa, 1995), antioxidant, anticancer (Ahmed *et al.*, 2001) and macrofilaricidal (Chatterjee *et al.*, 1992) activities. Roots of *Asteracantha longifolia* Nees. are used as a

diuretic (Joshi, 2000; Warriar, 1995), seeds as aphrodisiac tonic in Unani medicine (Rastogi and Mehrotra, 1991) and leaves are popularly used for hepatoprotection, against anemia and in female reproductive dysfunction. Whole plant powder of *Asteracantha longifolia* Nees. is used as a tonic against debility (Joshi, 2000; Warriar, 1995). Diuretic activity of *Asteracantha longifolia* Nees. is attributed to Lupeol (Elisandra and Diones, 2005). Lupeol is also a potential phytochemical in controlling arthritis (Geetha and Varalakshmi, 1999). It also acts as chemo preventive and immunomodulatory (Anton *et al.*, 1993). β -sitosterol has therapeutic action in female reproductive disorders (Ryokkynen *et al.*, 2005).

Formulations and Preparations

Panaviraladi bhasma (kshara), Gokshuradi modaka, Mahakameshwara modaka, Shrimadananada modaka, Brihat shatavari modaka, Rativallabha modaka, Mopharava, Brihat kushmandavaleha, Madhyama manjishthadi kwatha, Apamargadi sweda, Ras gauggulu, Paushtika churna etc.

Propagation and Cultivation

It is propagated by seeds; the plant tolerates variety of soils and can be cultivated in wet and marshy places.

Preliminary screening of Phytochemicals

The preliminary phytochemical studies were performed for testing the different chemical groups present in the drug. 10% (w/v) solution of extract was taken unless otherwise mentioned in the respective individual test.

General screening of various extracts of the plant material was carried out for qualitative determination of the groups of organic compounds present in them (Trease and Evan, 1983).

1. Alkaloids- Dragendorff's test: Dissolve a few mg of alcoholic or aqueous extract of the drug in 5 ml of distilled water, add 2 M hydrochloric acid until an acid reaction occurs, then add 1 ml of Dragendorff's reagent, an orange or orange-red precipitate is produced immediately.

Hager's test: To 1 ml of alcoholic extract of the drug taken in a test tube, add a few drops of

Hager's reagent. Formation of yellow precipitate confirms the presence of alkaloids.

Wagner's test: Acidify 1 ml of alcoholic extract of the drug with 1.5% v/v of hydrochloric acid and add a few drops of Wagner's reagent. A yellow or brown precipitate is formed.

Mayer's test: Add a few drops of Mayer's reagent to 1 ml of acidic aqueous extract of the drug. White or pale yellow precipitate is formed.

2. Carbohydrates- Anthrone test: To 2 ml of anthrone test solution, add 0.5 ml of aqueous extract of the drug. A green or blue colour indicates the presence of carbohydrates.

Benedict's test: To 0.5 ml of aqueous extract of the drug add 5 ml of Benedict's solution and boil for 5 mins. Formation of a brick red coloured precipitate is due to the presence of carbohydrates.

Fehling's test: To 2 ml of aqueous extract of the drug add 1 ml of a mixture of equal parts of Fehling's solution 'A' and Fehling's solution 'B' and boil the contents of the test tube for few minutes. A red or brick red precipitate is formed.

Molisch's test: In a test tube containing 2 ml of aqueous extract of the drug add 2 drops of a freshly prepared 20% alcoholic solution of β -naphthol and mix, pour 2 ml conc. sulphuric acid so as to form a layer below the mixture. Carbohydrates, if present, produce a red-violet ring, which disappears on the addition of an excess of alkali solution.

3. Flavonoids- Shinoda's test: In a test tube containing 0.5 ml of alcoholic extract of the drug, add 5-10 drops of dil. hydrochloric acid followed by a small piece of magnesium. In the presence of flavonoids a pink, reddish pink or brown colour is produced.

4. Triterpenoids- Liebermann-Burchard's test: Add 2 ml of acetic anhydride solution to 1 ml of petroleum ether extract of the drug in chloroform followed by 1 ml of conc. sulphuric acid. A violet colour coloured ring is formed indicating the presence of triterpenoids.

5. Proteins- Biuret's test: To 1 ml of hot aq extract of the drug add 5-8 drops of 10% w/v sodium hydroxide solution followed by 1 or 2

drops of 3% w/v copper sulphate solution. A red or violet colour is obtained.

Millon's test: Dissolve a small quantity of aqueous extract of the drug in 1 ml of distilled water and add 5-6 drops of Millon's reagent. A white precipitate is formed which turns red on heating.

6. Resins- Dissolve the extract in acetone and pour the solution into distilled water. Turbidity indicates the presence of resins.

7. Saponins- In a test tube containing about 5 ml of an aqueous extract of the drug add a drop of sodium bicarbonate solution, shake the mixture vigorously and leave for 3 mins. Honeycomb like froth is formed.

8. Steroids- Liebermann-Burchard's test: Add 2 ml of acetic anhydride solution to 1 ml of petroleum ether extract of the drug in chloroform followed by 1 ml of conc. sulphuric acid. A greenish colour is developed which turns to blue.

Salkowski Reaction: Add 1 ml of conc. sulphuric acid to 2 ml of chloroform extract of the drug carefully, from the side of the test tube. A red colour is produced in the chloroform layer.

9. Tannins- To 1 – 2 ml of plant extract, add a few drops of 5% FeCl₃ solution was added. A green colour indicates the presence of gallotannins while brown colour tannins.

10. Starch- Dissolve 0.015g of Iodine and 0.075g of Potassium Iodide in 5 ml of distilled water and add 2 – 3 ml of an aqueous extract of drug. A blue colour is produced.

11. Glycosides- Detection of glycoside on paper spray solution No. 1 (0.5 % aqueous sol. of Sodium metaperiodate) & wait for 10 minutes after then spray solution No. 2 [0.5 % Benzidine (w/v) in solution of Ethanol – acetic Acid (4:1)], white spot with blue back ground shows presence of *glycoside*.

Identity, Purity and Strength

Loss on drying at 105° C/Moisture content

Place about 10 gm of drug sample after accurately weighing it in a tared evaporating dish. After placing the above said amount of sample in a tared

evaporating dish dry at 105° C for 5 hours and weigh. After drying tared evaporating dish cool in desiccators for 30 minutes and then weight.

$$\text{The \% of Loss on drying} = \frac{\text{Difference in weight after heating} \times 100}{\text{Weight of sample taken}}$$

Determination of Ash

Determination of Total Ash

About 2.0g of powdered drug was incinerated in a tared silica dish at a temperature not exceeding 450°C until free carbon was left, cooled and final weight was taken. The percentage of ash calculated with reference to the air-dried drug (PASF, 1987).

Determination of Acid Insoluble Ash

The ash obtained as above method was boiled for 5 minutes with 25 ml of dilute hydrochloric acid and collected the insoluble matter on an ash-less filter paper, washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash with reference to the air dried drug was calculated (PASF, 1987).

Determination of Sulphated Ash

Heat a silica crucible to redness for 10 minutes; allow cooling in a desiccator and weighing. Put

about 2.0g of powdered drug into the crucible; ignite gently at first, until the sample is thoroughly charred. Cool, moisten the residue with 1 ml of *sulphuric acid*, heat gently until white fumes are no longer evolved and ignite at 800° ± 25° until all black particles have disappeared. Allow the crucible cool; add few drops of *sulphuric acid* and heat. Ignite as before, allow to cool and final weight was taken after two successive operation repetitions.

Determination of Extractable Matter in water and alcohol

About 4.0g of coarsely powdered air dried material, was accurately weighed in a glass stopper conical flask and macerated with 100ml of the solvent specified for the plant material concerned for 6 hours, shaking frequently, then allowed to stand for 18 hours. Filtered rapidly, taking care not to lose any solvent. The extracted matter was dried at 105°C for 6 hours, cooled in a desiccators for 30 minutes and then weighed. The percentage extractable matter was calculated.

Identity, Purity and Strength

Foreign matter	1.00 %
Moisture content	2.25 %
Total ash	8.2 %
Acid – insoluble ash	1.75%
Sulphated ash w/w	10.6 %
Alcohol – soluble extractive	5.5 %
Water – soluble extractive	15.8 %

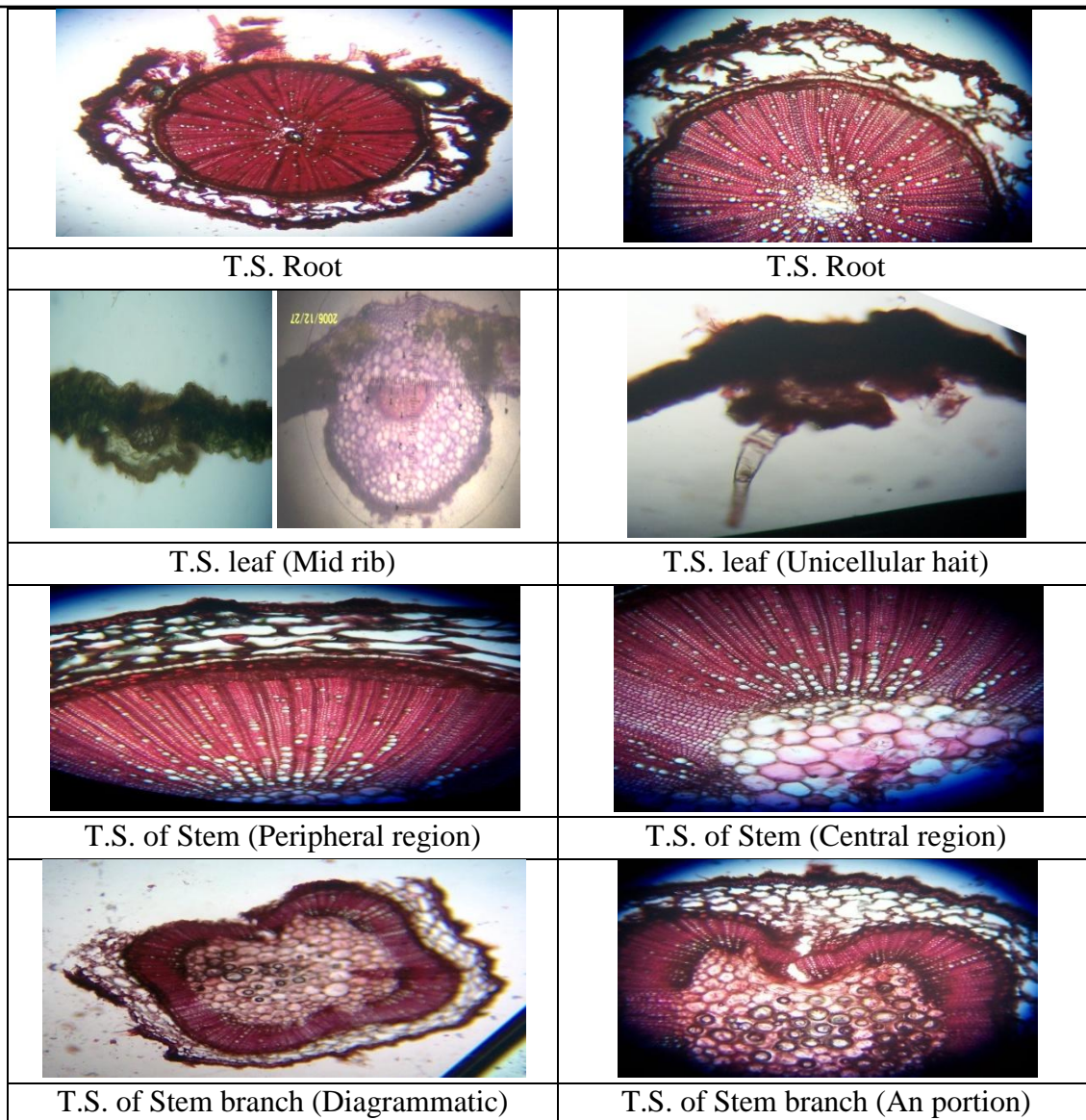
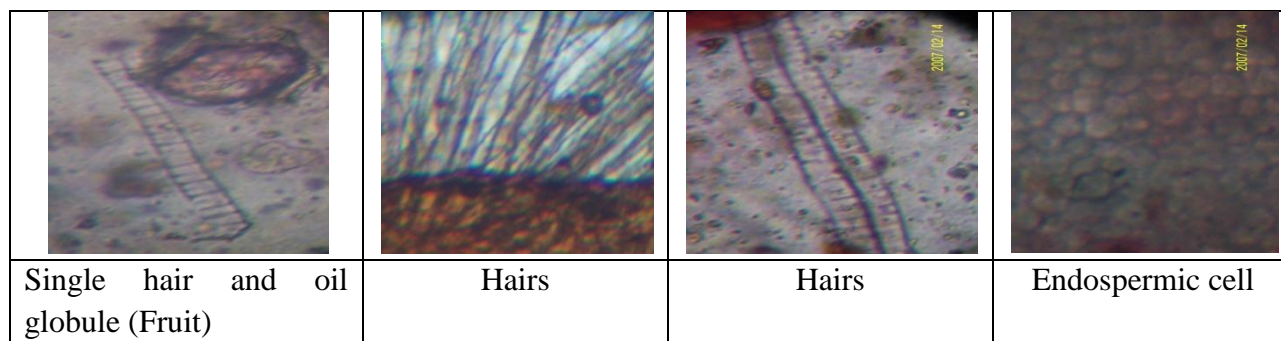


Fig. of Kokilaksh (Parts) T.S.



CHEMICAL TEST

Alkaloids	-	+ ve
Steroids	-	+ ve
Glycosides	-	+ ve
Saponins	-	+ ve

Fluorescence Study

[Chase & Pratt, 1949.,Kokaski, et al.,1958) with some modification]

S.NO.	Treatment	Under ordinary light	Under UV - Long (366nm)
	Drug as such	Dark brown	Cream
	Drug + Nitrocellulose	Brownish grey	Greenish cream
	Drug + Picric acid	Yellowish grey	Greenish brown
	Drug + HCl conc.	Brown	Black
	Drug + H ₂ SO ₄ conc.	Black	Light grey
	Drug + HNO ₃ (50%)	Dark brown	Grey
	Drug + 1 N Na OH in Me OH	Light brown	Violet
	Drug + 1 N Na OH in Water	Blackish brown	Grey
	Drug + NH ₄ OH	Blackish brown	Brown
	Drug + FeCl ₃	Greenish brown	Black
	Drug + Acetic acid Glacial	Reddish brown	Grey
	Drug + Sudan-III	Light brown	Grey

Thin Layer Chromatography

The principle underlying the separation of the compounds is their adsorption at the solid-liquid interface. For successful separation the compounds of the mixture should show different degrees of affinity for the solid support (or adsorbent) and the interaction between adsorbent and the component must be reversible. As the adsorbent is washed with the fresh solvent, the various components move down the column and arrange themselves in the order of affinity to the adsorbent. Those with the least affinity move down the column at a faster rate than those with greater affinity.

Materials used- All the TLC plates used for the analysis were prepared with silica gel containing binder. Most frequently used binder in silica gel is calcium sulphate (Silica gel 60 F₂₅₄, Merck). Precoated aluminium sheets were also used which is coated with silica gel 60 F₂₅₄ (Merck).

Preparation of TLC plates - Required quantity of silica gel was mixed in a glass mortar to as smooth consistency with the requisite amount of water and the slurry quickly transferred to the spreader. The mixture has been spreaded over the plates in thickness of 0.2mm and was allowed to set into a thin layer. The plates were transferred

carefully to a suitable holder and after 30 minutes, dried and heated at 100– 120°C for at least one hour. The plates were kept in a desiccates after cooling, until required for further use. The pre coated plates were also activated by heating them for 30 minutes at 100°C.

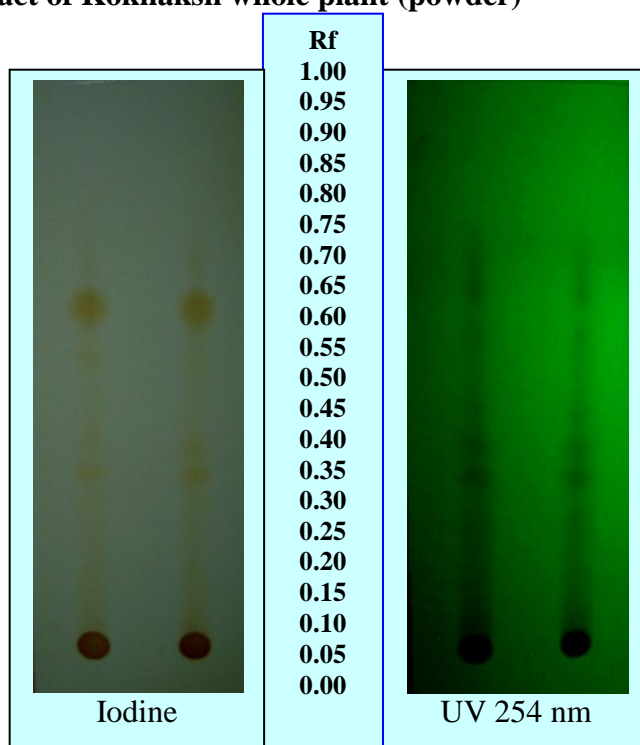
Application of sample- A known quantity of sample was dissolved in a known volume of solvent and the sample applied on pre coated TLC plates.

Selection of Solvent Systems- The choices of the solvents depend upon the nature of the substances to be separated and also in the material on which the separation is to be achieved. The solvents system was selected on the basis of trial and error method and by elutropic series. It has been found that combination of two solvents gave better separation than with a single solvent.

Chromatographic development (separation)- Development of the chromatogram is effected after the solvent of the applied sample is completely evaporated. Rectangular glass chambers or twin trough chambers are commonly used for TLC development.

Detection of spots- Detection of R_f. Value of spots done by using Iodine exposure and Vanillin Sulphuric Acid Reagent.

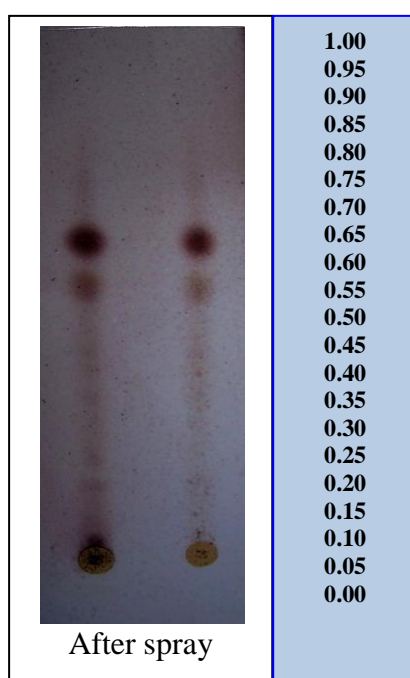
TLC of Acoholic extract of Kokilaksh whole plant (powder)



Stationary phase	TLC Aluminium sheet silica gel 60 F 254 plate
Mobile phase	Toluene – Ethyl Acetate – Methanol – Formic Acid (7: 3: 1: 0.3)
Rf value of spots visualized in Iodine	0.30, 0.35 & 0.60
Rf value of spots visualized in UV 254 nm	0.10, 0.20, 0.30, 0.35 & 0.60

TLC of Kokilaksh (powder)

Stationary phase	TLC Aluminium sheet silica gel 60 F 254 plate
Mobile phase	Toluene – Ethyl Acetate – Methanol – Acetic acid (7: 3: 1: 0.3)
Rf value of spots Visualized after spray of sulphuric acid reagent and heated 110 ⁰ C for 5 minutes	0.15, 0.20, 0.35, 0.40, 0.50 & 0.60



Results

TLC results indicate alcoholic extract of Kokilaksh (whole plant) Rf value of spots visualized in Iodine at Rf. 0.30, 0.35 & 0.60(3 spots). Rf value of spots visualized in UV 254 nm at Rf. 0.10, 0.20, 0.30, 0.35 & 0.60 (5 spots). Rf value of spots Visualized after spray of sulphuric acid reagent and heated 110⁰C for 5 minutes at Rf. 0.15, 0.20, 0.35, 0.40, 0.50 & 0.60.(6 spots).

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