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# Pharmacognostic evaluation and investigation of revitalizer herb *Evolvulus alsinoides* Linn.

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**Abstract**

Medicinal plants have been used from ancient time for the treatment and well-being of human beings. *Evolvulus alsinoides* Linn. is also been extensively used as traditional medicine in various culture. *Evolvulus alsinoides* Linn. is often prostrate, slender and wiry with long hairs. The plant is common in tropical and sub-tropical regions of the world. The metabolites of the plant are considered to be effective in treating many ailments. Various dosage forms and a wide array of extracts have been used in traditional system of medicine with potent therapeutic activity. It has anticonvulsant, nootropic, anti-inflammatory, antimicrobial, antioxidant, anxiolytic, cardio-protective effects. The present study deals the pharmacognostic evaluation and investigation of systematics, macro-microscopy, physico-chemical studies, phytochemical studies and TLC assay & HPTLC analytical studies of this medicinally important plant species. Study not only covers critical aspects of pharmacognosy but also important phytochemical investigation with reference to its known bioactive secondary metabolites. It helps in the quality evaluation and standardization of herbal drug. Pharmacognostic diagnosis is very helpful to improve cultivation procedure, plant safety, drug quality and its efficacy along with authentication of commercial samples, used in various formulations.



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## Introduction

The revival of interest in natural drugs and the herbal products started in the last few decades mainly because of the widespread belief that 'green medicine' is healthier than synthetic products. The global herbal medicine is about US $ 90 billion which is growing at the rate of 10-15% annually and is expected to cross 5 trillion US $ by 2030. These targets can be achieved by providing scientifically validated, safe and standardized herbal products in domestic and international markets by preventing herbal drugs generally lack proper quality, specification and standards. The World Health Organization, in a number of its resolutions, emphasized the need for quality control of herbal drugs. An ideal herbal drug and their rational use required quality, safety and efficacy.

*Evolvulus alsinoides* Linn., ( Family-Convolvulaceae) is a perennial, prostrate, hairy, small herb with woody root stock, found wild in most parts of India in the plainsand other tropical and subtropical countries [1]. In Ayurveda, the plant is considered to be a powerful brain stimulant, febrifuge, insanity, epilepsy, hysteria, to cure burns, cuts, wounds and scorpion stings etc [2]. Pharmacologically, it is reported to possess anticulcer and anti-catatonic [3], immunomodulator, anti-inflammatory [4] and nootropic activities [5]. *Evolvulus alsinoides* Linn. Contains several alkanes, alkaloids, fatty acids, flavonoids, organic acids, phenolics, phytosterol, saponins and tannins [6]. Some of

them are betain, shankhapushpine and evolvine [7], scopoletin, scopolin, umbellifereron, ferulic acid easters, kampferol-7-o-β-glucopyrenoside and caffeic acid [8]. Antioxidant properties of this plant used to treat low spirits and depression as shown in various *in vivo* experiments [9].

*Evolvulus alsinoides* Linn., is controversial in origin as several other plants including *Convolvulus pluricaulis* L., *Clitoria ternatea* L. and *Canscora decussata* L. are also known as *Shankhapushpi* in various parts of India [10, 11, 12]. Not only this , the Indian pharmaceutical industry constantly facing the serious problem of the adulteration and substitution of raw drugs and it is very difficult to authenticate the commercial crude drugs because these are available as dried whole plant or some part of it. Therefore, it is extremely important to establish the reference samples and determine the quality parameters of the medicinal plants by undertaking extensive and intensive study of the plant, for producing quality herbal medicine to maintain consistency and desirable therapeutic effects. So, considering all above the facts, the present study is plan out to investigate systematics, macro-microscopic characters, physico-chemical parameters, phytochemical analysis, TLC and HPTLC assay and fingerprinting for *Evolvulus alsinoides* with the aim to set the quality control parameters of this important medicinal plant.

## Material and Methods

Pharmacognostical studies of plant genera *Evolvulus alsinoides* Linn. (Convolvulaceae), were undertaken and following methods were followed for investigation.

**Collection of genuine plants material**

Specimens of plant genera *Evolvulus alsinoides* were collected from their natural habitat. The plant were identified with the help of floras and by matching them with the type specimens deposited in the institute's herbarium. The herbarium specimens were made and deposited to national herbarium of National Botanical Research Instatute (NBRI), Lucknow, India.

**Botanical name**- *Evolvulus alsinoides* Linn.
**Family** - Convolvulaceae
**Vernacular name** - Shankhapusphi
**Place of collection** - Banthra farm of NBRI, Banthra, Lucknow
**Voucher No.** - 262525
**Part used**- Whole plant

**Processing of plant material for study**

The plant materials were properly dried in shade at 40°C and powdered. The fresh material was preserved in FAA solution (formaldehyde: acetic acid: alcohol: water in a ratio of 10:5:50:35) for microscopic studies.

**Studies of organoleptic characters**

The study include surface markings, texture, fracture, internal appearance, cut surface, odour and taste of the crude drug.

**Microscopic methods for herbal raw material**

Microscopic evaluation deals with identification of the various characters of tissues, cells and cell contents by microscopic methods by preparing specimens of crude material. Microscopic studies vary, depending on the part used like, leaf, stem, root, bark, flower, and fruit and also on the nature of the material i.e. entire, cut orpowdered.

**A. Disintegration of hard and woody tissues**

Cut the material into small pieces and transfers few pieces to test tube containing 4ml of dil. $HNO_3$ and heat to boiling. Add powdered potassium chlorate warm it gently and allow to react. Tissue starts to disintegration, when completely bleached. Apply pressure with glass rod for complete disintegration of the tissues. Allow the material to settle down, decant the liquid and wash the bottled material repeatedly with waters until the acidity is removed.

**B. Preparation of sections**

For microscopically studies, the sections were cut by the razor/ blade or through microtome and double staining were performed in safranin and hematoxylin. The sections of 13-18 µm thickness were taken from the plant genera. The permanent slides (T.S./T.L.S.) were prepared by using dehydration method.

**C. Leaf surface preparation**

For the surface study and quantitative microscopy, boil pieces of leaves in a test tube with chloral hydrate for several minutes until completely clarified and then examine them in chloral hydrate solution after clarification, leaf pieces are placed on a microscopic slide and then divided into two parts with the help of scalpel or needle and carefully turn one part.

**D. Quantitative microscopy**

Draw a square with the help of microscope, stage micrometer scale and camera Lucida. Place transparent leaf fragments of about 5x5 mm in size on a microscope slide and prepare the mount, with 1 drop of safranin and 1 drop of glycerin.

(a) Stomatal number / density: Is the number of stomata present per $mm^2$.

(b) Stomatal Index: Is the percentage which the number of stomata forms to the total number of epidermal cell, each stoma being counted as one cell. Stomata Index can be calculated by using the following equation-

S.I. = S/E+S x 100

Where's

S.I. = Stomata Index

S = Number of stomata per unit area

E = Number of epidermal cells in the same unit area.

(c) Vein-islet number: Is the number of vein–islets per sq. mm of the leaf surface mid-way between the midrib and the margin.

(d) Vein- termination number: Is the number of vein let termination per sq. mm of the leaf surface midway between midrib and margin.

(e) Determination of palisade ratio: Is the average number of palisade cell beneath each epidermal cell. Count the palisade cells under the four epidermal cells where a cell is intersected. Calculate the average number of palisade cells beneath one epidermal cell, dividing the count by 4.

**Maceration**

To observe the shape, size and structure of isolated thick walled elements, small pieces of material are placed in a test tube and boil with 40% $HNO_3$ for 15-45 minutes. Wash thoroughly with water, place the material on the microscopic slide and then macerate with the help of a needle then add 1 drop of glycerine and 1 drop of safranin, cover with a cover slip.

**Powder Studies**

Different characters of powdered drugs like organoleptic characters viz. color, odour, fineness, degree of uniformity of the particles and sensation of smoothness were recorded. For examining characters of the powder, take sufficient amount of powder in chloral hydrate solution on a slide and cover it with a cover slip, warm over a low flame for a short time. Fluorescence test of powder (under UV light and visible light) were performed according to the method described by Chase and Pratt (1949) [13] and Kokoski et.al. (1958) [14]).

**Physico-chemical parameters for the standardization of crude drugs**

The physico-chemical analysis often plays an important role in herbal drug standardization. These tests are simple and quick to perform and give valuable information about the nature and purity of a crude drug. The values given in the results are replicate of six samples. The tests which are normally performed include:

**A. Determination of foreign matter**

Drug should be entirely free from visible sign ofcontamination by moulds or insects and other animal contamination. No abnormal odour, discoloration, slime or sign of deterioration should be detected. It is seldom possible to obtain marketed plant materials that are entirely free from harmful foreign mater or residue. Morphological examination can conveniently be employed for determining the presence of foreign matter in whole or cut plant materials. However, microscopy isindispensable.

*Procedure:*

100-500g of the drug sample to be examined weighed it and spread out in a thin layer. Detect the foreign matter by inspection with the unaided eye or by the use of a lens (6 xs). Separate the other material weight it and calculated the percentage present. The amount of foreign matter shall not be more than the percentage prescribed in the pharmacopoeia (2%).

**B. Determination of moisture content (loss on drying)**

Determination of the amount of volatile matter in the drug is measure of loss on drying for substances.

*Procedure:* 10 gram of drug were kept in oven at 100°C for 3h and made it moisture free, weighted till constant weight was attained and calculated the percentage of moisture by the following formula.

$$\text{Moisture percentage} = \frac{Pw - Fw}{W} \times 100$$

Where's,

Fw = Final constant weight of the sample

Pw = Pre weight of sample

W = Total weight of sample

**C. Ash Value**

Ash value is determined to estimate the total amount of the inorganic salts present in the drug. This includes total ash and acid insoluble ash.

(a) The total ash: Method is designed to measure the total amount of material remaining after ignition. This includes both "Physiological ash" which is derived from the plant tissue itself, and "Non Physiological ash" which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.

*Procedure:* Place 2 gm of ground air-dried material was accurately in a previously ignited and tarred crucible. Spread the material as an even layer and ignite it by gradually increasing the temperature not exceeding 450°C, until it become white, indicating the absence of carbon. Cool in desiccators and weight. If carbon free ash cannot be obtained in this manner, cool the crucible and moisten the residue with about 2ml of water. Dry on a plate and ignite to constant weight. Allow the residue to cool in suitable desiccators for 30 minutes, then weight without delay. Calculate the content of total ash of air-dried material.

$$\text{Total ash percentage} = \frac{Pw - Fw}{W} \times 100$$

Where's,

Pw = Pre weight of crucible
Fw = Final weight of crucible
W = Total weight of powdered plant material

(b) Acid insoluble ash: *Procedure:* Boil the ash obtained as total ash with 25 ml of dilute hydrochloric acid in the crucible, cover with a watch glass and boil gently for 5 minutes. Rinsed the watch glass with 5ml of hot water and add this liquid in the crucible. Collect the insoluble matter on an ash-less filter paper and wash with hot water until the filtrate neutral. Transfer the ash-less filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccators for 30 minutes, and then weight with delay. Calculate the content of acid-insoluble ash of air-dried material.

$$\text{Acid insoluble ash percentage} = \frac{FWb - FWa}{W} \times 100$$

Where's,
FWa = Final weight of crucible with acid insoluble ash
FWb = Final weight of crucible with total ash
W = Total weight of powdered plant material

**D. Extractive values:** It is the amount of soluble constitu- ents (active or otherwise) extracted with solvents like al- cohol, water, methanol, hexane and other solvents from a given amount of medicinal plant material. These are used to determine the amount of the matter, which is soluble in the solvents used; it includes alcohol soluble extractive, water soluble extractive, and hexane soluble extractive etc.

(a) Determination of alcohol soluble extractive: *Procedure*– Macerate 5 g of the coarsely powdered air- dried drug with 100 ml of alcohol in a closed flask for twenty-four hours, shaking frequently during six hour and loss of solvent. Take 25 ml of the filtrate in a tarred flat-bottomed shallow dish, evaporate and dry at 105°C to constant weight. Calculate the percentage of alcohol soluble extractive with reference to the air-dried [15].

(b) Determination of water soluble extractive: *Procedure* - Macerate 5 g of the coarsely powdered air-dried drug with 100ml of chloroform water (0.1%) in a closed flask for twenty-four hour, shaking frequently during six hours and allowing standing for eighteen hours. Filter rapidly, taking precaution against loss of solvent. Take 25 ml of the filtrate in a tare-bottomed shallow dish, evaporate and dry at 105°C to constant weight. Calculated the percentage of water–soluble extractive with reference to the air-dried drug [15].

(c) Determination of successive soxhlet extractive values: *Procedure*- Extract 5g of the air dried coarsely powder drug exhaustively with hexane, chloroform, acetone, alcohol and water in a successive order. Collect the hexane, chloroform, acetone, alcohol and water soluble extractives obtained separately, concentrate and dry. Calculated the percentage of each extractive with reference to the air dried drug.

**E. Sugar estimation** (Montgomery, 1957) [16] – Total amount of sugar present in the drug calculated as :

*Procedure:* Prepare 10 percent homogenate of the plant tissue in 80 percent ethanol. Centrifuge at 2000 rpm for 50 minutes. The supernatant obtained is made up to known volume (generally up to 10 ml or depending on the expected concentration of sugar). Take 0.1 ml aliquot and add 0.1 ml of 80 percent phenol and 5 ml conc. $H_2SO_4$. Cool and then read the absorbance at 490 nm. Calculate the percentage according to the absorbance.

$$\text{Total amount of sugar percentage} = \frac{3.1 \times \text{Absorbance}}{\text{Sample amount}}$$

**Phytochemical screening / tests (Qualitative analysis)** Determination of various class of primary (carbohy- drates, lipids, proteins, etc.) as well as secondary (alka- loids, glycosides, saponins, flavanoids, terpenoids, tan- nins etc.) metabolites was estimated. General screening of the alcoholic, aqueous and other extracts of the plant ma- terial is used for quantitative determination of the group of organic compound present in them. The preliminary phytochemical studies are used for testing the different chemical groups present in plant extracts. 10% (w/v) solution of extract is taken unless otherwise mentioned in the respective individual test. General screening of the extracts of the plant material is used for qualitative determination of the groups of organic compound present in them.

**A. Alkaloids-** *Dragendorff's test:* Dissolve few mg of alcoholic or aq. extract of the drug in 5 ml of distilled water , add 2 M hydrochloric acid until an acidic reaction occur, then add 1 ml of Dragendorff's reagent, an orange or orange - red ppt. produced immediately indicate the presence of alkaloid.

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

**C. Flavonoids:** *Schinoda 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 color is produced.

**D. 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 through the side. A violet color colored ring formed indicating the presence of triterpenoids.

**E. Proteins-** *Biuret's test:* To 1ml 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 color is obtained.

**F. Resins:** Dissolve the extract in acetone and pour the solution into distilled water. Turbidity indicates the presence of resins.

**G. Saponins:** In a test tube containing about 5 ml of an aqueous of the drug add a drop of sodium bicarbonate solution, shake the mixture vigorously and leave for 3 mnts. Honeycomb like forth formed indicates saponins.

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

**I. Tannins:** To 1-2 ml of extract of the drug add a few drops of 5% $FeCl_3$ solution. A green color indicates the presence of Gallo tannins while brown color indicates tannins.

**J. Starch:** Dissolve 0.015 g of iodine and 0.075 g of potassium iodide in 5 ml of distilled water and add 2-3 ml of an extract of drug. A blue color is product.

**Chromatographic Analysis**

**A. Thin layer chromatography (TLC)**

Thin layer chromatography (TLC) is frequently used for the rapid and positive analysis of herbal medicines. The time required for the demonstration of most of the characteristic constituents by TLC is very short and in addition to qualitative detection, the TLC also provides semi-quantitative information on the chief constituents of the plant drug and thus enables an assessment of drug quality. It is a open bed technique in two phases a stationary phase acting through adsorption and a mobile phase in the form of a liquid. Identification can be effected by adsorption of spots of identical Rf value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi- quantitative estimation.

TLC is used for the separation of simple mixtures where speed, low cost, simplicity are required. It provides a chromatographic drug fingerprints. It is therefore suitable for monitoring the identity and purify of drug. In TLC the various steps involved are.

1. Application of sample
2. Chromatographic development
3. Detection of spots
4. Quantification
5. Documentation

1. Application of sample- A known quantity of sample is dissolved in a known volume of solvent and the sample applied on percolated TLC plate in the form of a spot or a band.

2. Chromatographic development (separation)

Development of the chromatographic is affected after the solvent of the applied sample is completely evaporated. Rectangular glass chamber or twin through chamber is commonly used for TLC development.

3. Detection of spots- For detection of spot UV light is generally preferred.

4. Quantification and documentation- Densitometry is *in situ* instrumental measurement of visible UV absorbance, fluorescence quenching directly.The scanner convents the spot/band on the layer into a chromatogram consisting of peaks similar in appearance of HPLC. The portion of the scanned peaks on the recorder chart is related to Rf value of the spots on the layer and the peaks light or area is related to the concentration of the substance on the spot.

**B. High performance thin layer chromatography (HPTLC)**

HPTLC is an advanced versatile chromatographic technique for quantitative analyses with high sample throughout and is complementary to HPLC/GLC. It provides a chromatographic drug fingerprint. It is therefore suitable for monitoring the identity and purity of drugs. In HPTLC the various steps involved are

1. Application of sample.
2. Chromatographic development
3. Detection of spots
4. Quantification
5. Documentation

Applications of sample– An automatic applicator (Linomat) is used for sample application.

A known quantify of sample is dissolved in a known volume of solvent and the sample on percolated TLC plate either in the form of a spot or a band. However a band form is preferred because:

- Larger quantities of sample can be handled for application.
- Better separation because of rectangular area in which compounds are present on the plate.
- Response of densitometry in better due to variable concentration of substances in a spot.

1. Chromatographic development (separation):

Development of the chromatogram is affected after the solvent of the applied sample is completely evaporated. Rectangular

glass chambers or twin trough chambers are commonly used for TLC development.

2. Detection of spots: For densitometry scanning, detection under UV light is generally preferred. But post chromatographic derivatisation reactions are essentially required for detection when individual compounds does not respond to UV light or do not have intense fluorescence.

3. Quantification and Documentation: Densitometry is *in situ* instrumental measurement of visible, UV absorbance, fluorescence quenching directly. The scanner converts the spot/band on the layer into a chromatogram consisting of speaks similar in appearance to HPLC. The portion of the scanned peaks on the recorder chart is related to Rf values of the spots on the layer and the peak height or area is related to the concentration of the substance on the spot.

## Results

**Systematics (Taxonomy)**

Botanical name– *Evolvulus alsinoides* Linn.

Family–Convolvulaceae

Vernacular Names:

Hindi-Sankhapushpi

English-Dwarf morning glory

Gujrati-Shankhavali

Marathi-Shankhavela

Sanskrit-Vishnugandhi

Bengali-Shankhavalli

Malayalam-Vistnaclandi

Punjabi-Vistnaclandi

Tamil-Visnukarandi

Telgu-Vishnukarant

**Identification**

| | |
|---|---|
| Plants with seeds, ovules enclosed within the ovary | Angiosperms |
| Venation reticulate, flower pentamerous | Dicotyledonae |
| Petals fused | Gamopetalae |
| Carpals two, ovary superior | Bicarpellatae |
| Leaves alternate, expstipulate, flowers actinomorphic | Polemoniales |
| Gynoecium bicarpellary, syncarpous with basal ovules in each locule on axile placentation, fruit capsule | Convolvulaceae |
| Corolla lobes in duplicate contorted, flowers solitary in the axils, stamens | *Evolvulus* |
| Capsules 2- celled | *alsinoides* |

**Habit:** A much branched, diffuse, perennial, small, silky-pubescent, suberrect herbs.

**Habitat:** It is a common weed in open and grassy places almost throughout India, ascending to 6,000 ft. in the Himalayas.

**Root:** Short woody root stock, wiry, more or less pilose or sometimes almost glabrous.

**Stem:** Prostrate, unbranched, solid, cylindrical, hairy, herbaceous.

**Leaf:** Leaves variable, sessile, ¼ - 1 inch long, lanceolate to ovate, obtuse, mucronate, and acute to round at both ends, densely clothed with appressed white silky pubescence.

**Flower:** Bluewish-white, on 1 – 3 flowered filiform peduncles, bracts small, linear, hirsute, persistant, pedicels filiform. Calyx densely silky, segments 1/6 inch long, lanceolate, acute. Corolla subrotate, 1/5 inch long.

**Fruit:** Capsule 1/3 - 1/5 inch in diameter.

**Seed:** Four valved and usually 4 seeded.

**Flowering and Fruiting:** August-March.

Fig.1: Whole plant of *Evolvulus alsinoides* Linn.

**Microscopic study of crude drug**

**Root:** Outline is nearly circular, cork composed of 3-4 layer of tangentially elongated, elliptical, parenchymatous cells and yellowish brown, tanninferous, secretory cells present in this region. Phloem composed of sieve elements, phloem parenchyma and phloem rays. Xylem consisting of usual elements, vessels solitary or in groups of two with simple pits. Fibers and tracheids aseptate and pitted, medullary rays 1-3 cells. Wide and multicellular in length, starch grains solitary or in groups, present in cortex, phloem, xylem rays and parenchyma (Fig.-2.3).

**Stem:** Epidermis single layered, covered with thick cuticle, unicellular hairs, cortex is differentiated in many zones. Endodermis single layered, pericycle present in the form of single strand of fibers, phloem a narrow zone, mostly solitary with spiral thickening, fibers aseptate having pointed ends and narrow lumen (Fig.-2.4).

**Leaf:** Midrib– looks concave in upper side and convex in lower side, epidermis single layered, covered with thick cuticle, lower epidermis fallowed by 2-3 layers of chlorenchymatous cells, vascular bundle bicollateral, composed of usual elements of phloem and xylem, 4-5 layers of parenchymetous cells between chlorenchyma and vascular bundles (Fig.-2.5).

Lamina: Epidermis on both surfaces covered with thick cuticle, hairs unicellular, present on both surfaces. Palisade 2 layered, spongy parenchyma 3-6 layered. Few bicollateral vascular bundles present in spongy parenchyma (Fig.-2.6).

**Stomatal Number**

Upper surface- 182-227 per sq. mm

Lower surface- 167-234 per sq. mm

**Stomatal index**

Upper surface- 13-15 per sq. mm

Lower surface- 15-19 per sq. mm

**Vein-islet Number–**18-23 per sq. mm

**Vein-termination Number–**14-19 per sq. mm

**Palisade ratio–**7-9

**Powder studies**

**A. Organoleptic characters**

Following are the organoleptic characters of whole plant powdered drug.

Colour–Yellowish-brown

Taste–Slight characteristic

Odour Pungent

**B. Microscopic study**

On powder microscopy of *E. alsinoides* whole plant revealed the presence of fragments of critical parenchymatous cells, fragments of pitted vessels, fibers, fragment of cells, starch grains, in single or compound grains.

**C. Fluorescence analysis**

The behavior of the powdered with different chemicals reagents has been shown in the table.

| S.No. | Treatment | Day light | UV -254 mm | UV -336 mm |
|---|---|---|---|---|
| 1 | Powder (P) as such | Yellowish brown | Pale yellow | Whitish yellow |
| 2 | P + NaOH in water | Greenish black | Dark muddy | Brown |
| 3 | P+1N NaOH in methanol | Pale green | Black | Dull muddy |
| 4 | P + 50% KOH | Green | Blackish green | Muddy yellow |
| 5 | P + 1N HCl | Yellow | Green | Pale green |
| 6 | P + 50% $H_2SO_4$ | Light black | Greenish black | Dull black |
| 7 | P + 50% $HNO_3$ | Yellow | Green | Black |
| 8 | P + 50% Conc. $HNO_3$ | Dark yellow | Pale yellow | Yellow |
| 9 | P + Acetic acid | Light black | Pale yellow | Yellow |
| 10 | P + Conc. $H_2SO_4$ | Black | Black | Black |
| 11 | P + Iodine water | Yellow | Yellow | Pale yellow |

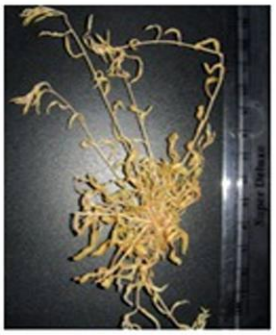
**Physico-chemical studies:** The different physico-chemical values obtained are recorded for identity, purity and strength.

| S. No. | Parameter | Range (in percent) | Mean (in percent) |
|---|---|---|---|
| 1 | Foreign matter | 0.80-1.20 | 1.00 |
| 2 | Moisture content | 12.17-13.15 | 12.66 |
| 3 | Total ash | 13.72-14.68 | 14.20 |
| 4 | Acid insoluble ash | 04.21-05.38 | 04.79 |
| 5 | Hexane soluble extractive | 02.50-02.00 | 2.25 |
| 6 | Alcohol soluble extractive | 02.31-03.52 | 2.91 |
| 7 | Water soluble extractive | 16.79-18.52 | 17.16 |
| 8 | Sugar | 0.478-0.584 | 0.543 |

**Phytochemical studies**

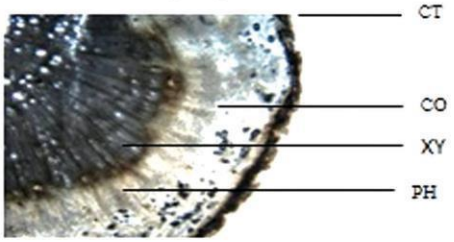
The preliminary phytochemical screening of whole plant drug are recorded for different chemical groups present in different extractives are as follows:

| S. No. | Phytochemicals | Water | Alcohol | Chloroform | Acetone | Hexane |
|---|---|---|---|---|---|---|
| 1 | Alkaloids | + | + | | + | |
| 2 | Carbohydrate | | | + | | |
| 3 | Flavnoides | | | | | |
| 4 | Triterpenoids | | + | | + | |
| 5 | Protein | + | | + | | |
| 6 | Resin | + | | + | | |
| 7 | Saponin | | | | | |
| 8 | Steroids | | | | | |
| 9 | Tannins | + | | | + | |
| 10 | Starch | | + | | | |

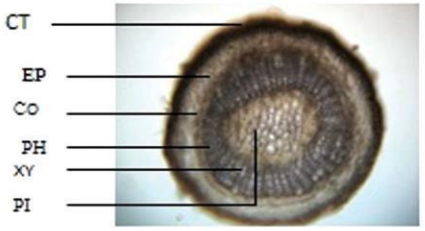


1. A flowering twig

2. Dried whole plant crude drug

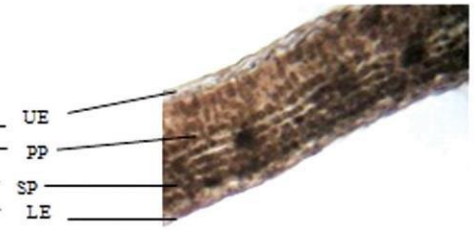



3. T.S. cellular structure of root (10X)

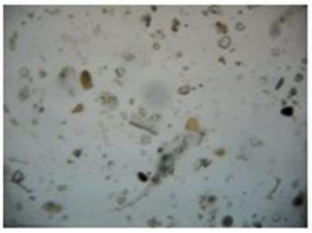



4. T.S. cellular structure of stem (4X)


5. T.S. cellular structure of leaf through midrib region (4X)




6. T.S. cellular structure of leaf through lamina region (4X)



7. Powdered elements (4X)

**Fig: 02 Macroscopic and microscopic characters of *Evolvulus pluricaulis* Linn.**

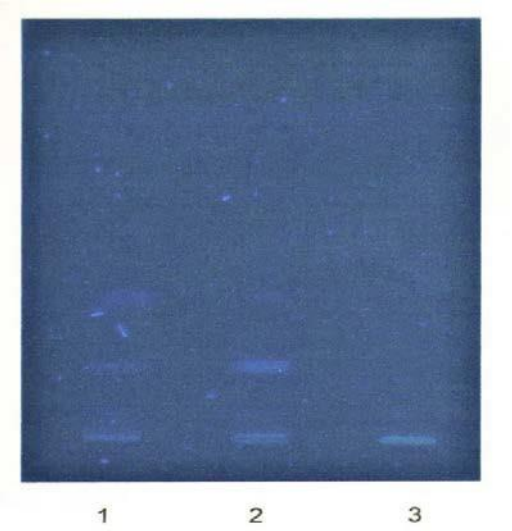



**Fig: 03 TLC profile of whole plant extract of *Convolvulus pluricaulis* (1), *Evolvulus alsinoides* (2), and *Clitoria ternatea* (3).**

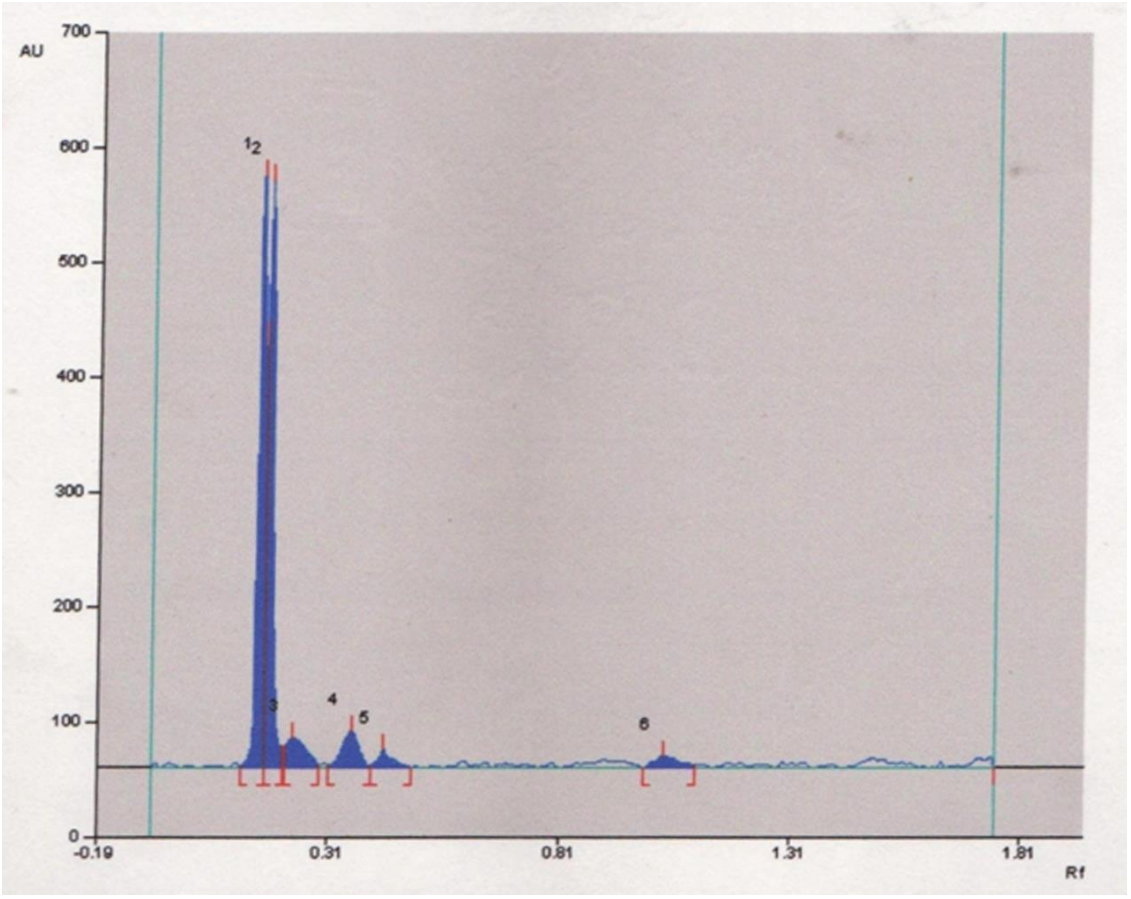



**Fig: 04 HPTLC chromatogram (densitimetric scan at 366 nm) of *Evolvulus alsinoides* whole plant extract.**

**TLC Assay and HPTLC Analytical studies**

Test solution– Extract 5gm of powdered drug in soxlet apparatus with methanol. Filtrate and concentrate the methanolic extract. Take 10 mg of the residue and dissolve in 1 ml of methanol and use the same for TLC and HPTLC analysis of the drug.

Solvent system– Toluene: Ethyl acetate: Formic acid (8:2:05).

*Procedure*– Apply 10ml of the test solution on pre-coated silica gel 60 f254 TLC plate (E. Merck) of uniform thickness of 0.2 mm. Develop the plate in solvent system at distance of 8 cm.

Visualization and Evaluation– Visualize the plate under UV light at 366nm (See Fig. 3,4) shows six fluorescence

zones at Rf 0.16, 0.18, 0.24, 0.36, 0.43 and 1.04 which are not identical and corresponding to substituent's like *C. pluricaulis* and *C. ternatea* etc., conforms in the variation of chemical contents.

## Discussion

To meet out the great demand of the botanicals for producing standardized and quality herbal drugs/products and to promote the export of Ayurvedic medicine. It is essential to maintain the quality of herbs used for the preparation of these products. So, realizing the importance of quality raw material and prevalence of spurious raw material is very important. Today quality assurance is thrust area for the evaluation of traditional used medicinal plant and herbal formulation. Plants collected from wild sources strictly required quality of herbal drugs.

The herbal drugs may be in broken conditions, matted together condition, transverse and longitudinal slices, cut forms, shredded forms, peeled conditions or in powdered forms. The systematic macro-microscopically studies along with systematics are the valuable parameters for the quality control and proper identification of crude herbal drugs. The physico-chemical analysis often plays an important role in determination of identity, purity and strength of plant crude drugs. The tests are simple and quick to perform and give valuable information about the nature and purity of a drug. Air dried material was used for qualitative determination of physico-chemical values. Some of the parameters like ash values indicate about the care being taken up while preparing the drug for com- merce and extractive values can also be useful for selec- tion of solvents for extraction.

The preliminary phytochemical studies are used for testing the different chemical groups present in plant extracts. Qualitative determination of various class of primary (Carbohydrate, protein etc.) as well as secondary (Alkaloids, resin, saponins, flavonoids, terpenoids, tannins etc.) metabolites can be determined by phytochemical screening. The quality control and quality assurance of herbal drugs determine by chromatographic techniques because of the high variability of chemical components of the plant. It is a physical method of separation in which the components to be separated are distributed between two phases.

## Conclusion

From the present studies, it can be concluded that the systematics, characteristic macro-microscopical features, physic-chemical parameters, phytochemical analysisand distinguishing bands in the TLC, HPTLC profiles are very important for pharmacognostic evaluation and investigation of revitalizer herb *Evolvulus alsinoides* Linn..

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## Abbreviations

| | | | |
|---|---|---|---|
| % | - Percentage | dr. wt. | -Dry weight |
| °C | - Degree centigrade | g | -Gram |
| aq. | - Aqueous | h | - Hours |
| cm | - Centimeter | l | - Liter |
| conc. | - Concentrated | ml | - Milliliter |
| mm | - Millimeter | COL | -Collenchyma |
| ppt | - Precipitate | CU | - Cuticle |
| s | - Second | EP | - Epidermis |
| sq. mm | - Square millimeter | LE | - Lower epidermis |
| TLC | - Thin Layer Chromatography | MR | - Medullary rays |
| µm | - Micrometer | PH | - Phloem |
| UV | - Ultraviolet | PI | - Pith |
| v | - Volume | PP | - Palisade parenchyma |
| v/v | - Volume per volume | RZ | - Rizome |
| w | - Weight | SC | - Scleroids |
| w/v | - Weight per volume | SG | - Starch grain |
| dil. | - Dilute | SP | - Spongy |
| sps. | - Species | TR | - Trichrome |
| CC | - Cork cambium | UE | - Upper epidermis |
| CHL | - Chlorenchyma | VB | - Vascular bundle |
| CK | - Cork | XY | - Xylem |
| CO | - Cortex | | |

## References

1. Kiritikar K.R., Basu B.D. (1994) *Indian Medicinal Plants,* 2nd Edn., Vol-III, Bishen Sigh Mahendrapal Singh: Dehradun; 1738.
2. Chopra R.N., Nayar S.L. & Chopra I.C., (1956) *Glossary of Indian Medicinal plants,* (Council of Scientific and Industrial research, New Delhi), 116.
3. Purohit M.G., Shanthaveerappa G.K. & Badami S. (1996) Antiulcer and anticatatonic activity of

alcoholic extract of *Evolvulus alsinoides* (Convolvulaceae), *Indian J Pharm Sci*, 58, 110–112.

4. Ganju L., Karan D., Chanda S., Srivastava K.K., Sawhney R.C. & Selvamurthy W. (2003) Immunomodulatory effects of agents of plant origin, *Biomed Pharmacother*, 57,296–300.
5. Nahata A., Patil U.K. & Dixit V.K. (2009) Anxiolytic activity of *Evolvulus alsinoides* and *Convolvulus pluricaulis* in rodents, *Pharm Biol*, 47(5), 444–51.
6. Mehta C.R. & Shah N.B. (1958) Chemical examination of *Evolvulus alsinoides*, *Sci Culture*, 24, 180–181.
7. Guruswami M.N., Krishnamurthy T.R. & Vadyanathan T.S. (1956) A preliminary notes on the pharmacology of Evolvine, *Curr Sci*, 25, 119-120.
8. Gupta P., Siripurapu A., K.B., Ahamad A., Palit G., Arora A. & Maurya R. (2007) Antistress constituents of *Evolvulus alsinoides* : An Ayurvedic crude drug, *Chem Pharm Bull*, 771-715.
9. Kumar, M., Ahmad, A., Rawat, P., Khan, M.F., Rasheed , N., Gupta, P., Sathiamoorthy, B., Bhatia, G., Palit , G., Maurya , R.(2010) Antioxidant flavonoid glycosides from *Evolvulus-alsinoides*. *Fitoterapia*, 81, 234–242.
10. Shah V., Bole P.V. (1961) Botanical Identity of Shankhapushpi. *Indian J Pharm*, 23:223-4.
11. Sethiya N.K., Nahata S.H., Dixit V.K. (2009)An update on Shankhpushpi, a cognition-boosting Ayurvedic medicine. *J. Chin Integr Med*, 7(11):1001-22.
12. Vasisht K., Dhobi M., Khullar S., Mandal S.K., Karan M. (2016) Norneolignans from the roots of *Clitoria ternatea* L. *Tetrahedron Lett* , 57:1758-62.
13. Chase C.R. and Pratt R.J. (1949) Fluorescence of powdered vegetable drugs under ultra-violet radiation, *J. Amer. Pharmacol. Assoc*., 38(6): 324-331.
14. Kokoshi J., Kolcoski R. and Slama F.J. (1958) Florescence of powdered vegetable drug under ultraviolet radiation, *J. Amer. Pharmacol. Assoc.*, 47(10):715-717.
15. Anonymous (1966) Indian Pharmacopoeia, 2nd eds., Government of India, New Delhi.
16. Montgomery R. (1957) Determination of glycogen, *Arch. Biochem. Biophysics, 67:378-386.*