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Research article ***Convolvulus pluricaulis choisy***

Quality evaluation and pharmacognostic diagnosis of memory boosting herbal drug *Convolvulus pluricaulis* Choisy

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ABSTRACT

Pharmacognostic evaluation is the first and foremost step to determine identity and to assess the quality and purity of crude drug *Convolvulus pluricaulis* Choisy belong to family Convolvulaceae, a perennial wild herb commonly found on sandy & rocky areas under xerophytic conditions in northern India. *Convolvulus pluricaulis* Choisy is reputed drug of Ayurvedic formulation for improving memory power & intellect, chronic cough, sleeplessness, epilepsy, hallucination, anxiety and other central nervous system disease and disorders. The present study deals the pharmacognostic evaluation including taxonomy, morphology, microscopic characters, fluorescence analysis, physico-chemical studies, phytochemical studies and chromatographic analysis. The transverse section of root, stem and leaves were performed. Physico-chemical studies showed the values of foreign matter, moisture content, total ash, sugar and extracts in different solvents. The preliminary phytochemical screening of whole plant drug are recorded for different chemical groups like alkaloids, carbohydrates, flavonoids, resins, tannins and steroids etc. Although, preliminary attempt to evaluate drug by some other researchers but to provide some additional parameters which may useful for authentication of *Convolvulus pluricaulis* Choisy. The result of the standardization may throw immense light on the botanical identification of *Convolvulus pluricaulis* Choisy which may furnish a basis of judging the authenticity of the plant and also to differentiate the drug from its allied species and adulterants.

Keywords: *Convolvulus pluricaulis* Choisy, Shankhpushpi, Pharmacognosy, Macroscopy, Microscopy, Phytochemistry, Physico-chemical, Chromatographic analysis.

INTRODUCTION

Medicinal plant drugs have been used as ethnomedicine for the treatment of wide range disease since time immemorial. Herbal medicines are becoming more and more popular in recent years with their over increasing acceptability in both developing and developed countries. The resurgence of herbal drugs is mainly due to realization of the harmful side effects of many modern drugs. The preventive and primitive aspects of the traditional medicine particularly prevailing in traditional systems of medicine such as Ayurveda, Siddha and Unani in India are finding increasing popularity in the world over. The over increasing demand of herbal drugs led to unprecedented demand for the raw materials of herbal drugs.

Over 80 % of the raw material required for traditional herbal medicines used to be collected from wild resources. With the increase in demand of medicinal plants for commercial herbal medicine sector led to the indiscriminate and unscientific collection without any consideration for the quality and identity of the material collected. Herbal drug industries and agencies generally face the problem of adulterants and substitution.

Sometimes, not only the various species of a particular genus but entirely different plant taxa are being sold under the same vernacular name. Such adulteration and substitution lead to poor quality of herbal products. (Mehrotra et al., 2001)

Sankhpushpi is well known Ayurvedic drug of *Rasayana* group. It is a Sanskrit word meaning the plant with flower shaped like a conch. The conch or sankha is one of the Lord

Shiva's sacred instruments often used in virtual worship. Sankhpushpi of the Ayurvedic pharmacopeia of India consists of the whole plant of *Convolvulus pluricaulis* Choisy (Convolvulaceae) synonym *Convolvulus microphyllus* Sieb. (MHFW 2001). Plants other than *C. pluricaulis* use the name Shankhapushpi in different parts of the country. These include *Evolvulus alsinoides* Linn. , *Clitorea ternatea* Linn. and *Canscora decussate* Schutt (Pandey et al., 2020).

Convolvulus pluricaulis Choisy is a prostrate spreading perennial wild herb commonly found on sandy or rocky ground under xerophytic conditions in northern India. In India, it is widely distributed in and grows on the waste land in the plains of Punjab, Bihar and Chhotanagpur and other parts of the country. The whole plant is used in various formulae as a nervine tonic for improvement of memory and intellect (Adams et al., 2007). It has been widely screened for its various pharmacological activities. It has relatively well documented neuropharmacological actions such as nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilising and sedative activities which justify its use in central nervous system diseases in the Ayurvedic system of medicine. It has antimicrobial, antipyretic, anti-inflammatory, analgesic, diuretic, antidiabetic and insecticidal properties. These are reported to contain several types of alkaloids, flavonoids and steroids as active chemicals that bring about its biological effects (Sethiya et al. 2010). The quality assurance of raw materials required for maintaining the desired therapeutic efficacy of and its finished products. Further, this will not only help in maintaining the credibility of but also assure importance for the effective enforcement of the provision of the act for detection of adulteration and substitution of these drugs. This will in turn assure the interests of the profession and public health.

The present study is designed and covers an integrated range of aspects and parameters of pharmacognostic line on quality evaluation and pharmacognostic diagnosis of economically important memory boosting herbal drugs *Convolvulus pluricaulis*. Study not only provides critical aspects of pharmacognosy but also important phytochemical investigations 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. It helps for the authetification of commercial samples of this important crude herbal drug with the aim to more clarify the present scenario of marketed Shankhapushpi.

MATERIAL AND METHODS

In the present investigation detailed pharmacognostical studies of plant genera *Convolvulus pluricaulis* (Convolvulaceae), were undertaken and following methods were followed for investigation.

Collection of genuine plants material

Specimens of plant genera *Convolvulus pluricaulis* were collected from their natural habitat of National Botanical Research Institute (NBRI), Aurawa, Lucknow, India. 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 Institute (CSIIR-NBRI)), Lucknow, India.

Botanical name - *Convolvulus pluricaulis* Choisy
Family - Convolvulaceae
Vernacular name - Shankhapusphi
Place of collection - Aurawa farm of NBRI, Aurawa, Lucknow
Voucher No. - 262524
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

This 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 or powdered.

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) and Kokoski et.al. (1958).

Physico-chemical parameters for the standardization of crude drugs

The physicochemical 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 of contamination 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 is indispensable.
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.

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

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 weigh. 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.

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

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.

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

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 constituents (active or otherwise) extracted with solvents like alcohol, 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 drug (Anonymous, 1966).

(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 (Anonymous, 1966).

(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) – Total amount of sugar present in the drug:

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.

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

Phytochemical screening/ tests (Qualitative analysis)

Determination of various class of primary (carbohydrates, lipids, proteins, etc.) as well as secondary (alkaloids, glycosides, saponins, flavanoids, terpenoids, tannins etc.) metabolites was estimated. General screening of the alcoholic, aqueous and other extracts of the plant material 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: 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

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.

RESULT

Systematics

Botanical name : *Convolvulus pluricaulis* Choisy
Family : Convolvulaceae

Vernacular names

Hindi	-	Sankhapushpi
Sanskrit	-	Sankhapushin
Bengali	-	Sankhapuspi
Gujrati	-	Shankhavali
Kannada	-	Bilikantisoppu, Shankhauli
Marathi	-	Sankhahuli, Shankhavela
Oriya	-	Sankhapuspi
Punjabi	-	Ksirapushi, Sankhahuli
Tamil	-	Kalkattam, Kakkanangudi
Telugu	-	Shankhapushpi

Identification

Plants with seeds, ovules enclosed within the ovary -Angiosperms
Venation reticulate, flowers pentamerous -Dicotyledonae
Petals fused, flowers with calyx and corolla, ovary superior, two carpals -Bicarpellatae
Flowers actinomorphic -Polemoniales
Plants prostate, carpals medially placed -Convolvulaceae
Stigma's filiform, ovary 2 celled, capsule 4 valved, hairs simple -*Convolvulus*
Branches prostrate or sub-erect not twining, Flowers sessile or nearly so, corolla 1/4-1/3 inch long -*pluricaulis.*

Habit: A diffuse hairy perennial herbs.

Habitat: A common perennial weed herb, found almost north India.

Root: Cylindrical, straight or somewhat tuberous, 7–12 cm long, 0.5–1.5 cm, breadth, externally the root is faintly longitudinally striated and exhibits small circular rootlet scars, yellowish-brown to light in color, fracture fibrous, bract used surface pale whitish yellow, odour indistinct, taste slightly bitter.

Stem: Numerous slender wiry, cylindrical, spreading, aerial stems densely covered with silky hairs with clear hairy nodes and internodes. The stem 2–6 mm in diameter and 30 –50 cm in length, arises from the crown, fracture splintery, light green, taste slightly bitter.

Leaf: Green, simple, alternate, sessile, linear, oblong, shortly petiolate, entire, acute, pubescent, tapering at the base of varying in size ranging from 15–30 mm in length, 5– 10 mm in width, lateral vein 3–4 pairs, hairy on both surfaces.

Fig 1: Plant of *Convolvulus pluricaulis* Choisy

Flower: White, solitary in short peduncle with pair of hairy bracts, infundibulum, rotate, deltoid. Sepals narrowly linear-lanceolate, sparsely hairy. Corolla shortly discoid, stamens 5, free, epipetalous, alternate with the petal lobes, inserted deep in the corolla tube. Ovary superior and bicarpellary.

Fruit: Capsule, oblong, globase with coriaceous, pale brown pericarp.

Seed: Brownish, minutely puberulous.

Flowering and Fruiting: August-March.

Microscopy of the Crude Drug

Root: Transverse section of root shows outer cork composed of 8–12 layers of elongated, thick- walled suberized cells. Cortex many layered parenchyamatous cells with starch grains and tanniniferrous secretory cells. Pholem composed of phloem parenchyma, pholem rays and sieve elements. Xylem vessel in groups of 2–6, tracheids and parenchyma pitted, medullary rays 1–3 seriate, starch grains oval to round in shape in single and groups(Fig.-2.3).

Stem: Transverse section of stem shows a layer of epidermis covered with thick striated cuticle, bearing long unicellular thick walled, simple trichomes and stomata. Cortex composed of 3–5 layered outermost chlorenchyma fallowed by 2 layers of collenchyma and finally by 2 layers elongated parenchymatous tissue. Endodermis distinct, pericycle with lignified fibers (Fig.-2.4).

Leaf: Transverse section of leaf shows convex midrib on the lower side and flat but with a small centrally placed notch at the upper side. Epidermis single layered and covered with thick striated cuticle and long unicellular trichomes. Beneath the epidermis layer of the lamina lies two layered palisade region on both the surface, in continuation with that of midrib, except at few central region of midrib where it becomes collenchymatous. A layer of spongy parenchyma is seen in between the upper and lower palisade tissue. The anitclinal walls of the lower epidermis are sinuous, and shows cruciferous type of stomata in surface view (Fig.-2.5, 2.6).

Stomatal Number- Upper surface–200-240 per sq. mm
Lower surface-188-256 per sq. mm

Stomatal Index - Upper surface-14-17 per sq. mm
Lowersurface-17-20 per sq. mm

Vein-islet Number- 20-25 per sq. mm

Vein-termination Number- 18-23 per sq. mm

Palisade ratio- 6-9

Powder studies

A. Organoleptic characters: Fallowing are the organleptic characters of whole plant powdered drug.

Color – Brown
Taste – Spicy bitter
Odour – Characteristic

B. Microscopic study: On powder microscopy of *C. pluricaulis* whole plant drug shows fragments of spiral annular and pitted vessels, unicellular trichome, pitted xylem parenchyma, triporate, smooth walled pollen grains, fibers, fragment of cork, parenchymatous cells with starch grains, transversely cut fragments of lamina, lower epidermal cells with striated cuticle, another cells in surface view (Fig.- 2.7).

C. Fluorescence analysis: The behavior of powdered drug with different chemical reagents has been shown in the table.

S. No.	Treatment	Day light	UV - 254 nm	UV - 336 nm
1	Powder (P) as such	Brown	Greenish yellow	Whitish yellow
2	P+NaOH in water	Greenish brown	Greenish brown	Greenish yellow
3	P+1N NaOH in methanol	Light brown	Greenish brown	Greenish yellow
4	P+50% KOH	Light brown	Greenish brown	Greenish yellow
5	P+1N HCl	Light brown	Greenish brown	Greenish yellow
6	P+50% H_2SO_4	Greenish yellow	Light black	Greenish yellow
7	P+50% HNO_3	Light red	Light black	Greenish yellow
8	P+ Conc. HNO_3	Red	Yellow	Black
9	P+ Acetic acid	Light black	Light black	Greenish yellow
10	P+ Conc. H_2SO_4	Black	Black	Greenish brown

11	P+ Iodine water	Light brown	Yellow	Greenish yellow

Physico-chemical studies

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

S.No.	Parameters	Range(in percent)	Mean(in percent)
1	Foreign matter	1.20-1.60	1.40
2	Moisture content	9.15-11.11	10.13
3	Total ash	12.65-15.10	14.17
4	Acid insoluble ash	4.43-6.25	5.64
5	Hexane soluble extractive	1.15-1.70	1.48
6	Alcohol soluble extractive	4.70-6.10	5.40
7	Water soluble extractive	17.55-19.75	18.43
8	Total Sugar	0.249-0.353	0.290

Phytochemical studies

The preliminary phytochemical screenings of whole plant drug are recorded for different chemical groups present in different extractives are as fallows.

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

TLC Assay and HPTLC Analytical studies

Test solution–Extract 5 gm 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). Apply 10 ml of the test solution on precoated silica gel 60 f 254 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 366 nm (Fig. 3,4) shows nine fluorescence zones at Rf 0.16, 0.35, 0.43, 0.85, 0.94, 1.03, 1.13, 1.45 and 1.54. Which are not identical and corresponding to substituents like *E. alsinoides* and *C. ternatea* etc., conforms in the variation of chemical contents.

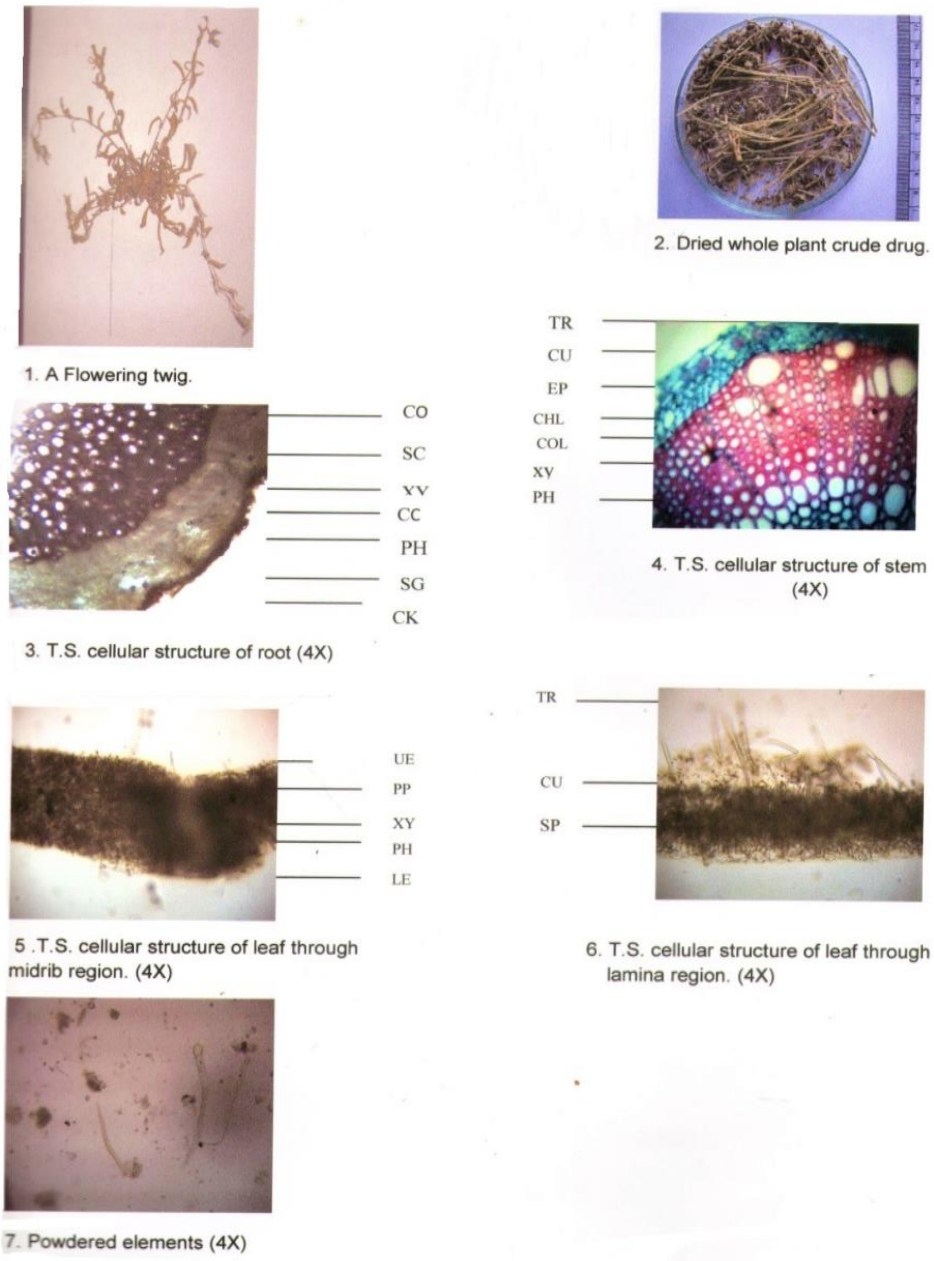

Fig 2: Macroscopic and microscopic characters of *Convolvulus pluricaulis* Choisy.

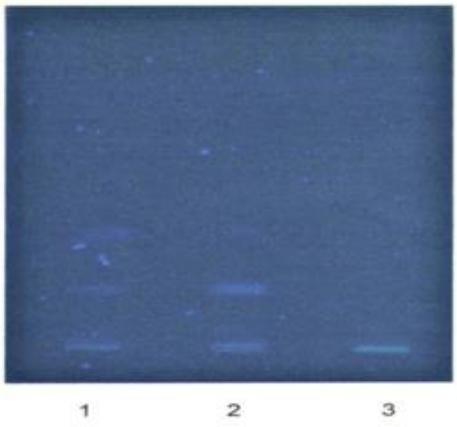

Fig 3: TLC profile of whole plant extract of *Convolvulus pluricaulis* (1), *Evolvulus alsinoides* (2), and *Clitoria tenatia* (3).

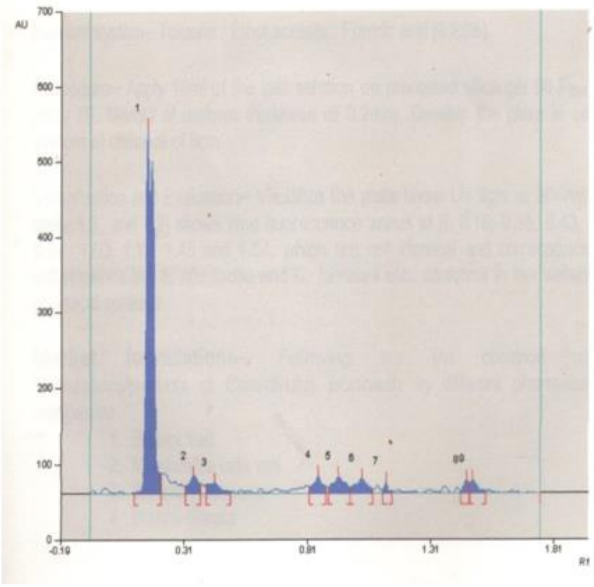

Fig 4: HPTLC chromatogram (densitometry scan at 366 nm) of *Convolvuluv pluricaulis* whole plant extract

DISCUSSION

Taxonomic and morphological assessment of crude drug helps in identification of plant as well as detection of substitution and adulteration. Preliminary, the quality of crude drug can be checked only on the basis of morphology (Prasad et al., 2016). The powder and powder microscopy of crude drug allows more information of a drug and it can be used to identify the unorganized drugs by their known histological characters (Rungsung et al., 2014).The powder microscopy as standard for authentication of this valuable powder form of crude drug. Phytochemical screening help in evaluating for presence and absence of phyto-constituents. Standardisation of medicinal plant is a complicated process, so different physico-chemical parameter together help in better understanding. Physico-chemical parameter can be used as standard to ensure the quality of crude drug. High ash values of shows the presence of very high inorganic content. Lower value of the acid insoluble ash suggests the greater physiological availability of drug. Extractive value gives information about availability of soluble phytoconstituents in particular solvent (Deshpande et al. 2014). Generally, alcohol soluble extractive is more as compared to aqueous extractive value suggesting alcoholic extract would be more beneficial as compared to aqueous extract as for therapeutic aspect. Phytochemical analysis showed the presence of important classes of phytoconstituents like alkaloid, flavonoids, terpenoids and steroids. This indicates that the plant can be useful for treating different diseases because the therapeutic activity of a plant is due to the presence of particular class of compounds. Low value of moisture content does not promote microbial contamination as the general requirement of moisture content in crude drug is not more than 14 % (w/w). Thin layer chromatography can be a valuable tool in standardization of medicinal plant. The TLC and HPTLC chromatogram and Rf value of phytoconstituents is preliminary tool of evaluation of crude drug (Wagner et al. 1996).

CONCLUSION

The present study pertains to pharmacognostic, phytochemical and physiochemical investigation of the medicinal plants. The standardization was according to International standardization criteria. The Qualitative as well as Quantitative analysis of the raw material has been investigated through which we came to know the active constituents present in the plant. From this study we have the above parameters are very useful for the proper identification and authentication of the species. The results of the present study will also be helpful in preparation of monograph. The study also provided some additional parameters for future researcher in this plant and revalidation of its use in Ayurveda.

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Abbreviation

%		Percentage	dr.	-	Dry weight
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.	-	Square millimeter	LE	-	Lower epidermis

TLC	- Thin Layer	MR	- Medullary rays	dil.	- Dilute	SP	- Spongy
µm	- Micrometer	PH	- Phloem	sps.	- Species	TR	- Trichrome
UV	- Ultraviolet	PI	- Pith	CC	- Cork cambium	UE	- Upper epidermis
v	- Volume	PP	- Palisade	CHL	- Chlorenchyma	VB	- Vascular bundle
v/v	- Volume per volume	RZ	- Rizome	CK	- Cork	XY	- Xylem
w	- Weight	SC	- Scleroids	CO	- Cortex		
w/v	- Weight per volume	SG	- Starch grain				

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