

PHARMACOGNOSTIC STUDIES ON SOME MEDICINAL PLANTS OF AURANGABAD DIST. OF MAHARASHTRA

FINAL REPORT OF MINOR RESEARCH PROJECT (BOTANY)



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CERTIFICATE

This is to certify that a copy of the final report of Minor Research Project entitled as *“Pharmacognostic studies on some medicinal plants of Aurangabad Dist. Of Maharashtra.”* completed by *Dr. Sutar Sangeeta Sahebrao*, Assistant Professor, Department of Botany has been kept in the library of Botany Department and an executive summary of the report has been Posted on the website of the Sir Sayyed College, Aurangabad

Principal

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Introduction

Medicinal Plants are of great value in the field of treatment and cure of diseases over the years. In ayurveda definite properties of drug obtained from plants and their uses have been given in some detail most of the drug plants are wild and few of them have been cultivated.

The medicinal importance of a plant is due to the presence of some special substances like alkaloids, glycosides, resins, volatile oil, gums, tannins etc. The active principles usually remain concentrated in the storage organ of the plant viz root, stem, leaves, bark, seeds etc.

Medicinal plants are of great value in the field of treatment & cure of disease over the years. Scientific research has expanded our knowledge of the chemical effects & composition & active constituents which determine the medicinal properties of plants. It has now been an universally accepted fact that the plant drugs & remedies are more safe than synthetic medicines for curing the complex diseases. large number of alkaloids, glycosides & antibiotics have been isolated, identified & used as curative agents.

In Allopathy, where in mostly pure compounds are used to treat various ailments, a number of plant products are regularly prescribed, A survey of the most common plants used in medicine shows that 45% of medicinal plant contain alkaloids as their active principle. 255 plant contain terpenoids, while 165 plants phenolics. An analysis of the data on the plant products exhibiting biological activity indicate that alkaloids, triterpenoids, & phenolics are the three major compounds which show curative properties including terpenoids (Wagner & Wolf 1977)

Worldwide realization of the medicinal plants in various traditional healing systems of developing countries is increasing. The world Health Organization (WHO) revealed that about 80 % of the people in developing countries rely on traditional remedies to treat various ailments & about 855 traditional medicines include about 30,000 botanical species are now recorded for their medicinal properties. (Tripathi et. al.2003)

In current age, modern medicines offers an unparalleled opportunity to relieve disease symptoms and save lives. modern surgical techniques, such as keyhole surgery and plastic surgery, and the whole range of diagnostic and life support machinery now available can all be used to improve the chances of recovery from serious illness or injury. Since ancient time, orthodox pharmaceutical medicines were the only solution to sustain life and counter infections. Despite of the dramatic advances and advantages of conventional medicine. or biomedicines it has been established that herbal medicine has offered a great solution to cure diseases of human being. From last fifty years or so many have relied almost entirely on plants to treat all manner of illness from minor problems such as cough & colds to life threatening diseases such as tuberculosis & malaria. Today, herbal remedies are coming back into prominence because the efficacy of conventional medicines such as antibiotics, which once had near-universal effectiveness against the serious infection, is on the wane (Prajapati et al.2003.)

The rate of growth of medicinal plants in relation to their economic prospects is not at all satisfactory. Perhaps, insufficient organization, lack of research, unplanned exploitation of natural resources, failure to grow them in large scale, inferior method of production, malpractices and adulteration are some of the reasons for our present state of affairs. It is unfortunate that with almost all types of climate and soil existing in our great country, the possibilities of raising large scale

plantation of medicinal plants on scientific lines has not been explored. It is a pity that interspaces of forests banks and the lands termed as barren, waste and marginal are being allowed to remain idle (Kattimani et al.,2003)

Due to varied geographical locations where medicinal plants grow coupled with the problems of different vernacular names, these plants are known by a great deal of adulteration or substitution which is encountered in the commercial markets. Therefore, reproducible standards of each plant are necessary for effective quality control (Patel D. 1985)

Marathwada, a region of the state Maharashtra comprises of eight districts i.e. Aurangabad, Beed, Jalgaon, Parbhani, Hingoli, Nanded, Latur & Osmanabad. It is one of the important agricultural and industrial divisions of Maharashtra state which is the third largest state in area and population in India. The climate of the region, as stated, supports the vegetation that can be conveniently divided into tropical dry deciduous forests, open scrub jungles and vast tracts of grassland (Naik et al. 1998)

Today, the exhaustive use of allopathic medicines and their side effects is well known. Many allopathic drugs are affecting resistance and immunity in human beings. In order to minimize the effect of allopathic medicines on human body, herbal medicines are gaining popularity. There is a great deal of interest in the ayurvedic system of medicines for the reason that many chronic disorders which are not easily cured by allopathic medicines are being recovered by herbal treatment. Thus the demand for various commonly used medicinal plants in the production of ayurvedic medicine is ever increasing.

In view of this, the present study has been undertaken to exploit the potential of some medicinal plants of the region as a cure for various human disorders. Also the cultivation of this group of plants will help in diversifying our

agriculture for new cash crop. These plants have a large potential for all occasions. It was therefore though worthwhile to undertake such a study.

In the present investigation five species belonging to different families have been dealt with for scientific determination and evaluation as drug resources the study is expected to adduce data of considerable importance which may prove to be relevant in the practical utilization of our natural resources nationally and internationally as well as novel source of drugs.

The present work some of the medicinal plants of the region will provide an initiative step towards better understanding of the knowledge of the regional medicinal plant wealth. This will also help the cultivators that it's of equally importance to cultivate readily available medicinal plants along with the traditional cultivation of various crops.

Materials and Methods

The plant material of five plants belonging to Amaranthaceae and scrophulariaceae were collected from various places according to their occurrence and flowering season *Alternanthera pungens* H.B.&K. and *Alternanthera sessilis* (L.) Br. (*A. triandra* Lamk.) were collected locally from open place near roshangate. *Bacopa monneri* (L.) Wett. was collected from Siddharth Garden, Aurangabad, *Kickxia ramosissima* (wall) Janchen was collected from Bhadkal gate Aurnagabad and *Verbascum chinense* (L.) santapau. was collected from History museum garden, Dr. Babasaheb Ambedkar marathwada university, Aurangabad.

The whole plant was uprooted, cleared from the soil and dust, the leaves, stem, and root were separated and dried in air. The dried material was finely powdered, sieved through muslin cloth and stored for chemical analysis few uprooted plants of each species, were preserved in 70% alcohol. Leaf epidermal studies were carried out on fresh specimens. For which the peels were stained with safranin (1 %) mounted in glycerin and made semipermanent by ringing with DPX solution. Stomatal index (SI) Was calculated as defined by Salisbury (1927, 1933), Viz, $SI = \frac{S}{E+S} \times 100$. Where 'S' is the number of stomata per unit area, and 'E' is number of epidermal cells in the area. Stomata per unit area, and 'E' is the number of epidermal cells in the area. stomatal frequency and stomatal index have been expressed as an average of ten reading. Palisade ratio (PR), was calculated at the average of palisade cells (P) beneath each epidermal cell (E) as defined by Zoning and Weiss: (1925), as $PR = \frac{R}{E}$ Small areas of the green tissues outlined by the vein lets are termed as vein-islets or areoles. the vein-islets number is defined as the number of vein-islet per mm^2 of the leaf surface midway between the midrib and the margins. Levin (1920), determined vein let number of vein let number of

several dicot leaves. Vein termination number is defined as the number of vein let terminations per mm² of the leaf surface midway between the midrib and margin. A vein termination is ultimate free termination of vein let.

For study of vessels, fragments of plant organs like root and stem were macerated using a mixture of nitric acid (10%) and potassium dichromate (10%) solution in equal proportion. The vessel elements were stained with aqueous safranin (1%), dehydrated and mounted in DPX. some vessel members were also examined in glycerin.

The line and cellular sketches of the figure were drawn using a camera lucida. The range of length and width of vessel elements was determined by the measurement of 20-25 vessel elements, and were classified as per the classification given by Radford et. al. (1974), which is reproduced as below.

Class-A, extremely short-less than 175 um

Class-B, Very short-175to 250 um

Class-C, Moderately short- 251 to 350um

Class-E, Moderately long- 801 to 1100um

Class-F, very long- 1101 to 1900um

Class-G, extremely long- over 1900um

Transsection of fresh and preserved materiel of leaf, petiole, node, stem, and root were taken by free hand section. The sections were stained with safranin (1 %), light green, (1 %), and mounted in DPX after the customary dehydration. Some hand sections were also examined in glycerin. Microphotographs camera affixed to Olympus microscope. For leaf architecture, leaves were first cleared in 10 to 20% aqueous sodium hydroxide solution followed by trichloroacetic acid and

phenol solution (2:1 by weight) and then stained with Kores stamp pad purple ink (Rao et. al. 1980)

PHYSICAL EVALUTIONS

DETERMINATION OF MOISTURE CONTENT

Freshly plucked leaves were washed, blotted dry and weighed as initial weight (IW), These leaf-bits were dried in oven at 100^oC for 24 hours and then the dry weight (DW) was taken as per the method described in A.O.A.C (1970). Water or moisture contents= $(IW - DW) / IW + 100$.

CHLOROPHYLL DETERMINATION IN LEAF

Chlorophyll a, b, and total chlorophyll content were determined according to Yoshida et. al. (1976), using 80 % acetone as solvent for extraction of pigments. The O.D. was recorded at 663 and 645 nm.

DETERMINATION OF ASH VALUES

Total ash :

The residue after incineration of sample at 500-600^oC is ash. For this purpose, the sample was subjected to a high temperature up 600^oC and then the ash content was subjected. During ignition to such a high temperature, organic compounds got oxidized and passed of in the form of gases, while the mineral elements remained in the form of ash. Ash content was calculated using formula given in A. O. A. C. (1970)

Acid insoluble ash (A. I. A.) :

50 ml 5N hydrochloric acid was added to the ash obtained as above and heated for 30 minute in hot water bath. This mixture was allowed to cool and filtered through previously weighed whatman filter paper no 42. Filter paper was washed with water until the washing was free from acid. The filter paper along

with acid insoluble portion of ash was dried in an oven at 100°C overnight and then weighed. A.L.A. per unit weight was used for the determination of ash.

Water insoluble ash (W.I.A.) :

50 ml of distilled water was added to the total ash obtained and heated for 30 minute in hot water. This mixture was allowed to cool and filtered through previously weighed filter paper No 42. Filter paper along with water in soluble portion of ash was dried in an oven at 100°C overnight and then weighed W.I.A. per unit weight of the sample used for ash was determined.

DETERMINATION OF EXTRACTIVE PERCENTAGE:

Solubility of the air-dried drug in alcohol and water was studied by (Anon, 1985).

- **Water Soluble Extractive (W.S.E) :**

Suitable amount of air-dried material was coarsely powdered and macerated with 100 ml distilled water in a closed flask for 24 hours with frequent shaking. The solution was filtered and 52 ml of filtrate was evaporated in a weighed flat bottom shallow dish, further dried at 100°C and weighed. The percentage of W.S.E. was calculated with respect to air-dried material.

2) Alcohol Soluble Extractive (A.S.E.):

Air dried drug was coarsely powdered, weighed and macerated with 100 ml alcohol in a closed flask for 24 hours, with frequent shaking. the solution was filtered and 25 ml of filtrate was evaporated in a weighed flat bottom shallow dish, further dried at 100°C The percentage of to A.S.E. was calculated with respect to the air-dried material.

PHYTOCHEMICAL METHODS

HISTOCHEMISTRY

Histochemical tests were performed on fresh plant material according to Johanson (1940) and Gurr (1965). The tests were taken as follows.

1) STRACH :

0.3 g Iodine was dissolved in 100 ml distilled water. A drop of this solution added on the fresh section and observed for the presence of blue violet starch grains.

2) PROTEINS :

i) Saturated aqueous solution of picric acid is an excellent precipitating agent for protein. On standing for 24 hours in this solution, was stained yellow, indicating presence of proteins.

ii) For localizing proteins, a reagent was prepared by dissolving 0.1 gm potassium Ferro cyanide in 20 ml distilled water and 100 ml glacial acetic acid. Sections were kept in this solution for one hour and washed with 60% alcohol. To this, a few drops of ferric chloride solution were added. The blue colour indicated the presence of proteins.

3) TANNINS :

10 % Aqueous ferric chloride made alkaline with sodium carbonate produced blue-green colour with tannin.

4) SAPONINS :

The section was placed in saturated barium hydroxide solution for 24 hours. Then it was washed with calcium choride solution followed by

potassium dichromate solution. Yellow colour indicates the presence of saponins.

5) FATS :

0.5 gm of Sudan Iv dye was dissolved in 100 ml 70% alcohol. Section were kept in this solution for 20 minutes, rinsed quickly with 50 % alcohol and mounted in glycerin for observations. Blue, red, pink precipitate indicated presence of fats.

6) GLYCOSIDES (Grignard's Tests.) :

Section were immersed in 1% aqueous picric acid for 30 minutes, washed with water and placed in a drop of 10% aqueous sodium carbonate. A red colour section with hydrochloric acid indicated presence of glycoside.

For localization, section were placed in solution composed of 20 parts of 20 % aqueous KOH and 80 parts of 90% alcohol for a few minutes. In a small watch-glass a mixture of aqueous ferrous sulphate and 20 % aqueous ferric chloride solution was taken in equal proportions, heated to a boil, sections were put in this solution for a few minutes and then transferred to a slide holding a drop of 20% HCL. A dark blue or deep blue precipitate indicated presence of glycosides.

TOTAL ALKALOIDS :

- **Mayer's reagent**

H_gcl₂ (1.36 gm) in 60 ml distilled water and 5gm of potassium iodide in 10 ml distilled water both solution mixed and diluted to 100 ml with water, the section was treated with few drops of reagent. A white precipitate indicated the presence of alkaloids.

- **Wagner's reagent :**

Iodine (1.27gm) and Potassium iodide (2 gm) was dissolved in 5 ml distilled water and the solution made up to 100 ml with distilled water. Golden yellow or brown precipitate colour was taken by alkaloids containing cell.

- **Drangendroff's reagent:**

Bi (NO₃)₃·5H₂O (Bismuth sub nitrite), 8 gm was in 20 ml of concentrate nitric acid and 27.2 gm potassium iodide in 50 ml of distilled water and made up to 100 ml with distilled water. This reagent gave orange-red precipitate with a alkaloid containing cells.

PHYTOCHEMICAL METHODS

ESTIMATION OF REDUCING AND NON-REDUCING SUGARS

Sugars were estimated, following the procedure given by Folin we and outlined by Osier. (1979).

5gm Sample was boiled with 50-70 ml distilled water. cooled and filtered. The filtrate was diluted to 100 ml with distilled water. Two ml this sample solution was taken in a Fol in-Wu to be. To it alkaline copper solutir was added. These tubes were heated in a water bath for 8 minutes: and cooled to each tube, mixed well, to gave a blue colour. Each tube was measured at 420 nm. Reducing sugar was calculated, using the values of O.D obtained for glucose solution. For total sugar, 50 ml of the sample solution was acid hydrolyzed by boiling with 5 ml of 1N. HCl cooled and then 5 ml 1N. NaOH was added. Difference in the total and reducing sugars gave the amount of non-reducing sugars in the amount of powder taken.

ESTIMATION OF TOTAL EXTRACTABLE PHENOLIC COMPOUNDS:

The ethanolic extractable phenolic compounds were estimated by folin-phenol method by Malick and Singh (1980). The compounds were extracted by grinding 50 mg dry sample of tissues using chilled 80% (v/v) ethanol. The homogenate was centrifuged at 10,000rpm for 20 min. The supernatant was saved and residue was evaporated to dryness. Residue dissolved was in a known volume of distilled water to (3ml) in a test tube and after adding 0.5 ml of Folin-ciocalteu reagent, the contents were mixed thoroughly, placed the tubes in 650 nm uv-visible (spectrophotometrically). Standard curve was prepared using catechol (0.5mg/ml) and all the concentration were expressed in terms of mg/mg of this compound.

Morphology and medicinal uses

I) *Alternanthera pungens* H.B.& K (Plate 1-A)

Genus- *Alternanthera* forsk.

Species- *pungens*

Family - Amaranthaceae

Common name - Chubuk Kata.

Part used- leaves.

Alternanthera pungens is a branched prostrate herb. The leaves are broadly ovate, elliptic as oblanceolate. the flowers have spiny bracts and bracteoles and are born in axillary and terminal heads.

Medicinal uses: *Alternanthera pungens* plant have diuretic property Its decoction is taken to treat gonorrhea it is purgative.

II) *Alternanthera sessilis*. (L.) Br (Plate 1-B)

(*Alternanthera triandra*. Lamk)

Genus - *Alternanthera* forks.

Species- *sessilis* (L.) Br

Family - Amaranthaceae.

Common name- Mukun wenna

Part used - whole plant.

Alternanthera sessilis is a much branched prostrate herb, branches often frequently rooting at the nodes, Leaves are simple, opposite, somewhat fleshy lanceolate ,flowers small in axillary clusters.

Medicinal uses :

The plant is bitter, sweet, astringent digestive it is useful in vitiated condition of kappa and pitta, diarrhea, liprosy, skin diseases' and fever.

III) *Bacopa monnieri* (L.) wett. (Plate 1-C)

Family- Scrophulariaceae

Genus - *Bacopa* Aublet

Species - *monneri* (L.) Wett.

Common Name -Neer Bramhi

Part used- Whole plant and leaves.

The plant is prostrate, creeping or procumbent herb, stem fleshy, glabrous, leaves opposite or the upper alternate, sessile, fleshy narrowed at base, obtuse or sub acute, glabrous, Flowers are purple in colour, axillary solitary, of Fruit capsule. leaves are used in ayuevdic medicine and preparation of hair oil.

Medicinal uses:

Bacopa Monneri is a bitter in taste has been used in ayurvedic medicine for centuries It has long history of use in the ayurvedic medicine tradition in the treatment of number of disorders particularly those involving Anxiety, intellect and poor memory.

Traditionally, it was used as a brain tonic to enhance memory development learning and concentration.

Bramhi has been found to be very beneficial in the treatment of anxiety, neurosis and mental fatigue. It has been found to be significant improve IQ level, general ability, behavioural pattern and mental concentration in children. Bramhi is

also used in the treatment of epilepsy, insomnia, asthma and rheumatism. Bramhi has antioxidant, cardiotonic and Anticancer properties.

The plant is also used for all sort of skin problem, eczema, psoriasis etc.

Bramhi is better in flavour. In India it is used in salads, soups, as a cooked vegetable or pickled.

IV) *Kickxia ramosissima* (Wall.) Janchen. (Plate 2-A)

Family - *Scrophulariaceae*.

Genus- *Kickxia* Dumort.

Species: *ramosissima*.

Common name : Banvel (Branching cancerwort)

Part used - Whole plant.

The plant is diffuse or pendent glabrous or perennial herb, branching from the base, branches slender, leaves alternate or the lower opposite, triangular hastate., or 3-7 lobed, the Upper ones lanceolate saligata, flowers axillary solitary, yellow in colour. fruit capsule.

Medicinal Uses:

The plant is astringent inflammatory and diuretic. The plant is used as a remedy for diabetics. The plant is diuretic and shows antidiabetic activity, major chemical constituents. found positive in the plant are carbohydrate. steroid saponins aspirins, flavonoids, alkaloids, tannins, phenols etc.

V) ***Verbascum chinense*** (L.) santapau (Plate 2-B)

Genus-*Verbascum* L.

Species - *chinense* (L.) Santapau.

Family- Scrophulariaceae.

Common name- Kutki. (Chinese mullein)

Parts used- Whole plant

The plant is scapigous annual herb. scapules often branched near the top leaves or lanceolate, narrowed at base, lyram or pinnatest acute create 2-10 cm long, upper ones gradually smaller passing into sessile, coarsely dentate, hairy on both surfaces, flowers in simple or branched rcemes, flowers, yellow in colour, fruit capsule.

Medicinal uses:

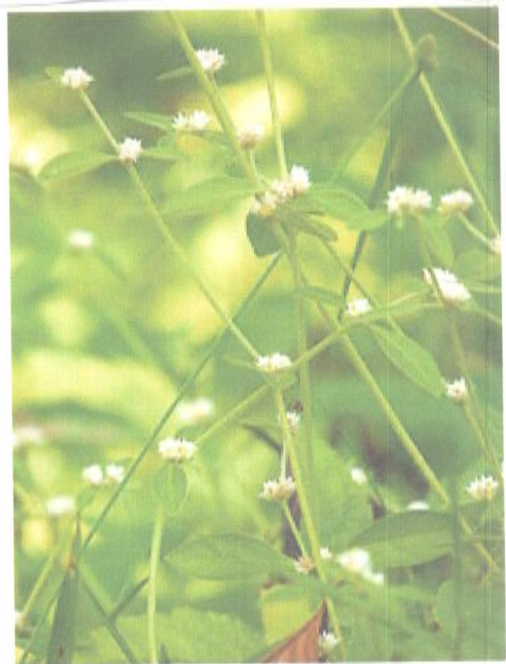
Verbascum chinese is a medicinal plant, it is commonly called as Chinese mullein and verbasum thapsus . (Mullein) the mullein plant is respiratory sedatives and demulcent, it is highly regared as safe lung tonic of particular use for respiratory ailments. such as asthan, bronchitis and dry cough.

Mullein is used in inflammatory symptoms. Skin orders and tumor formation.

It was used traditionally in the treatment of diarrhea rheumatism, it contain sapohin glycoside.



A *Alternanthera pungens*



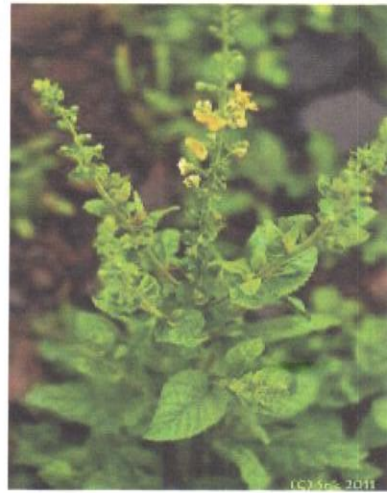
B *Alternanthera sessilis*



C *Bacopa monneri*



A *Kickxia ramosissima*



B *Verbascum chinense*

Observations

Alternanthera pungens (Plate 3. 1-7)

Vessel elements in root: (Fig.1)

- Shape - cylindrical
- Lateral wall thickening - simple pitted.
- Perforation plate -simple

Vessel element in stem: (Fig.2)

- shape- cylindrical
- Lateral wall thickening- Simple pitted
- Perforation plate - simple

T. S. of root (Fig.3)

It is circular in outline ,secondary growth is prominent .

cork is composed of 4-5 layers of tangentially elongated suberised rectangular cells followed by loosely arranged parenchymatous cortex Medullary rays are present Phloem is narrow and consist of phloem fibre. xylem is exarch and composed of radially arranged tracheary elements .

Node : (Fig.4)

The leaves are opposite at the nodal region the steler structure sends a prominent and an arc shaped trace laving behind gap.

The two traces for axillary bud are and given out next, which unites to form a ring like structure. The main trace remains unbranched and enters into the petiole .

Leaf : (Fig.5)

The leaf is dorsiventral and amphistomatic.the adaxial epidermis is of large unequal cells. The abaxial epidermal cells are comparatively small, the stomata are present on both the surfaces. They are more in number at lower epidermis

Mesophyll is differentiated into a palisade and spongy tissue. The palisade is 2-3 layered and spongy tissue is of loosely arranged cells.

The vascular bundles are collateral with xylem oriented towards upper side. The bundle sheath is present.

Epidermis: (Fig.6,7)

The leaves are anisostomatic, the numbers of stomata is less on the upper surface as compared to lower surface. The stomata is of the anacytic type. glandular hairs are common.

Alternanthera sessilis (*A. triandra*) (plate 4. 1-6)

Vessel elements in root: (Fig. 1)

- shape - cylindrical
- Lateral wall thickening - Simple pitted
- Perforation plate : simple

Vessel elements in stem :

- Shape - cylindrical
- Lateral wall thickening simple pitted
- Perforation plate - Simple

T.S.of root (Fig. 2) It is circular in outline in transverse section, secondary growth is prominent. cork is composed of 4-5 layers of tangentially elongated suberised rectangular cells followed by 8-10 layers of compactly arranged thin walled parenchymatous cortex. Primary xylem is triarch and cambium forms a complete ring of xylem surround of phloem at centre.

Node : (Fig. 3)

The leaves are opposite at nodal region, the stellar structure sends a prominent an arc shaped trace leaving behind a gap.

The two traces for axillary bud are given out next, which unite to form a ring like structure. The main trace divide into three and these traces enter into the petiole.

Leaf : (Fig.4)

The leaf is dorsiventral and amphistomatic, The adaxial epidermis is of larger unequal cells. The abaxial epidermal cells are comparatively smaller. The stomata present on both the surfaces, they are more in number at lower epidermis.

Mesophyll as differentiation into a palisade and spongy tissue, The palisade is 1-2 layered and spongy tissue is of loosely arranged cells.

In the midrib region three vascular bundles are present vascular bundle are collateral with xylem facing upwards. Bundle sheath is absent.

Epidermis : (Figs. 5,6)

The leaves are amphistomatic The number of stomata is less on the upper surface as compared to lower surface. The stomata are diacytic type, glandular trichomes are common

Bacopa monnieri (Plate 5. 1- 10)

Vessel elements in root : (Fig.1)

- Length of vessel element - 166.6 μ m to 499.8 μ m
- Diameter of vessel element - 16.6 μ m to 24.9 μ m
- Shape - cylindrical
- Lateral wall thickening - Bordered pitted.
- Perforation plate - simple

Vessel elements in Stem: (Fig.2)

- Length of vessel element - 148.9 μ m to 383.1 μ m
- Diameter of vessel element - 16.6 μ m to 33.3 μ m
- Shape - cylindrical
- Lateral wall thickening - Bordered pitted.
- Perforation plate - simple

Node : (Figs.3-5)

The leaves are developed in opposite manner at each node. The node has vascular tissue in the form of ring, a horse shoe shaped median trace is emerges out leaving behind a gap, upward this horse shoe shaped trace gives rise to two lateral traces towards inner side for axillary bud, The median trace then extends into the leaf unbranched, These two traces united to form a vascular cylinder which enters into axillary bud.

Leaf : (Fig.6)

The leaves are isobilateral and amphistomatic. The adaxial epidermal cells are larger, the outer walls are thick with thick cuticle. The cells of abaxial epidermis are smaller, the cuticle is thin. The stomata are confined to both the surfaces, the guard cells are with outer ledges. The glandular trichomes are common, They are generally sunken.

The mesophyll is composed of only spongy cells, the spongy cells are unequal in size with intercellular spaces.

The vascular bundles are many with xylem facing towards upper surface. The bundle sheath is parenchymatous.

The midrib vascular bundle is an arc-shaped with a parenchymatous bundle sheath.

Leaf architecture : (Figs.7-9)

Leaves are simple, opposite, venation is pinate, comptodromous - brochidodromous. Marginal venation incomplete. The vein termination show variation and include conventional and dilated tracheids. The bundle sheath is parenchymatous and occurs on the primary and secondary veins.

Epidermis : (Figs. 9,10)

The leaves are amphistomatic, the number of stomata is less on the upper surface as compared to lower surface. The stomata are anomocytic type and the glandular trichomes are common.

Kickxia ramosissima (Plate 6. 1-11)

Vessel elements in root: (Figs.1,2)

- Length of vessel element - 99.9 -449.8 um
- Diameters of vessel element 33.3 -44.9 um
- Average length -296.5 um
- Shape-cylindrical, conical.
- Lateral wall thickening -Bordered pitted.
- Perforation plate - simple.

Vessel elements in stem: (Figs.3,4)

- Length of vessel element 283.2 um - 446 um
- Diameters of vessel element -33.3 um 64.3 um
- Average length -388 um
- Shape - cylindrical
- Lateral wall thickening - Bordered pitted
- Perforation plate - simple.

Node : (Figs.5,6)

The leaves are alternate at nodal region the stellar structure sends a prominent an arc shaped median trace leaving behind a gap.

The two traces for the maxillary bud are given out next which unite to form a ring like structure. The median trace remains unbranched and enters into the petiole.

Leaf : (Fig.7)

The leaf is dorsiventral and amphistomatic, The adaxial epidermis is of larger unequal cells. The abaxial epidermal cells are comparatively smaller. The stomata are present on both surfaces they are more in number at lower epidermis. The guard cells with outer led.

Mesophyll is differentiated into a palisade and spongy tissue, the palisade is one layered and spongy tissue is of loosely arranged cells.

The vascular bundles are collateral with xylem oriented towards upper side. The bundle sheath is parenchymatous and one layered.

The hypodermis is parenchymatous in the midrib region. The vascular bundles are three in number and each is surrounded by bundle sheath.

Leaf Architecture : (Figs.8,9)

leaves are simple.

Venation - reticulate, actinodromous

Marginal venation- looped

Primary and secondary veins terminate in a loop as a main vein and apical vein join with the tip of the principal vein. Aerenchyma are empty. and well developed and variable in size.

Epidermis : (Figs.10-11)

The leaves are amphistomatic, the number of stomata is less on upper surface as compared to lower surface. the stomata is anisocytic type glandular. Glandular hairs are common.

Verbascum chinense (Plate 7. 1-13)

Observations:

Vessel element in root: (Fig - 1,2)

- Length of vessel element - 199.9um - 566.4um.
- Diameter of vessel element - 16.6um - 41.6um.
- Average Length - 326.5 um
- Shape-cylindrical.
- Lateral wall thickening - Bordered pitted and reticulate
- Perforation plate - Simple.

Vessel elements in stem: (Fig.3,4)

- Length of vessel element - 249.9 um to 1082.9 um
- Diameter of vessel element - 24.9 um to 33.3 um.
- Average length - 673.06 um.
- Shape - cylindrical.
- Lateral wall thickening - Bordered pitted
- Preforation plate - simple.

Node: (Fig.5,6,7)

The leaves arise in alternate manner at the nodal region, from the axial cylinder an arc shaped; median trace is given out at the nodal region leaving behind a prominent gap. It is three lobed, upward this median trace increases in size and gives rise to two lateral traces The lateral trace splits further into two at the base of the petiole, Thus one median and four lateral traces enter into the petiole. The node is unilacunar one traced.

After the emergence of the median trace, two traces from the area come out and then the gap is filled up. The two traces join to each other forming a cylindrical structure for an axillary bud.

Leaf : (Fig.8,9)

The leaves are dorsiventral or isobilateral and amphistomatic. The upper epidermis is of rather larger cells. The outer cell wall is thick. The lower epidermis is of smaller cells, the cuticle is rather thick at adaxial surface. The stomata are confined to both the surface, both glandular and non-glandular trichomes are common.

The mesophyll consists of palisade and spongy tissue. In dorsiventral leaf the palisade is three layered and spongy cells are loosely arranged. In isobilateral leaves two layered palisade occur at both the sides, while spongy cells are situated in between.

The vascular bundles are inversely oriented and extend through the spongy tissue. In the midrib region the epidermis is followed by one to two layered collenchymatous cortex in dorsiventral leaf and parenchymatous cortex in isobilateral leaf. A large arc shaped vascular bundle occur in the centre of midrib, it is surrounded by two to three layered thick walled bundle sheath.

Leaf architecture: (Fig.10,11)

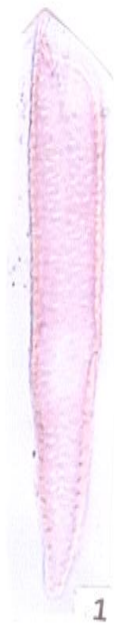
Leaves are simple, Venation is pinnate semi-craspedodrmous marginal venation Incomplete.

Primary and secondary veins terminate in a lobe as a main vein and apical vein join with the tip of the principal vein. A variation in teeth vasculature is found to be interesting. The accumulation of tracheids is a constant feature.

The marginal region of the serration show a number of blind vein endings each of these is expanded at the tip and shows an increase in the amount of tracheids. Aerenchyma are imperfect.

Epidermis : (Fig.12,13)

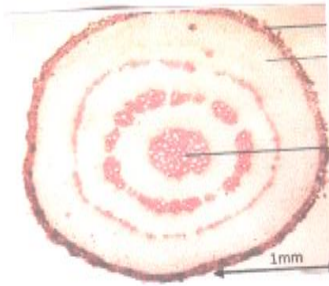
The leaves are amphistomatic, the number of stomata is less on the upper surface as compared to lower surface, cuticular striations are present on adaxial surface, the stomata are anomocytic type, glandular and non-glandular trichomes are common.



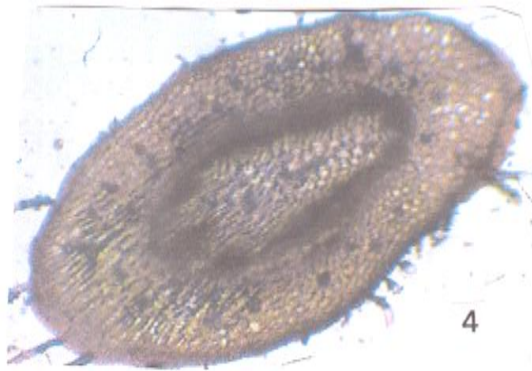
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2



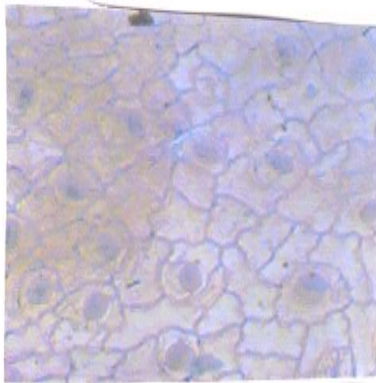
3



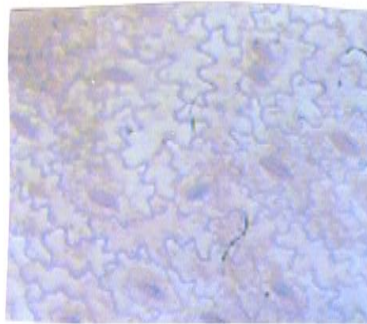
4



5



6



7

Plate 3

Alternanthera pungens

Fig. 1- Vessel element in root

Fig. 2- Vessel element in Stem

Fig. 3 T.S. of Root

Fig. 4- T.S. of node

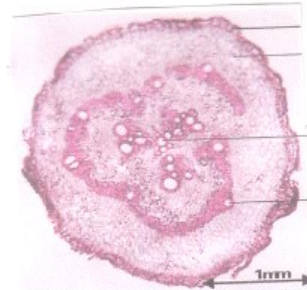
Fig. 5 T.S. of leaf

Fig. 6 Adaxial epidermis

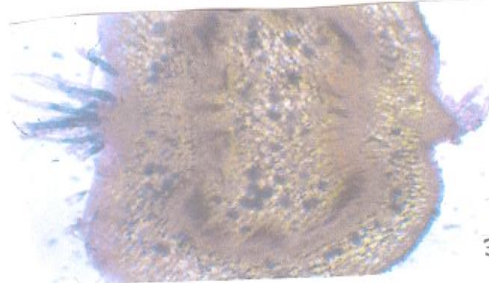
Fig. 7 - Abaxial epidermis



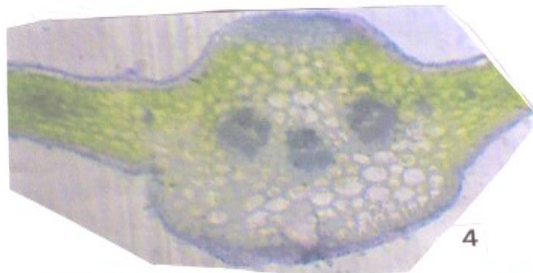
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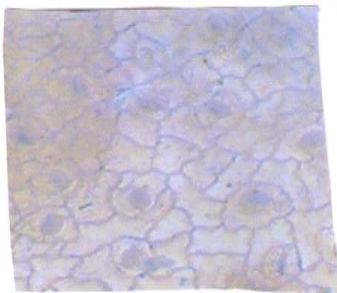
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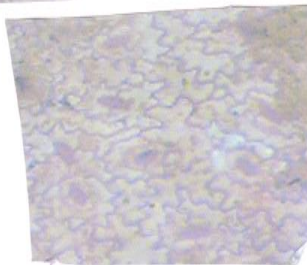
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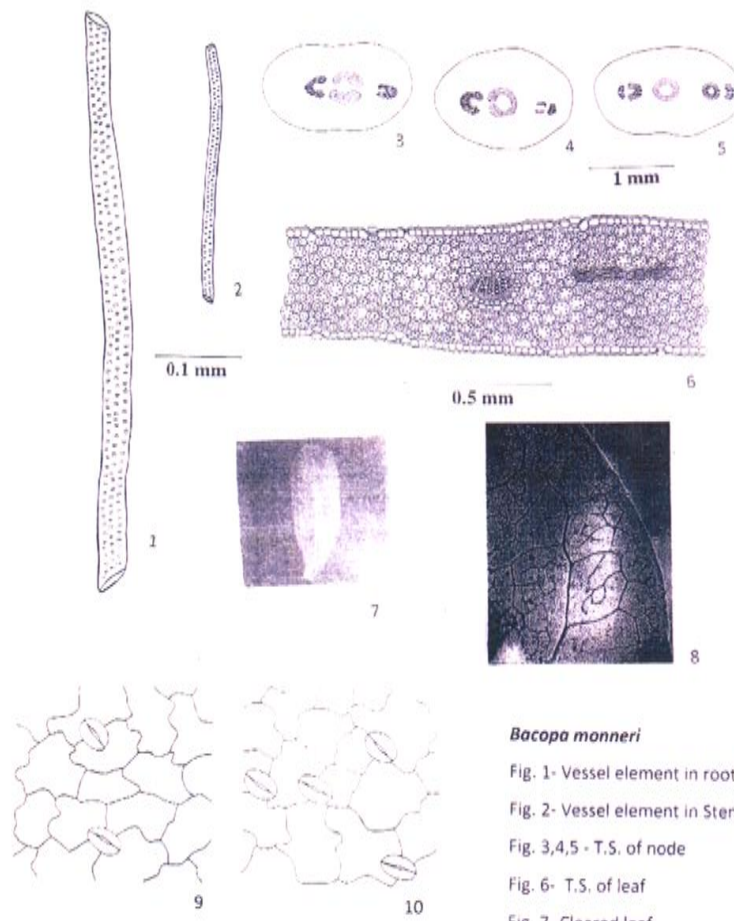
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6

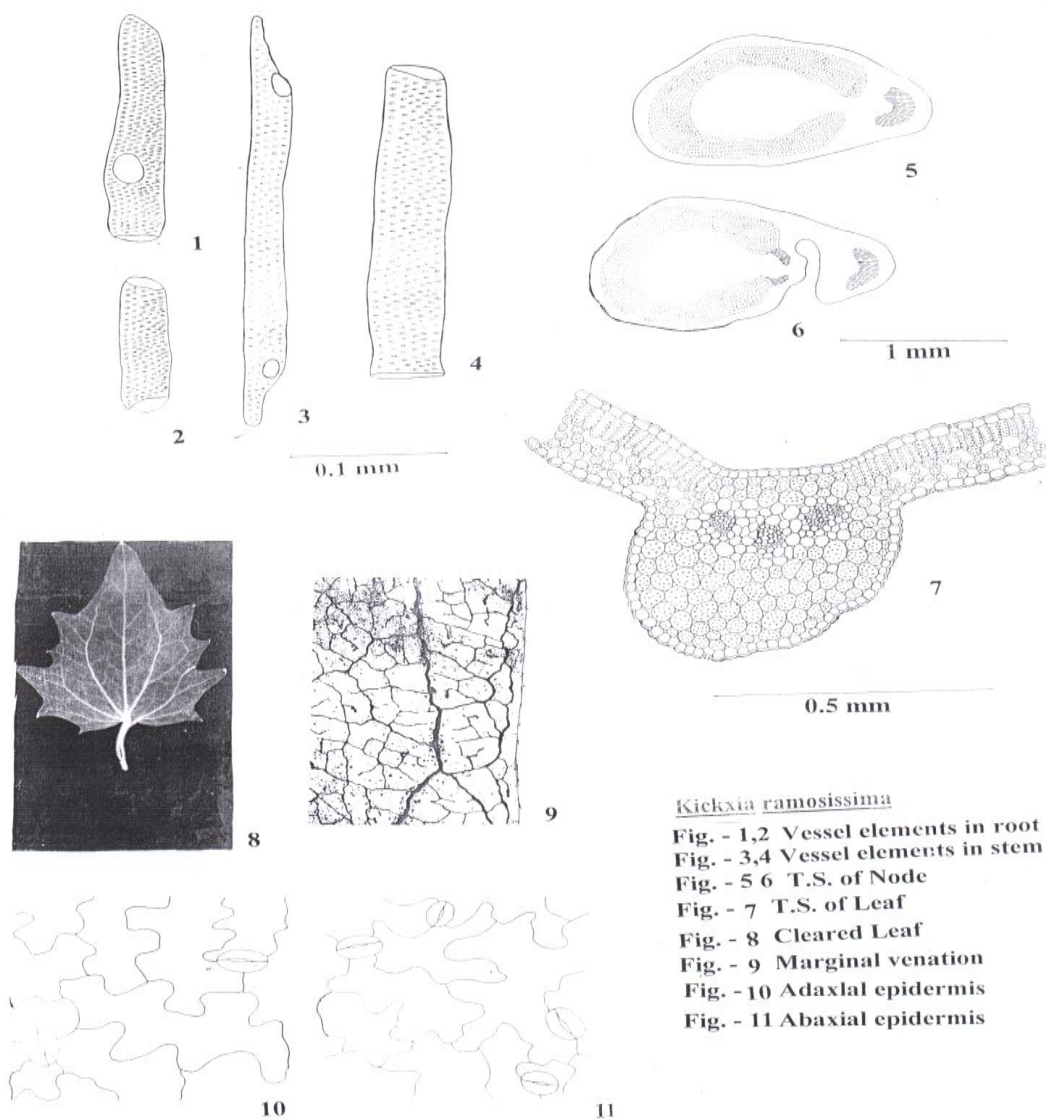
Alternanthera sessilis

- Fig. 1- Vessel element in root
 Fig. 2 T.S. of Root
 Fig. 3 T.S. of node
 Fig. 4 T.S. of leaf
 Fig. 5 Adaxial epidermis
 Fig. 6 - Abaxial epidermis



Bacopa monnieri

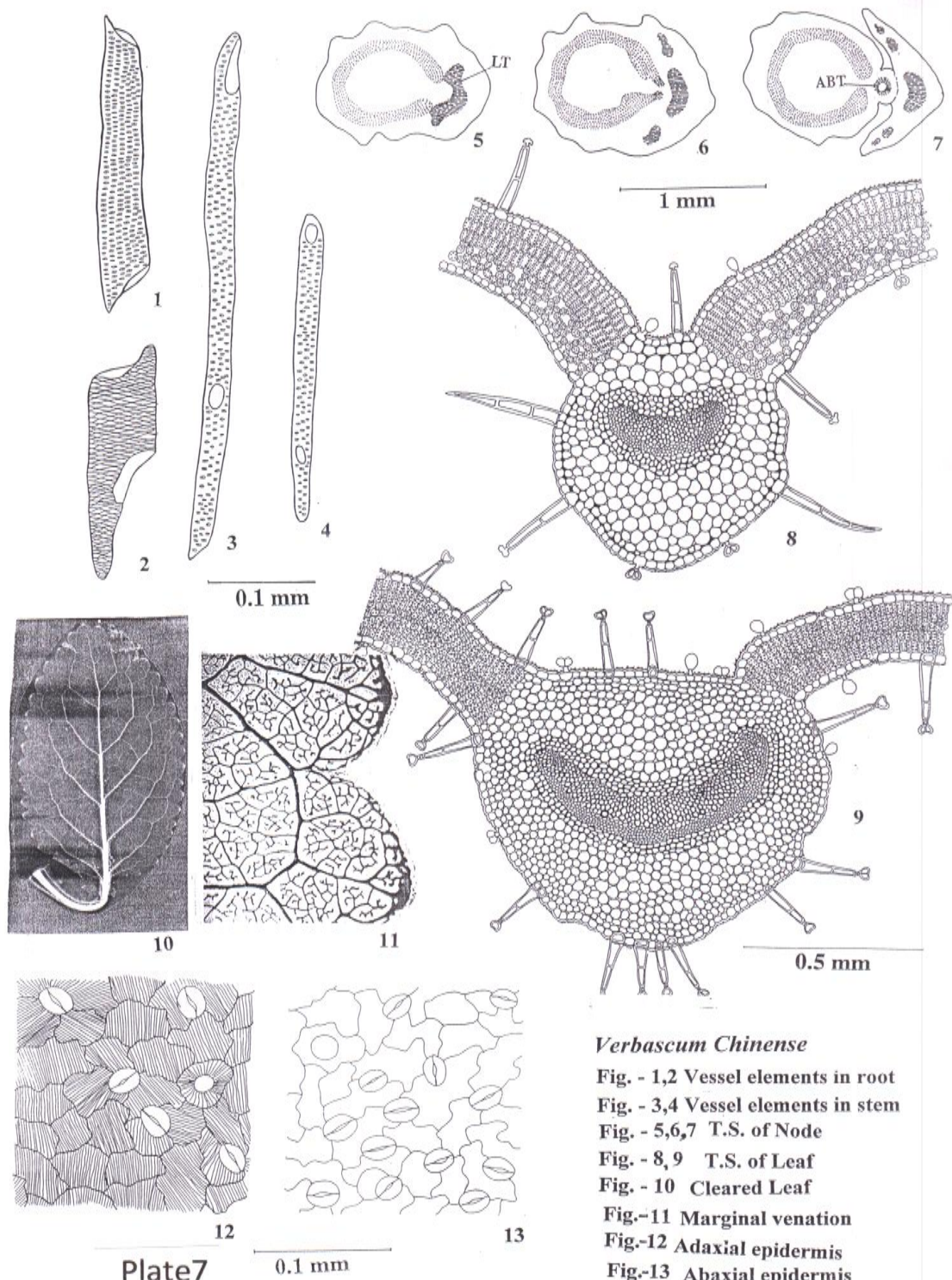
- Fig. 1- Vessel element in root
- Fig. 2- Vessel element in Stem
- Fig. 3,4,5 - T.S. of node
- Fig. 6- T.S. of leaf
- Fig. 7- Cleared leaf
- Fig. 8- Marginal venation
- Fig. 9- Adaxial epidermis
- Fig. 10- Abaxial epidermis



Kickxia ramosissima

- Fig. - 1,2 Vessel elements in root
- Fig. - 3,4 Vessel elements in stem
- Fig. - 5 6 T.S. of Node
- Fig. - 7 T.S. of Leaf
- Fig. - 8 Cleared Leaf
- Fig. - 9 Marginal venation
- Fig. - 10 Adaxial epidermis
- Fig. - 11 Abaxial epidermis

Plate 6



Verbascum Chinense

Fig. - 1,2 Vessel elements in root

Fig. - 3,4 Vessel elements in stem

Fig. - 5,6,7 T.S. of Node

Fig. - 8,9 T.S. of Leaf

Fig. - 10 Cleared Leaf

Fig.-11 Marginal venation

Fig.-12 Adaxial epidermis

Fig.-13 Abaxial epidermis

B) Histochemistry (Table No. 1,2,3)

The ergastic substances remain localized in a particular organ or a tissue or remain distributed in the plant body, generally metabolites are found in metabolically active tissue . metabolites like tannins, glycosides,saponins and alkaloids in leaves stem and root were studied, results are tabulated in the all five species studied.

Reactivity of powder with different reagents (Table No.4,5,6,7,8)

Reactivity of powder with different of root, stem, leaves tested with different reagent powder gave specific colour reaction on the basis of which the presence and absence of active chemical compound can be detected.

Phytochemical analysis :

Phytochemical analysis of *Alternanthera pungens* is given in the Table No. 1

Test	Stem	Root	Leaves
Starch Test	Present	Absent	Present
Proteins	Present	Present	Present
Tannins	Present	Absent	Present
Saponins	Present	Present	Present
Fats	Present	Present	Present
Glycosides	Absent	Absent	Absent
Alkaloids	Present	Present	Present

Phytochemical analysis *Alternanthera sessilis* is given in the Table No. 2

Test	Stem	Root	Leaves
Starch Test	Present	Absent	Present
Proteins	Present	Present	Present
Tannins	Present	Absent	Present
Saponins	Present	Present	Present
Fats	Present	Present	Present
Glycosides	Absent	Absent	Absent
Alkaloids	Present	Present	Present

Phytochemical analysis of *Bacopa monneri* is given in the Table No 3

Test	Stem	Root	Leaves
Starch Test	Present	Present	Present
Proteins	Present	Present	Present
Tannins	Present	Present	Present
Saponins	Present	Present	Present
Fats	Present	Present	Present
Glycosides	Absent	Absent	Absent
Alkaloids	Present	Present	Present

Phytochemical analysis of *Kickxia ramosissima* is given in the Table no 4

Test	Stem	Root	Leaves
Starch	Absent	Absent	Present
Proteins	Present	Present	Present
Tannins	Absent	Absent	Absent
Saponins	Present	Present	Present

Fats	Present	Present	Present
Glycosides	Absent	Absent	Absent
Alkaloids	Present	Present	Present

Phytochemical analysis of *verbascum chinense* is given in the Table No 5

Test	Stem	Root	Leaves
Starch	Prebsent	Present	Present
Proteins	Present	Present	Present
Tannins	Present	Absent	Present
Saponins	Present	Present	Present
Fats	Present	Present	Present
Glycosides	Absent	Absent	Absent
Alkaloids	Present	Present	Present

Table No. 6
Effect Of Chemical On Powdered Drug Of *Alternanthera pungens*

Sr.No.	Reagent	Leaf	Stem	Root
1	Powder	Dark green	Light	Light brown
2	Powder + iodine	Brown	Brown	Light brown
3	Pd+5% Ferric Chloride	Brown	Light brown	Brown
4	Pd + NaoH	Light brown	Light brown	Dark brown
5	Pd + Acetic Acid	Light Brown	Brownish	Yellow brown
6	Extracts + Acetic acid +50% H2So4	Light brown	Yellow green	Faint Yellow
7 ,	Pd + 50% H2So4	Greenish	Dark green	LightGreen
8	Pd +50% Concentrate HCL	Green	Light Yellow	Green
9	Pd + Ammonia	Brown	Light Yellow	Light brown
10	Pd + Ammonia + Pot.	Light brown	Yellow brown	Light brown

	Ferrocyanide			
11	Extracts +4% + NaoH+1% CuSo4	Dark brown	Faint brown	Dark yellow
12	Extracts + 4% + NaoH+1% Lead Acetate	Yellowish	Yellowish	Dark Green
13	Pd+50% + Nitric Add + Picric Acid	Redish brown	Lemon Green	Lemon Yellow
14	Pd+ Saturated picric acid.	Orange brown	Light orange	Light Yellow

Table No. 7
Effect Of Chemical On Powdered Drug Of *Alternanthera sessilis*

Sr.No.	Reagent	Leaf	Stem	Root
1	Powder	Olive green	Light	Light brown
2	Powder + iodine	Brown	Brown	Light brown
3	Pd+5% Ferric Chloride	Brown	Light brown	Brown
4	Pd + NaoH	Light brown	Light brown	Dark brown
5	Pd + Acetic Acid	Light Brown	Brownish	Yellow brown
6	Extracts + Acetic acid +50% H2So4	Light brown	Yellow green	Faint Yellow
7 ,	Pd + 50% H2So4	Greenish	Dark green	Light Green
8	Pd +50% Concentrate HCL	Green	Light Yellow	LightGreen
9	Pd + Ammonia	Light brown	Light Yellow	Light brown
10	Pd + Ammonia + Pot. Ferrocyanide	Light brown	Yellow brown	Light brown
11	Extracts +4% + NaoH+1% CuSo4	Dark brown	Faint brown	Dark yellow
12	Extracts + 4% + NaoH+1% Lead Acetate	Yellowish	Yellowish	Dark Green
13	Pd+50% + Nitric Add + Picric Acid	Redish brown	Lemon Green	Lemon Yellow
14	Pd+ Saturated picric acid.	Orange brown	Light orange	Dark Yellow

Table No. 8
Effect Of Chemical On Powdered Drug Of *Bacopa monneri*

Sr.No.	Reagent	Leaf	Stem	Root
1	Powder	Olive green	Light	Light brown
2	Powder + iodine	Brown	Brown	Light brown
3	Pd+5% Ferric Chloride	Brown	Light brown	Brown
4	Pd + NaoH	Light brown	Light brown	Dark brown
5	Pd + Acetic Acid	Brown	Brownish	Yellow brown
6	Extracts + Acetic acid +50% H2So4	Light brown	Yellow green	Faint Yellow
7 ,	Pd + 50% H2So4	Greenish	Dark green	Green
8	Pd +50% Concentrate HCL	Green	Light Yellow	Green
9	Pd + Ammonia	Light brown	Light Yellow	Light brown
10	Pd + Ammonia + Pot. Ferrocyanide	Light brown	Yellow brown	Light brown
11	Extracts +4% + NaoH+1% CuSo4	Dark brown	Faint brown	Dark yellow
12	Extracts + 4% + NaoH+1% Lead Acetate	Yellowish	Yellowish	Dark Green
13	Pd+50% + Nitric Add + Picric Acid	Redish brown	Lemon Green	Lemon Yellow
14	Pd+ Saturated picric acid.	Orange brown	Light orange	Dark Yellow

Table No. 9
Effect Of Chemical On Powdered Drug Of *Kickxia ramosissima*

Sr.No.	Reagent	Leaf	Stem	Root
1	Powder	Olive green	Light	Light brown
2	Powder + iodine	Brown	Brown	Light brown
3	Pd+5% Ferric Chloride	Brown	Light brown	Brown
4	Pd + NaoH	Light brown	Light brown	Dark brown
5	Pd + Acetic Acid	Brown	Brownish	Yellow brown
6	Extracts + Acetic acid +50% H2So4	Light brown	Yellow green	Faint Yellow
7 ,	Pd + 50% H2So4	Greenish	Dark green	Green
8	Pd +50% Concentrate HCL	Green	Light Yellow	Green

9	Pd + Ammonia	Light brown	Light Yellow	Light brown
10	Pd + Ammonia + Pot. Ferrocyanide	Brown	Yellow brown	Light brown
11	Extracts +4% + NaoH+1% CuSo4	Dark brown	Faint brown	Dark yellow
12	Extracts + 4% + NaoH+1% Lead Acetate	Yellowish	Yellowish	Dark Green
13	Pd+50% + Nitric Add + Picric Acid	Redish brown	Lemon Green	Lemon Yellow
14	Pd+ Saturated picric acid.	Orange brown	Light orange	Dark Yellow

Table No. 10
Effect Of Chemical On Powdered Drug Of *Verbascum chinense*

Sr.No.	Reagent	Leaf	Stem	Root
1	Powder	Olive green	Light	Light brown
2	Powder + iodine	Brown	Brown	Light brown
3	Pd+5% Ferric Chloride	Brown	Light brown	Brown
4	Pd + NaoH	Light brown	Light brown	Dark brown
5	Pd + Acetic Acid	Brown	Brownish	Yellow brown
6	Extracts + Acetic acid +50% H2So4	Light brown	Yellow green	Faint Yellow
7 ,	Pd + 50% H2So4	Greenish	Dark green	Green
8	Pd +50% Concentrate HCL	Green	Light Yellow	Green
9	Pd + Ammonia	Light brown	Light Yellow	Light brown
10	Pd + Ammonia + Pot. Ferrocyanide	Light brown	Yellow brown	Light brown
11	Extracts +4% + NaoH+1% CuSo4	Dark brown	Faint brown	Dark yellow
12	Extracts + 4% + NaoH+1% Lead Acetate	Yellowish	Yellowish	Dark Green
13	Pd+50% + Nitric Add + Picric Acid	Redish brown	Lemon Green	Lemon Yellow
14	Pd+ Saturated picric acid.	Orange brown	Light orange	Dark Yellow

C) Physical Evaluation

i) Moisture contents -

Shows Moisture and chlorophyll contents *Bacopa monneri* shows highest percentage of Moisture content and *kickxia ramosissima* shows lowest percentage of Moisture content

1) *Kickxia ramosissima* -

The plant showed 52.20% Moisture content. Total chlorophyll content was 2.884 while chlorophyll -a and chlorophyll -b content were 1.674 & 1.21 Mg/g.

2) *Alternanthera pungens* -

The plant showed 80.55% Moisture content. Total chlorophyll content was 1.957 Mg/g while amount chlorophyll -a was 95.57 Mg/g and chlorophyll -b was 97.4 Mg/g.

3) *Alternanthera sessilis* -

The plant showed 82.13% Moisture content. Total chlorophyll content was 1.960 mg/g chlorophyll - and chlorophyll - b were 0.840 mg/g and 4.12 mg/g respective.

4) *Bacopa monneri*-

The plants showed 83.72% Moisture content. Total chlorophyll content was 1.850 mg/g while chlorophyll - a and b content were 0.690 mg/gm & 1.16 mg/g.

5) *Verbascum chinense* -

The plant showed 80.14% Moisture content. Total chlorophyll content was 1.632 mg/gm chlorophyll -a 0.808 mg/g and chlorophyll - b was 0.824 mg/g.

ii) **Ash Values** - Ash value gives the amount of inorganic substances present in the drug. Maximum ash value is observed in *Verbascum Chinese* and minimum ash value is observed in *Kickxia ramosissima*

***Kickxia ramosissima* -**

The total amount of ash in the leaf of this plant was 4.78%. The acid soluble ash was 2.367 and acid insoluble ash is 2.42% water soluble ash 3.73% and water insoluble ash as 1.05%.

The total ash content in the stem is 3.20% and acid soluble ash was 2.40% and acid insoluble ash is 0.80% Water soluble ash 2.48% and water insoluble ash as 0.80%

The total ash content in the root is 2.72% and acid soluble ash was 0.50% and acid insoluble ash was 2.20%. Water soluble ash 2.07 and water insoluble ash is 0.65%. :

***Alternanthera pungens*-**

The total amount of ash in the leaf of this plant was 3.00%. The acid soluble ash was 2.75% and acid insoluble ash is 0.25%. Water soluble ash was 2.80% and water insoluble ash was 0.20%

The total amount of ash in the stem is 4.05%. the acid soluble ash was 3.20% and acid insoluble ash was 0.85%. Water soluble ash was 3.107% and water insoluble ash was 0.95%

The total amount of ash in the root is 4.25%. The acid soluble ash was 3.25% and acid insoluble ash was 1.00%. Water soluble ash was 3.15% and water insoluble ash was 1.10%

***Alternanthera sessilis*-**

The total ash content of the leaf is 3.3% acid soluble ash was 2.8% while acid insoluble ash was 0.50%. Water soluble ash was 2.70% and water insoluble ash was 0.60%.

The total amount of ash in the stem is 3.6% acid soluble ash was 3.30% and acid insoluble ash was 0.30%. Water soluble ash was 3.00% and water insoluble ash was 0.60%.

The total amount of ash in the root is 4.5% acid soluble ash was 3.35% and acid insoluble ash was 1.15%. Water soluble ash is 3.9% while water insoluble ash was

***Bacopa monneri* -**

The total amount of ash in the leaf is 6.58% acid soluble ash was 4.34% and acid insoluble ash was 2.24%. Water soluble ash was 5.58% and water soluble ash was **1.00%**.

The total amount of ash in the stem is 6.06% acid soluble ash was 4.44% and acid insoluble ash was 1.62%. Water soluble ash was 5.14% and water in soluble ash was 0.82%.

The total amount of ash in the root is 2.1% acid soluble ash was 0.18% and acid insoluble ash was 1.92%. Water soluble ash was 1.36% and water in soluble ash was 0.75%.

***Verbascum chinense* -**

The total amount of ash in the leaf is 7.0% acid soluble ash was 4.52% and acid insoluble ash was 2.48%. Water soluble ash was 5.70% and water insoluble ash was 1.30%.

The total amount Of ash in the stem was 8.0% acid soluble ash was 6.14% and acid insoluble ash was 1.26%. Water soluble ash was 5.96% and water insoluble ash was 2.04%.

The total amount of ash in the root was 10.0% acid soluble ash was 7.20% acid insoluble ash was 2.80%. Water soluble ash was 9.20% and water insoluble ash was 0.80%.

Moisture and Chlorophyll content. Table No.11

Sr. no.	Name of the plant	Moisture content (%)	Total Chlorophyll-a mg/gm	Total Chlorophyll-b mg/gm	Total Chlorophyll-t mg/gm
1.	<i>Kickxia ramosissima</i>	52.20	1.674	1.21	2.884
2.	<i>Verbascum chinense</i>	80.55	96.57	97.4	195.7
3.	<i>Alternanthera sessilis</i>	82.13	0.840	1.12	1.960
4.	<i>Bacopa monneri</i>	83.72	0.690	1.16	1.850
5.	<i>Alternanthera pungens</i>	80.14	80.8	82.4	163.2

Conclusions

Anatomical studies of root, node, leaf, leaf architecture and epidermis has been carried out. Vessel element in root and stem was studied. Vessel elements are bordered pitted in *Bacopa*, *Kickxia*, *Verbascum* and vessels are simple pitted in *Alternanthera* species. Node is unilacunar one traced in all plant species. Leaf is dorsiventral and amphistomatic in all plant species except *Bacopa*, In *Bacopa* leaves are isobilateral and amphistomatic. Leaf architecture of plant species studied of scrophulariaceae shows major pinnate type of venation pattern. sub-type venation is Brochidodromous in *Bacopa*, Actinodromous in *Kickxia* and Camptodromous-brochidodromous in *Verbascum*, Marginal venation is complete in *Kickxia* and incomplete in *Bacopa* and *Verbascum*. leaves are amphistomatic, the number of stomata is less on the upper surface as compared to the lower surface. stomata is anomocytic type in *Bacopa*, *Kickxia* and *Verbascum*, stomata is anomocytic and diacytic type in *Alternanthera* species. Glandular and non glandular trichomes are common.

Phytochemical analysis root, stem and leaves has been carried out. In *Alternanthera pungens* and *A. sessilis* proteins, saponins, fats and alkaloids are present in root, stem and leaves. starch and tannins are present in stem and leaves, absent in root and glycosides are absent in all plant parts studied. In *Bacopa monneri* starch, proteins, tannins, saponins, fats and alkaloids are present in root, stem and leaves and glycosides are absent. In *Kickxia ramosissima* proteins, saponins, fats are present in root, stem and leaves, alkaloids are present in root, stem absent in leaves, starch is present in leaves and absent in root and stem and glycosides are absent in all plant parts studied. In *Verbascum chinense* starch, proteins, saponins, fats and alkaloids are present root, stem and leaves tannins are

present in stem and leaves and absent in root and glycosides are absent in all plant parts studied.

Shows Moisture and chlorophyll contents *Bacopa monneri* shows highest percentage of Moisture content and *kickxia ramosissima* shows lowest percentage of Moisture content

Ash value gives the amount of inorganic substances present in the drug. Maximum ash value

is observed in *Verbascum chinense* and minimum ash value is observed in *Kickxia ramosissima*

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