

**PHARMACOGNOSTICAL STUDIES ON ACALYPHA INDICA
LINN. AND HEDYOTIS PUBERULA LINN.
(ANTI - ASTHMATIC PLANTS)**

By

GEETHA S.

Reg. No. 92PLS02

A THESIS SUBMITTED TO
THE AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER
EDUCATION FOR WOMEN (DEEMED UNIVERSITY) COIMBATORE-641 043
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE IN LIFE SCIENCES.

APRIL, 1994.

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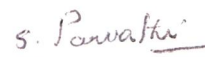
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the Guide

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Introduction

INTRODUCTION

The origin of botanical science may be traced to the investigation of the medicinal properties of plants. Search for the healing properties of plants to mitigate the misery of human beings caused by various ailments led to the serious study of the plants around them. Thus developed the science of Ayurveda which forms an important part of Atharva veda the most ancient and celebrated treatise on Hindu medicine, although the use of some plants is mentioned earlier in Rig veda.

Herbalism has been one of the main branches of medicine for centuries in all parts of the world, and the science of therapeutics as we know it is comparatively recent. And, since it is the fashion to ascribe to Europe every advance in the scientific field, let us causally, note that in Europe some of our grandmothers herbal remedies are still effective, that in some cases they are as good as those of modern medicine, and that many modern European medicines are modifications or survivals of ancient herbalism (Biswas & Chopra, 1982).

The use of western drugs is getting decreased day by day due to the side effects in their long course of administration. This leads to the use of the drugs of Ayurvedic, Unani, siddha and other traditional systems of medicine. With the views, the present day pharmacutists are

depending only on the plants for their drug preparation.

Hippocrates prescribed eating garlic as treatment for uterine tumors, and the Bower manuscript, dating from about AD 450, in India, recommended garlic as a cure for abdominal cases. From the National Cancer Institute Central Files, Hartwell reported that cancer in France is supposed lowest where garlic consumption is greatest, that garlic eaters in Bulgaria do not have cancer (Lewis and Elvin Lewis, 1977).

The school of chemists obtained raw materials from plants in order to obtain potent new drugs. In India, dramatic relief from trigeminal neuralgia is obtained by prescribing an indigenous drug recipe along with isonicotinic acid hydrazide. The potent drug taken in tablet form includes : Capparis moonii, Caesalpinia digyna, Withania somnifera, Tinospora cordifolia, Allium sativum and other species (Lewis and Elvin - Lewis, 1977).

One of the outstanding developments in the field of natural products in recent times has been the discovery of therapeutic properties of Rauvolfia serpentina for the treatment of hypertension and mental disease. This discovery stimulated investigators the world over to re-emphasize the research for medicinal plants.

Because of the adverse side effects of the present day conventional synthetic drugs, people are afraid of using

them. In spite of multifaceted and multidimensional progress of medical science, the germ theory and the anti-germ drugs have failed to cure several ailments. Not able to successfully isolate the causative organism, some diseases are classified under functional or metabolic group.

For most of these diseases, only a substitute or a supplement is administered which no doubt provides temporary relief. The patient has to depend upon these supplements for his life till these are found to be inadequate as well as ineffective and he suffers from the adverse consequences of these drugs. At this stage, both the doctor and the patient feel helpless and the unfortunate patient succumbs to his ailments. This shortcoming of the conventional therapy is gradually being appreciated more and more, and thus, there is intensive search for an alternative. This leads them to the exploration of herbal medicines.

The Greeks of old used herbs such as mustard, cinnamon, Gentian, Rhubarb and many others. A pupil of Aristotle wrote ten books on the history of plants and Alexander the Great made a number of expeditions into Africa, Persia and India and brought back herbs in use in those centuries. An infusion of the leaves of Ammi visnaga Lam. has been used in Egypt and neighbouring countries as a folk remedy for cough and colic. For bronchial asthma, lobeline from Lobelia inflata and ephedrine from chinese plant Ma

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Huang were introduced in medicine in 1925 by Chen of Ei Lilly and co., U.S.A. This takes us to an example of development of a totally synthetic drug based upon a natural product mode (Kadans, 1983).

Man used the parts of the plants such as roots, stem and bark, etc., for the treatment of various diseases. The leaves of Aegle marmelos, Azardacta indica and Catharanthus roseus etc., have been found to be used as anti-diabetic in action. The seeds of Lawsonia sps and the leaves of Eclipta alba were found to have antisterility effects. Similarly the whole parts such as root, stem and leaves of the plants Acalypha indica Linn. (Euphorbiaceae) and Hedyotis puberula Linn. (Rubiaceae) were believed to act as anti-asthmatic plants (Nadkarni, 1954).

Acalypha indica Linn. and Hedyotis puberula Linn. is useful against asthma and pneumonia (Rastogi and Mehrotra, 1990). Apart from asthma both the plants, acts against, bronchitis, rheumatism, pneumonia, bronchial catarrh, and snake bite (Kirtikar & Basu, 1981).

The leaves of Acalypha indica Linn. are used in scabies & mixed with chunam in other cutaneous diseases (Drury, 1873).

The powder of dry leaves is given to children in worm cases, with the addition of little garlic. When the

juice of the leaves is mixed with small portion of margosa oil, & rubbed on the tongues of infants for the purpose of clearing their stomachs of viscid phlegm. A decoction of the leaves is given in earache. The leaves is applied as a local application to syphilitic ulcers and also useful in relieving obstinate constipation of children. The plant is used in relieving head symptoms by causing haemorrhage from the nose (Kirtikar & Basu, 1975).

When the juice of Acalypha indica Linn. is made into a liniment with oil, it acts against rheumatism, venereal pains and earache. The roots, leaves and tender shoots are used in medicine by the Hindus (Dymock, 1890). A decoction of the leaves and roots of Hedyotis Puberula Linn. one in 10 was given in doses of half to one ounce a day in cases of asthma & bronchitis (Kirtikar and Basu, 1975). The leaves are dried and powdered and mixed with flour and made into cakes which are eaten by those who are suffering from asthmatic and consumptive affections (Dymock, 1891). It is also used in snake-bite cases.

Other important anti - asthmatic plants include Adhatoda vasica, Achyranthes aspera, Allium sativum, Cassia sps, Coleus sps, Ephedra vulgaris, Euphorbia sps, Tylophora asthmatica, Terminalia sps and Indigofera tinctoria (Nadkarni, 1954).

Hence the present investigation was undertaken with the main objective of screening the powder of anti - asthmatic plants such a Acalypha indica Linn. and Hedyotis puberula Linn. for their pharmacognosical characters through the parameters : taxonomy, anatomy, organoleptic, microscopic and fluorescence evaluation ; biochemical, phytochemical and physical constant determination.

Review of Literature

REVIEW OF LITERATURE

The literature pertaining to the work entitled pharmacognosical studies on Acalypha indica Linn. (Euphorbiaceae) and Hedyotis puberula Linn. (Rubiaceae) (anti - asthmatic plants) is reviewed under the following heads :

- i) Pharmacognosical studies on the experimental plants.
- ii) Pharmacognosical studies on the plants belonging to the family Euphorbiaceae and Rubiaceae.
- iii) General researches to present investigation.

i) PHARMACOGNOSICAL STUDIES ON THE EXPERIMENTAL PLANTS

A pilot study of Kuppaimeni (Acalypha indica) in the treatment of Swasakasam (Bronchial asthma) was discussed by Ganapathi et al., (1979). The drug Acalypha indica is used in Siddha medicine for treating bronchitis, pneumonia, asthma, scabies etc., The drug in 30 ml to 60 ml doses was given twice or thrice daily to 38 patients suffering from wheezing, cough, desponoea etc., 60 % of the cases were relieved from wheezing within two weeks, while 90 % got relief from severe cough, the raised eosinophyl count was also normalised.

Chemical investigation of Acalypha indica was done

by Manzoori - khuda et al., (1985). The chemical constituents of the roots and leaves of Acalypha indica yielded stigmasterol and an ester acalyphol acetate.

From the pharmacognosical studies, it was found that the drug " peh-hue-juwa-chi-chhau " have been originated from Hedyotis diffusa, H. corymbosa, H. tenelliflora and Mollugo pentaphylla (Lin et al., 1987).

ii) PHARMACOGNOSICAL STUDIES ON THE PLANTS BELONGING TO
THE FAMILY EUPHORBLACEAE AND RUBIACEAE

Datta et al., (1981) discussed the pharmacognosy of Excoecaria agallocha L. bark.

Observations on pharmacognostic, physiochemical and pharmacological characteristics of Croton penduliflorus were made by Shetty et al., (1983).

Ayurvedic Medicines in Kerala - Thamalaki has been reported to be a mixture of two different closely related species, phyllanthus amarus and phyllanthus debitis. Their botanical identification was also made, (Sivarajan et al., 1984).

Sinha et al., (1984) studied the pharmacognosical characters of Euphorbia prostrata and compared it with that of Euphorbia thymifolia.

Euphorbia hirta was reported to be the correct name for " Dudhi " which is referred to in the literature for

Unani drug (Ghauri, 1985).

Sinha et al., (1986) differentiated the various species of phyllanthus by foliar diagnostic characters.

Mukherjee et al., (1986) discussed the morphological characters, range of occurrence and medicinal uses of 16 plants of the family Rubiaceae.

In 1986 Ahmad studied the morphological, anatomical and preliminary phytochemical characters of bark and leaf samples of plants viz., Jatropha curcas and J. gossipifolia.

Patil (1988) described the nodal anatomy of seven genera in Euphorbiaceae.

Pharmacognosical and phytochemical characters phyllanthus fraternus Web. and Jatropha glandulifera Roxb. was discussed by Ramchandani et al., (1988). They isolated a crystalline compound from P. fraternus and determined the physical constant.

Pharmacognosical and phytochemical studies on Jatropha podagrica was made (Kotain et al., 1990). Microscopic characters, ash values, extractive values, fluorescence analysis and leaf constants were recorded.

Datta (1990) studied the macromorphology, micromorphology, histochemical and physical pharmacognostic characters of phyllanthus amarus.

Lal et al., (1991) explored Euphorbia geniculata by botanical and phytochemical studies.

The roots of Ixora coccinea was studied to fix the parameters for pharmacognosical characters (Seetha Devi et al., 1991).

Ixora coccinea (Paranti in sanskrit) was identified botanically and its diagnostic features and therapeutic usage was discussed (Raghunathan et al., 1992).

Bagchi et al., (1992) studied and identified phyllanthus amarus, P. fraternus, P. urinaria and P. virgatus to avoid confusion and adulteration in commerce.

iii) GENERAL RESEARCHES TO PRESENT INVESTIGATION

Under ultraviolet radiation after treatment with several reagents, numerous powdered vegetable drug and some powdered animal drugs were examined. The characteristic fluorescence of each drug was observed and data pertaining to colour and intensity were recorded. A similar observation was made by Chase et al., (1949) and a study on " A Nomenclature of colours for Naturalists and Compendium of useful knowledge for ornithologist " was made by Ridgeway (1886) (see : Kokoshi et al., 1958).

The procedure for the preparation of AnnaPavalaSindhooram (APS), a drug based on the concepts of Indian medicine for the prevention and reversal of atherosclerosis, is described in detail. The ingredients

used are green vitriol (Annabedi or ferrous sulphate) coral reef (corallium rubrum) leaves or Acalypha indica, Lippia nodiflora, vinca rosea, Lawsonia alba, Cynodon dactylon, flowers of Hibiscus rosa-sinensis and ripe fruits of phyllanthus emblica. The possibility of iodine, calcium, copper, iron and magnesium present in the APS, acting metabolically to reduce hyper cholesterolemia. " Sindhooram " is the cheif therapeutic form of herbo - mineral preparation used in the sidha system of Indian medicine (Shanmugasundaram et al., 1984).

A bibliographic survey on the anatomical study of 312 Indian medicinal plants for the period 1970 - 1984 has been given. For each plant, the botanical names, family, common Indian names, plant part investigated and the reference number, have been incorporated in a tabular form. Various short comings in the research papers on anatomical studies of plant parts have also been discussed from pharmacognostic point of veiw and suggestions have been made for the proper botanical description. So that the details are helpful in the proper authentication of crude drugs(Bagchi and Puri 1985).

A phytochemical study of South Gujarat forests plants with special reference to the medicinal plants were discussed by Joshi et al.,(1989). The paper deals with phytochemical screening for alkaloids, saponins and tannins

of 182 samples consisting of 147 species which are used as medicines either in indigenous system or by aboriginal tribes of South Gujarat forests.

Herbal drugs for asthma - a review of clinical evaluation of anti-asthmatic drugs were discussed by Aulakh et al., (1989). Work carried out on the clinical evaluation of 22 single component herbal drugs and 5 composite herbal drugs for asthma has been reviewed. Mode of their administration duration of treatment, clinical response, active constituents and side effects of these drugs have been discussed.

Methodology

METHODOLOGY

Different parts of the following species of medicinal plants were used in the "pharmacognosical studies on Acalypha indica Linn. and Hedyotis puberula Linn (Anti - asthmatic plants).

Table. 1

S. No.	Plant	Part studied
i)	<u>Acalypha indica</u> Linn.	Whole plant
ii)	<u>Hedyotis puberula</u> Linn.	Whole plant

Specimens of the above plants were collected at their flowering stage, from different parts of Coimbatore. The plant specimens were made into herbaria and identified from authentic specimens at the Botanical Survey of India, Southern Circle, Coimbatore. Their voucher specimens were kept at the Herbarium, Botany Department, Sri Avinashilingam Deemed University, Coimbatore.

The fresh specimens - Leaf, stem and root of Acalypha indica Linn. and Hedyotis puberula Linn. were fixed in FAA. Free hand and microtomic sections were taken. The sections were stained with safranin (aqueous) and fast green by adapting alcohol - Xylol series and mounted in DPX

mountant (Johanson, 1940).

The above mentioned plant parts were shade dried and powdered by using Wiley Mill (0.5 mm) at Sugarcane Breeding Institute, Coimbatore. These plant powders from the experimental plants were taken for further studies. The powder was sieved through 40 mesh sieve plate and proceeded for pharmacognosical studies (Jackson and SnowDown, 1968).

The various methods used in the study include :

1. Organoleptic Evaluation.
2. Microscopic Evaluation
3. The behaviour of the powder with various chemicals
4. Fluorescence Analysis
5. Biochemical Analysis
6. Physical constant Determination
7. Preliminary phytochemical screening.

1. Organoleptic evaluation

This includes the study of the colour, texture and taste of the powdered samples. The results of the above study are presented in Table.2.

2. Microscopic evaluation

The powdered plant samples were bleached by boiling in 5 % chloralhydrate, and were mounted in glycerine. Later, the slides were observed under the microscope to find its constitutents. The materials seen were drawn in

cameralucide diagrams (figure 7 and 8).

3. Behaviour of the powder with different chemicals

The sieved plant powders were treated with a number of chemicals in order to study their behaviour. The different chemicals used include :

1. 1N NaoH in Methanol
2. Picric acid
3. Acetic acid
4. Conc. Hcl
5. 5 % Iodine solution
6. 5 % Ferric Chloride
7. Conc. HNO₃
8. Iodine

The behaviour of the powders with different chemical reagents were studied and the results are presented in Table.3.

4. Fluorescence analysis

To study the fluorescence properties of the powdered samples they were exposed to the visible light as well as uv radiation as per kokoshi et al., (1958) and chase and pratt (1949) method and the colour of fluorescence emitted by the powder's is identified by employing the colour chart of Ridgeway (1886). The following reagents were used to study the fluorescence property :

1. 1N NaoH

2. 1N HCl
3. 1N NaOH in Methanol
4. 50 % $\frac{H}{2} \frac{SO}{4}$

Fluorescence analysis of the powder of Acalypha indica Linn. and Hedyotis puberula Linn. were carried out and the results, are presented in Table 4.

5. Biochemical analysis

This includes :-

- a. Estimation of Total proteins and
- b. Estimation of Total Free Sugars

For the estimation of total proteins from the dried drug Lowry et al., (1951) method was followed. This method is based on the principles that different proteins contain different amounts of aromatic residues, which react with folin - Ciocalteau reagent giving a blue colour and this is read in a colorimeter.

The estimation of total free sugars present in the experimental powders were done using Yemm and Willis (1954), method.

The results of protein and sugar estimation are presented in Table.5 and 6, respectively.

6. Determination of physical constant Ash analysis

To determine the ash values, alcohol, water and acid soluble extractives, the procedure recommended by Anonymous (1966) and Indian pharmacopeia were followed.

The results of the above test are presented in Table. 7.

7. Phytochemical screening

Preliminary phytochemical screening of the powdered samples were carried out in order to find out the presence or absence of phenols, tannins, Juglone, Quinone, oils, flavonoids, aldehydes, saponin, sterols, triterpenoids, suberin etc.

The results of the preliminary phytochemical screening of the two experimental plant powders are presented in Table. 8.

Results

Taxonomy and Histology

TAXONOMY AND HISTOLOGY

Acalypha indica Linn.

Systematic Position :

- Class : Dicotyledonae
- Subclass : Monochlamydeae
- Series : Unisexuales
- Order : Curvembryae
- Family : Euphorbiaceae

There is much difference of opinion regarding systematic position of the family. Hallier included it in his order passionales. Bentham and Hooker placed this family in Unisexuales, just before the Urticaceae. Wetestein as well as Rendle created an order (Tricoccae) for it. Engler included the family in Geraniales close to Malvales.

There are about 283 genera and 7,300 species in this family (Shukla and Misra, 1979).

Distribution and habit of the plant

Distribution

A common weed throughout the hotter parts of India, Ceylon, Tropical Africa and Philippiness.

Habit : (Photo 1 and 2)

Annual erect herb 30 - 75 cm high ; branches numerous, long, ascending, angular, finely pubescent. Leaves 2.5 to 7.5 by 2 to 4.5 cm, ovate, acute, serrate, glabrous, thin, base cuneate, 3 nerved; petioles usually longer than the

blade, slender ; stipules minute. Flowers in numerous, elongate axillary spikes, the males minute, clustered near the summit of the spike, the females scattered, 3 to 5 surrounded by a shortly pedunculate large leafy truncate, dentate, cuneiform many nerved bract 6 to 8 mm diameter ovary hispid. Capsules small, hispid, quite concealed by the bract, often only one seeded. Seeds ovoid, smooth, pale brown, 1.2 mm long (Kirtikar and Basu, 1981).

Morphological nature of the root

Size, colour and behaviour

Taproot, branched, 14 to 16 cms long, creamy white in colour, tough in behaviour.

Transverse section of the root (Fig.1)

The T.S. of the root of *Acalypha indica* Linn. shows the following regions :

Corkcells: In older roots the epidermis is replaced by the formation of cork cambium which gives cork cells on the outside and secondary cortex on inside.

Secondary cortex: This consists of thin walled parenchyma cells with intercellular spaces. The endodermis and the pericycle are not clear in the mature root.

Vascular region: This consists of secondary xylem and phloem and primary xylem and phloem.

The secondary phloem occurs in the form of radial

rows. They are formed of sievetubes, companion cells and phloem parenchyma. Primary phloem is seen in a crushed state. The secondary xylem is made up of tracheids, vessels and xylem parenchyma. The primary xylem is seen in the centre. The protoxylem is pointing towards the epidermis. Pith is very small and becomes completely obliterated in older roots.

Morphological nature of the stem

Size, colour and behaviour 44 to 55 cms in height ; 0.5 mm to 0.8 mm in diameter ; green in colour ; herbaceous and hairy.

Transverse section of the stem (Fig. 2)

The T.S of the stem shows the following regions :

Primary structure

Epidermis : is made up of single layered, regular, rectangular, compactly arranged parenchyma cells, outer wall cutinised. Numerous uniseriate hairs arise from the epidermal cells.

Cortex : Heterogenous, made up of Collenchyma, chlorenchyma and parenchyma cells.

Endodermis : Single layered, compactly arranged with casparian strips. In the region of pericycle thick walled sclerenchyma cells are present.

Stele : Collateral, endarch, open.

Secondary structure

After secondary thickening, the cortex shows, cork, phellum, phellogen and phellogen.

The primary phloem disintegrates above the secondary phloem and the sclereids are distributed irregularly. The secondary medullary rays occurs in between the secondary xylem. The primary xylem is pushed towards the pith.

The pith is large and parenchymatous.

Morphological nature of the leaf

Duration : Deciduous

Orientation : Leaf mosaic (to get maximum sunlight)

Lamina

(1) Petiole : Petioles of the lower leaves are longer than those of the upper ones, so that all the blades are fully exposed.

(2) Shape : Ovate

(3) Leaf base : Cuneate

(4) Venation : Reticulate, three nerved

(5) Margin : Serrate

(6) Apex : Acute

(7) Surface : Green, Pubescent

(8) Texture : Smooth

Transverse section of the leaf (Fig.3)

The T.S of the leaf shows the following regions :

Epidermis : Both upper and lower epidermis is present. They are made up of uniseriate, compactly arranged barrel shaped cells. Both the epidermis are protected by cuticle externally.

Stomata are found to be present only on the lower epidermis. They are rubiaceous. Non-glandular uniseriate trichomes are present on both the upper and lower epidermis.

Mesophyll : is differentiated into two portions

- a) Upper columnar, uniseriate, compactly arranged cells with chloroplasts forming the palisade parenchyma.
- b) Multilayered loosely arranged parenchyma cells with starchgrains forming the spongy parenchyma.

Vascular region: The protoxylem is found facing towards the upper epidermis and metaxylem towards the lower epidermis. The phloem consists of sieve tubes, companion cells and phloem parenchyma and are found below the xylem, facing the lower epidermis.

Below the upper epidermis collenchyma cells are present which give mechanical strength to the leaf. The vascular tissues are surrounded by a layer of parenchyma cells. Crystals are present just above the lower epidermis.

Hedyotis puberula Linn.

Systematic position

Class : Dicotyledonae
Subclass : Gamopetalae
Series : Inferae
Order : Rubiales
Family : Rubiaceae

The systematic position of the family according to most of the taxonomists just preceds the order Campanuales to which compositae belong. Engler placed this family under the Rubiales between the plantaginales and cucurbitales. Bentham and Hooker included it under Rubiaceae in the series inferae and just before compositae.

Family Rubiaceae is closely related to umbellales (cornaceae) in having cymose umbel like inflorescence. It also have relationships with Adoxnaceae and caprifoliaceae (Takhtajan).

This family includes about 400 genera and 4,800 to 5,500 species (Shukla and Misra, 1979).

Distribution and Habit of the plant

Distribution

India, Orissa, Bengal, Burma, Deccan and Carnatic.

Habit (photo 3 and 4)

Annual herbs, stem semi-woody at the base, 15 to 25 cms high, much branched from the base, branches more or

less angular and rough. Leaves numerous, often fascicled, rather close, sessile, 13 to 25 by 3 to 4 mm linear, flat, very acute, stipules short, pectinate. Flowers 3 to 10 in small irregular axillary peduncled umbels, peduncles usually longer than the leaves, stout, erect, pubescent, pedicels very short, pubescent calyx (in flower) 3 mm long ; teeth 2 mm long, triangular, cuspidate, ciliolate. Corolla 3 mm long, glabrous on both sides, lobes triangular, oblong, equalling the tube. Capsules globose, 2.5 mm, glabrous, crowned with not very distant calyx-teeth the top of the capsule not protruded (Kirtikar and Basu, 1975).

Morphological nature of the root

Size, colour and behaviour

Taproot, branched, semi-woody ; 5 to 7 cms long ; brown in colour ; tough.

Transverse section of the root (Fig. 4)

The T.S of the root of Hedyotis puberula Linn. shows the following regions :

Corkcells : In older roots the epidermis is replaced by the formation of cork cambium which gives corkcells on the outside and secondary cortex on the inside.

Secondary cortex

This consists of thin walled parenchyma cells with intercellular spaces. The endodermis and the pericycle are

not clear in the mature root.

Vascular region

Consists of secondary xylem and phloem and primary xylem and phloem.

The secondary phloem occurs in the form of radial rows. They are formed of sieve tubes, companion cells and phloem parenchyma. Primary phloem is seen in a crushed state.

The secondary xylem is made up of trachieds, vessels and xylem parenchyma. The primary xylem is seen in the centre. The protoxylem is pointing towards the epidermis.

Pith : is absent

Morphological nature of the stem

Size, colour and behaviour

15 to 18 cms in height ; 0.3 to 0.4 mm in diam.
pale green in colour ; tough

Transverse section of the stem : (fig.5)

The T.S. of the stem shows the following regions :
primary structure.

Corkcells : In older roots the epidermis is replaced by the formation of cork cambium which gives corkcells on the outside and secondary cortex on inside.

Cortex : Heterogenous ; made up of 2 types of cells :

- a) Outer chlorenchymatous cortex and
- b) Inner parenchymatous cortex

Endodermis : Innermost layer of the cortex ; single layered ; made up of compactly arranged barrel shaped cells with casparian strips.

Stele : Collateral, endarch, open.

Secondary structure

In the stelar region, the secondary xylem and secondary phloem are formed by the interfascicular cambium. The secondary phloem is formed of sievetubes, companion cells and phloem parenchyma.

The pith is large and parenchymatous.

Morphological nature of the leaf

Duration :· Deciduous

Orientation : Fascicled

Lamina

- (1) Shape - Linear
- (2) Nature - Stipulate
- (3) Venation - Reticulate
- (4) Apex - Acute
- (5) Surface - Green, flat
- (6) Texture - Smooth

Transverse section of the leaf (fig.6)

The T.S of the leaf shows the following regions :

Epidermis : The upper epidermis consists of single layer of elongated columnar cells. The lower epidermis consists of small rectangular parenchyma cells. Unicellular trichomes arise from the upper epidermis. Both the upper and lower epidermis are covered by cuticle externally. Stomata is found only on the lower epidermis.

Mesophyll : is differentiated into upper uniseriate compactly arranged short palisade cells and loosely arranged spongy parenchyma cells with raphides, here and there. The midrib is vertically transcurrent by collenchyma cells.

Vascular region : Xylem and phloem are well developed. The protoxylem is facing towards the upper epidermis and the metaxylem towards the lower epidermis. The phloem is found below the xylem.

Photo - 1



Photo - 2



Photo - 4



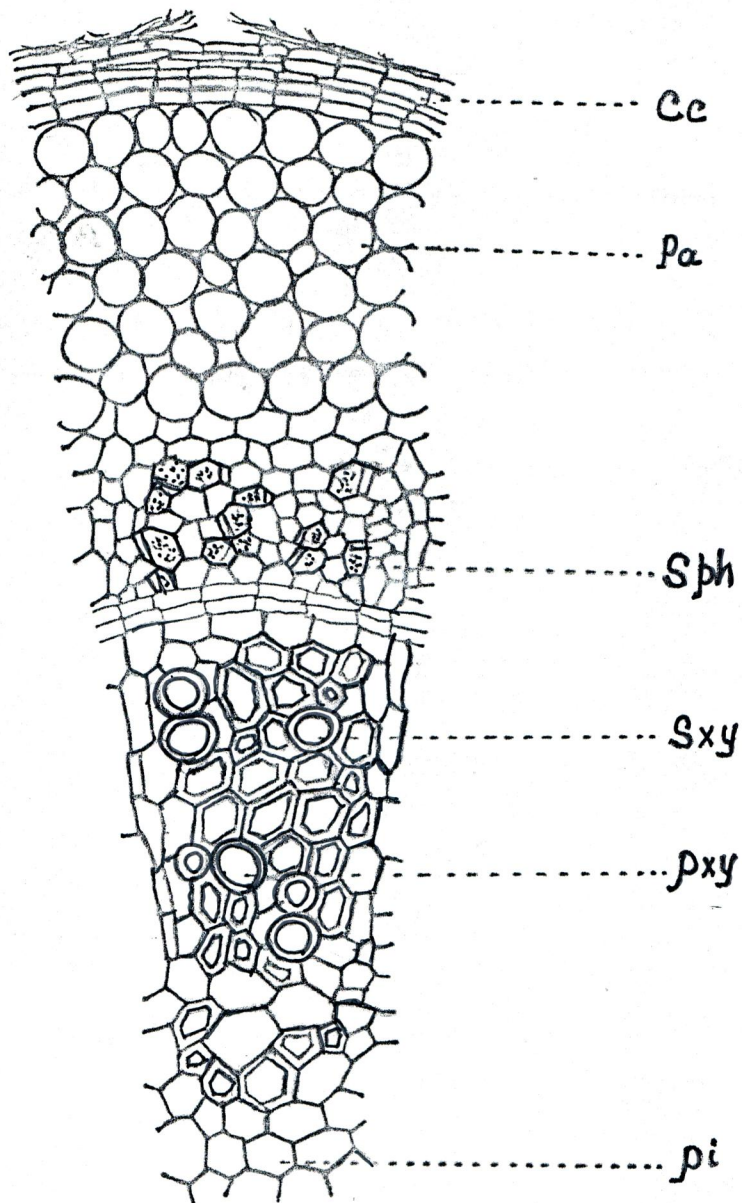
Photo - 3



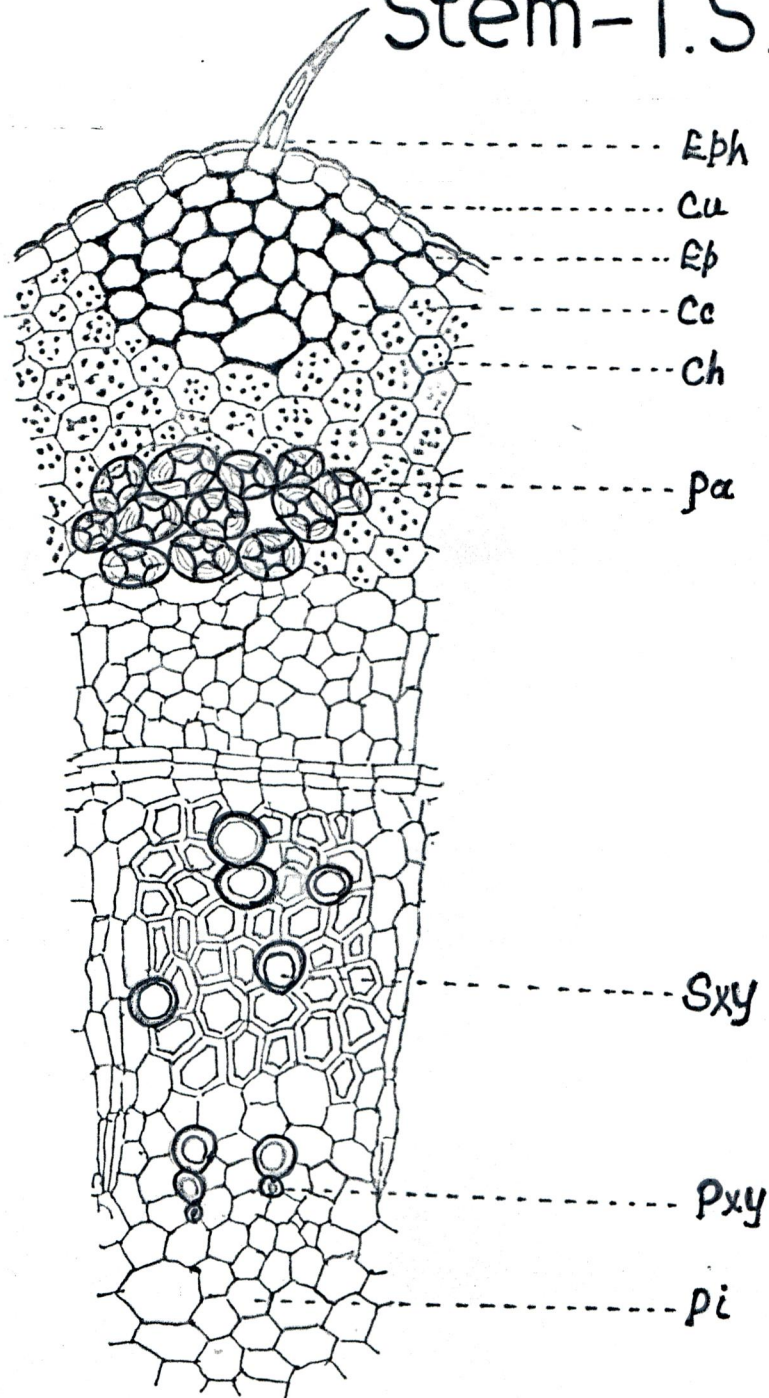
Legend

Cc	-	Cork cells
Pa	-	Parenchyma
Sph	-	Secondary pholem
Sxy	-	Secondary xylem
Pxy	-	Primary xylem
Pi	-	Pith

Acalypha indica Linn.
Root - T.S. Fig. 1

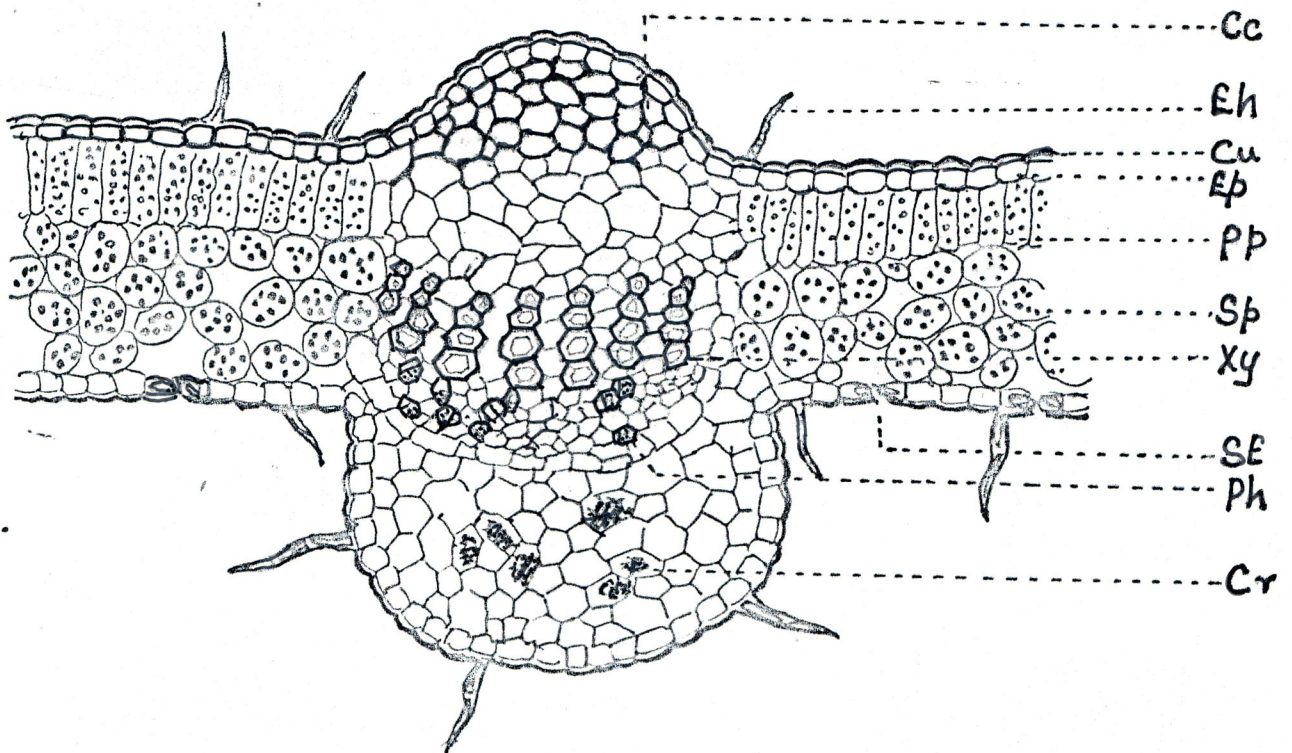


Acalypha indica Linn.
Stem-T.S. Fig. 2

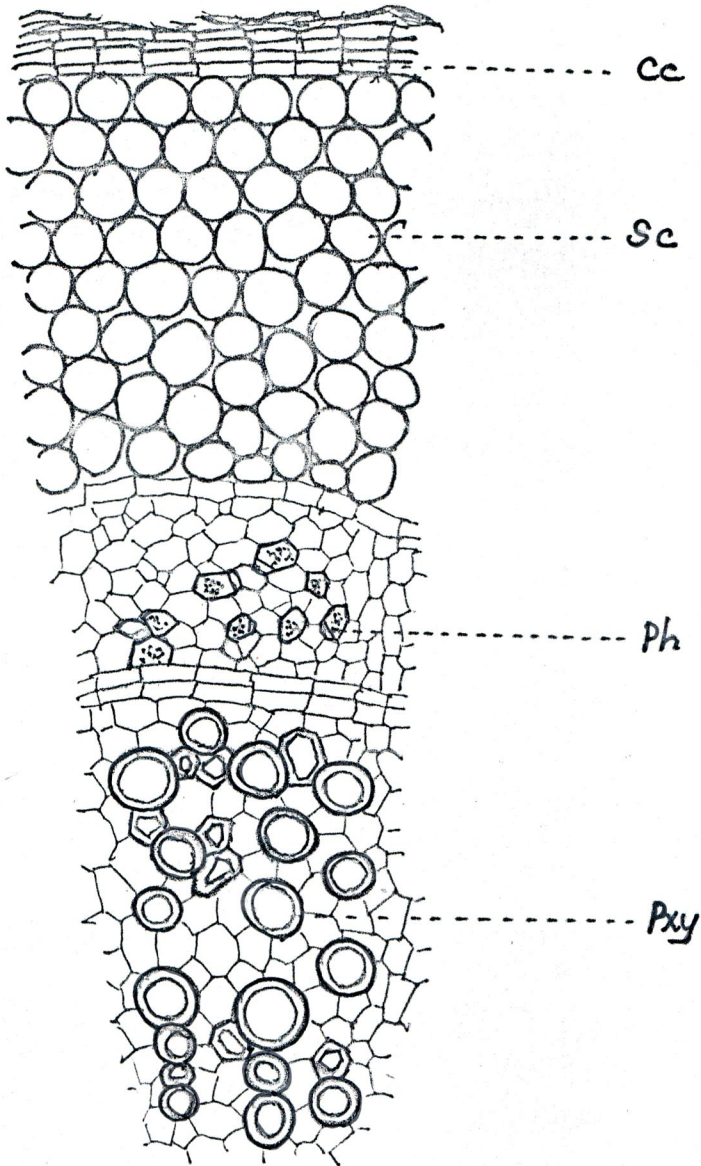


Acalypha indica Linn.

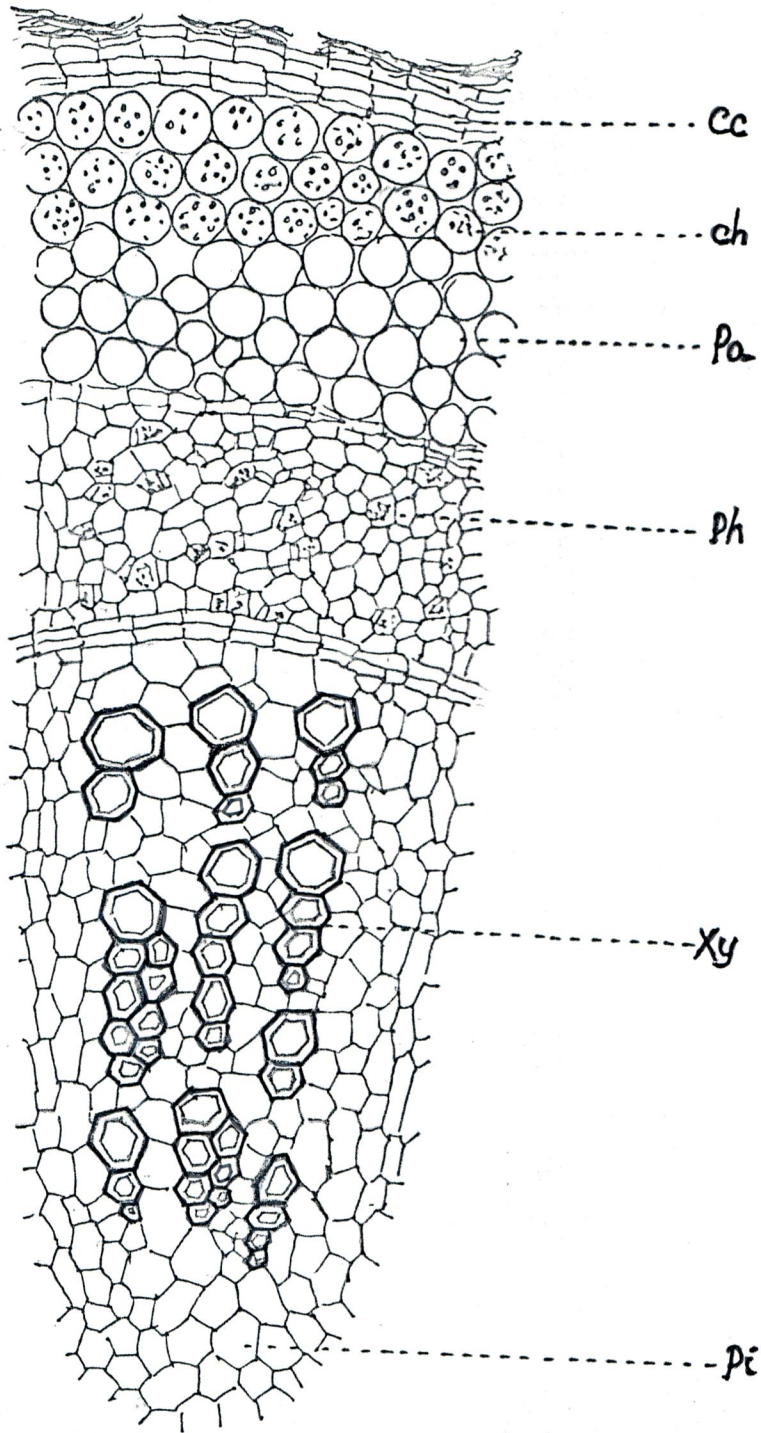
Leaf - T.S. Fig. 3



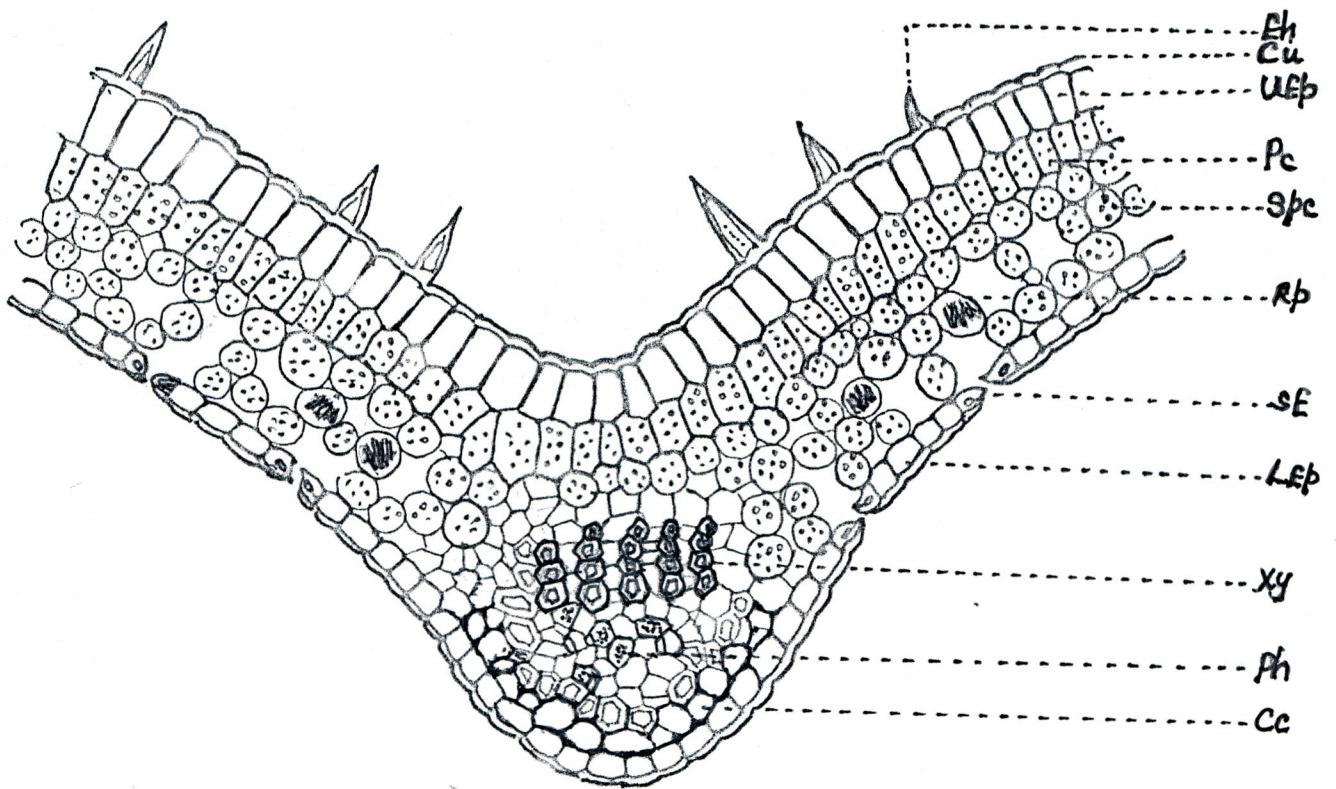
Hedyotis puberula Linn.
Root - T.S. Fig. 4



Hedyotis puberula Linn.
Stem-T.S. Fig. 5

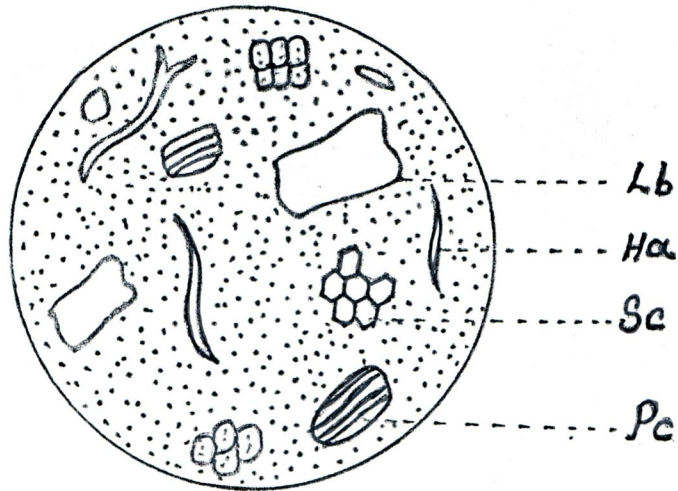


Hedyotis puberula Linn.
Leaf-T.S. Fig. 6



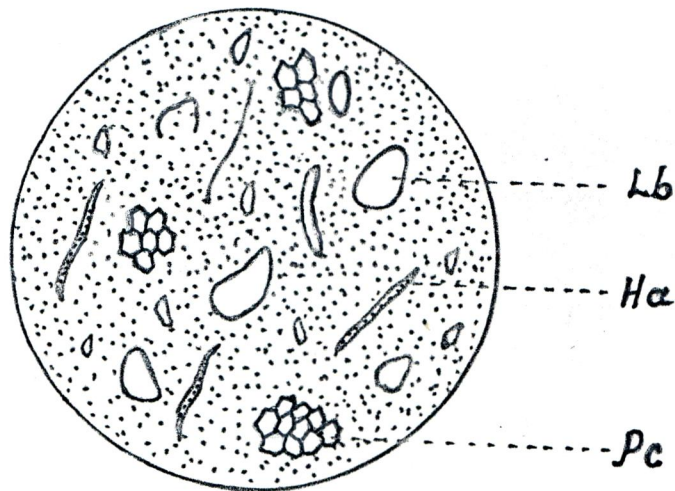
Acalypha indica Linn.

Fig. 7



Hedyotis puberula Linn.

Fig. 8



RESULTS

ORGANOLEPTIC STUDY OF THE POWDER

TABLE . 2

S.NO.	TESTS	<u>Acalypha indica</u> Linn.	<u>Hedyotis puberula</u> Linn.
1.	Colour	Green	Pale Green
2.	Texture	Coarse	Coarse
3.	Taste	Bitter	Bitter

THE BEHAVIOUR OF THE POWDER WITH DIFFERENT CHEMICALS

TABLE . 3

S.NO.	Treatment with chemicals	<u>Acalypha indica</u> Linn.	<u>Hedyotis puberula</u> Linn.
1.	Powder as such	Green	Pale Green
2.	Powder + IN NaOH in Methanol	Brownish yellow	Brownish yellow
3.	Powder + picric acid	Greenish yellow	Greenish yellow
4.	Powder + Acetic acid	Brownish yellow	Brownish yellow
5.	Powder + Conc. Hcl	Green	Dark Green
6.	Powder + 5% iodine solution	Brown	Greenish Brown
7.	Powder + 5% ferric chloride	Blackish Green	Blackish Green
8.	Powder + Conc. HNO ₃	Brown	Pale Brown
9.	Powder + Iodine	Brownish Green	Brownish Green

FLUORESCENCE ANALYSIS

TABLE . 4

S.NO. Treatment with chemicals	<u>Acalypha indica</u> Linn.		<u>Hedyotis puberula</u> Linn.	
	Visible light	U.V. Light	Visible Light	U.V. Light
1. Powder as such	Green	Green	Pale Green	Pale Green
2. Powder + IN Hcl	Green	Brownish Green	Dark Green	Brownish Green
3. Powder + IN NaOH	Dark Green	Brownish Green	Brown	Brownish Green
4. Powder + IN NaOH in methanol	Greenish yellow	Dark Green	Greenish yellow	Dark Brown
5. Powder + 50 % H ₂ SO ₄	Green	Greenish Black	Pale Brown	Greenish Black

BIOCHEMICAL STUDIES

ESTIMATION OF TOTAL PROTEINS (LOWRY et al., 1952)

TABLE . 5

S.NO.	Plant Name	% of protein / gram
1.	<u>Acalypha indica</u> Linn.	9.4 %
2.	<u>Hedyotis puberula</u> Linn.	5.2 %

* Average value of 2 replicatives per gram of the dry plant powder.

BIOCHEMICAL STUDIES

ESTIMATION OF TOTAL FREE SUGARS (YEMM AND WILLIS 1954)

TABLE . 6

S.NO.	Plant Name	% of sugars / gram
1.	<u>Acalypha indica</u> Linn.	9.3 %
2.	<u>Hedyotis puberula</u> Linn.	10.3 %

* Average value of 2 replicatives per gram of the dry plant powder.

PHYSICAL CONSTANT DETERMINATION

ASH ANALYSIS

TABLE . 7

S.NO.	Name of the plant	Total Ash %	Acid insolubility of ash %	Water solubility of Ash %	Ash solubility of Alcohol %
1.	<u>Acalypha indica</u> Linn. *11		4.9	10.1	12.3
2.	<u>Hedyotis puberula</u> Linn. *8.5		5.0	4.6	14.8

* Average value of 2 replicatives dry powder 10 grams.

PHYTOCHEMICAL SCREENING

TABLE . 8

Phytochemical Test	Plant Name	Observation	Results
<u>Hot water test</u>			
Mature leaf is dipped part way into hot water 85° C steadily for 5 seconds.	<u>Acalypha indica</u> Linn.	Blackish Brown band develops	Presence of phenols
	<u>Hedyotis puberula</u> Linn.	Blackish Brown band develops	presence of phenols
<u>Hcl Methanol test</u>			
A small amount of the powder is put into a test tube and covered with methanol and conc. Hcl (4 :1) and stoppered. Then the tube is allowed to stand occasional shaking for 4 to 5 hours.	<u>Acalypha indica</u> Linn.	Blackish Green band develops	Presence of Tannin
	<u>Hedyotis puberula</u> Linn.	Blackish Green band develops	Presence of Tannin

Phytochemical Test	Plant Name	Observation	Results
<u>Test for juglone</u>			
2 gms offinely chopped powder is put in a test tube and chloroform is added till it covers. It is stoppered and kept for several hours. The chloroform extract is filtered off, evaporated to dryness over a water bath and the residue is taken up in a few ml. of ether and equal volume of dil. Ammonia (1 Vol, of conc. Ammonia + 9 vol. of water) and the mixture is shaken gently.	<u>Acalypha indica</u> Linn.	No characteristic reaction	Absence of Juglone
	<u>Hedyotis puberula</u> Linn.	No characteristic reaction	Absence of Juglone
<u>Syringinaldehyde test</u>			
freshly hand cut leaf sections are mounted in a drop of aqueous H ₂ SO ₄ (50 %) 2 4	<u>Acalypha indica</u> Linn.	Disapperance of Green colour	Presence of syringinaldehyde
	<u>Hedyotis puberula</u> Linn.	Green colour partly disappeared	Presence of syringinaldehyde

Phytochemical Test	Plant Name	Observation	Results
<u>Saponin test</u>			
A small amount of fresh leaves are finely chopped, placed in small glass stoppered test tube and 5 ml of water is added. The contents are then boiled for 2 minutes, cooled, shaken vigorously and set aside for 5 minutes.	<u>Acalypha indica</u> Linn.	No characteristic reaction	Absence of saponin
	<u>Hedyotis puberula</u> Linn.	No characteristic reaction	Absence of saponin
<u>Liebermann - Burchard test</u>			
50 % H ₂ SO ₄ is added to a mixture of Methanolic extract and Acetic anhydride	<u>Acalypha indica</u> Linn.	Blackish Green band develops	Presence of Triterpenoids
	<u>Hedyotis puberula</u> Linn.	Blackish Green band develops	Presence of Triterpenoids

Phytochemical Test	Plant Name	Observation	Results
<u>Salakowski reaction</u>			
Chloroform and conc. H ₂ SO ₄ are added to the alcoholic plant extract	<u>Acalypha indica</u> Linn.	Blackish brown colour develops	Presence of steroidal nucleus
	<u>Hedyotis puberula</u> Linn.	Blackish brown colour develops	Presence of steroidal nucleus
<u>Phenolic test</u>			
Plant extract + ferric chloride solution	<u>Acalypha indica</u> linn.	Blackish green precipitate develops	Presence of phenols
	<u>Hedyotis puberula</u> Linn.	Blackish green precipitate develops	Presence of phenols
<u>Test for cellulose</u>			
To the powder was added Iodine solution followed by H ₂ SO ₄	<u>Acalypha indica</u> Linn.	Purplish black colour develops	Presence of cellulose
	<u>Hedyotis puberula</u> Linn.	Brown colour develops	Presence of cellulose

Phytochemical Test	Plant Name	Observation	Results
<u>Test for fixed oils and fats</u>			
To the powder was added sudan III.	<u>Acalypha indica</u> Linn.	Reddish brown colour develops	Presence of fixed oils and fats
	<u>Hedyotis puberula</u> Linn.	Reddish brown colour develops	Presence of fixed oils and fats
<u>Test for flavonoids</u>			
To the powder was added 10 % NaoH	<u>Acalypha indica</u> Linn.	Yellow band develops	Presence of flavonoids
	<u>Hedyotis puberula</u> Linn.	Yellow band develops	Presence of flavonoids
<u>Test for quinone</u>			
To the powder was added conc. Hcl.	<u>Acalypha indica</u> Linn.	No characteristic reaction	Absence of quinone
	<u>Hedyotis puberula</u> Linn.	No characteristic reaction	Absence of quinone

Phytochemical Test	Plant Name	Observation	Results
<u>Test for saponin</u>			
a) Lead acetate solution was added to the powder	<u>Acalypha indica</u> Linn.	No characteristic reaction	Absence of saponin
	<u>Hedyotis puberula</u> Linn.	No characteristic reaction	Absence of saponin
b) The powder was shaken well with water	<u>Acalypha indica</u> Linn.	No frothing	Absence of saponin
	<u>Hedyotis puberula</u> Linn.	No frothing	Absence of saponin
<u>Test for suberin</u>			
The powder was heated with conc. H ₂ SO ₄	<u>Acalypha indica</u> Linn.	Blackish brown precipitate develops	Presence of suberin
	<u>Hedyotis puberula</u> Linn.	Blackish brown precipitate develops	Presence of suberin
<u>Test for starch</u>			
Iodine solution was added to the plant powder	<u>Acalypha indica</u> Linn.	Reddish brown band develops	Presence of starch
	<u>Hedyotis puberula</u> Linn.	Reddish brown band develops	Presence of starch

Discussion

DISCUSSION

The dawn of human life is an association with the origin of pathogens. The primitive man resorted to the world around him to derive remedies which could alluviate pain and cure illness. The knowledge of drugs has developed together with the evaluation of scientific and social progress.

Man's knowledge of plants presumably grew in much the same way. At first it may have been only a craving or instinct, from which followed empirical knowledge giving traditional usage to many plants. This knowldge was handed down by all tribes and civilisations from century to century from all parts of the world. Some plants were deliberately grown for their virtues by the monks in their monastery herb gardens.

Herbs were recorded in use as early as 2,560 B.C. Ancient day tablets found reveled that the ancient sumerians used them. Also, the ancient Assyrians knew about the virtues of approximately 250 herbs. The ancient Egyptians, as early as 1,600 B.C. used Elderberry, pomegranate bark, wild lettuce, worm wood, hemlock and other herbs for health (Kadans, 1983).

Throughout the history, man has used plants as prophylactic and therapeutic aids to health. The primitive data he acquired, largely on a trial and error basis, have led to the development of many useful medicinal agents.

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Surveys in the 1960 showed that almost half of all prescriptions filled in the United States contained one or more active ingredients of natural origin. In India of the 250 most frequently prescribed products 75 contained one or more active constituents of higher plant origin (Chandler and Hooper, 1979).

Due to the growing interest in phytopharmaceuticals and their ever widening use in therapy in recent years, emphasis has been placed on the importance of both of the crude drugs themselves and the preparations obtained from them. This standardisation, necessary to ensure the reproducibility of the pharmacological action, is frequently based on the identification and determination of the content of the active component (White and Kennedy, 1985).

Even though so many drugs of western system have come into effect for the treatment of asthma no one is found suitable to approve the disease completely.

Ancient and recent literature reveals that there are many plants which find their place in the treatment of asthma. Among them Acalypha indica Linn. and Hedyotis puberula Linn. are predominant (Kirtikar and Basu, 1975). Hence an attempt is made to characterize the drug pharmacognosically with the following parameters ; taxonomy, anatomy, powder study, biochemical estimation, physical constant determination and phytochemical screening.

Identification and naming of drugs based upon some fundamental characteristics will bring drugs into large groups, each having some particular feature in common. Moreover pharmacognosy arises out of classification proper. The identification of the source of the material forming the drug and to determine its morphological nature are the two main functions of the pharmacognosist (Wallis, 1985).

In the present study, Acalypha indica Linn. was identified to be a annual erect herb, where as Hedyotis puberula Linn. is a annual herb with semi-woody stem. The leaves of the former is toothed, glabrous, petioles longer than the blade and has axillary spikes, and that of latter is sessile, fascicled, linear, flat and with short stipules. The flowers of the former is monoecious, in numerous lax erect, the males minute, clustered near the summit of the spike, and the females scattered, 3 to 5 surrounded by a shortly pedunculate large leafy truncate, dentate, cuneiform many nerved bract. The flowers of the latter is white in colour, small, irregular, axillary peduncled umbels, and pubescent.

Anatomical characters are most important to check adulterations and substitutions, and have been instrumental to establish guilt or innocence of suspected criminals. Furthermore, these characteristics make the identification of

the plant possible even without the flowers (Metcalfe and Chalk, 1972). Correct conclusions can be arrived at only when more than one parameter are studied.

In the present study it was found that the roots of Acalypha indica Linn. and Hedyotis puberula Linn. showed the presence of cork cells and secondary cortex. Pith is small and completely obliterated in the former and absent in the latter. (Fig. 1, 4)

Anatomical studies on the stem of Acalypha indica Linn. showed secondary thickening with the formation of periderm and Sclereids. Epidermis consists of numerous uniseriate hairs. The cortex is made up of collenchyma, chlorenchyma and parenchyma cells. The secondary medullary rays occurs in between the secondary xylem. The primary xylem is pushed towards the pith. (Fig. 2)

The stem of Hedyotis puberula Linn. showed only periderm. Cortex is made up of only chlorenchyma and parenchyma cells. In the stelar region, the secondary xylem and secondary phloem are formed by the interfascicular cambium. (Fig. 5)

The leaf of Acalypha indica Linn. showed the presence of upper and lower epidermis with non-glandular uniseriate trichomes on both the epidermis. Below the upper epidermis collenchyma cells are present. The presence of rubiaceous stomata is seen only in the lower epidermis.

presence of crystals are found just above the lower epidermis. Whereas the leaf of Hedyotis puberula Linn. showed the presence of upper epidermis with elongated columnar cells and lower epidermis with rectangular parenchyma cells. Unicellular trichomes are arising only from the upper epidermis. The presence of raphides is seen in the spongy parenchyma where as the leaf of former contains starch grains in the spongy parenchyma. The midrib is vertically transcurrent by collenchyma cells in the latter. The crystals are absent in Hedyotis puberula. (Fig. 3, 6)

To investigate the potency, purity and freedom from admixture, the powders were evaluated microscopically. The following components were observed : leaf bits, hair and palisade cells in both the plants and sclereids are found only in Acalypha indica. (Fig. 7, 8)

Pandey et al., (1984) and Gupta, (1985) treated the plant powders with different chemical reagents and observed their behaviour. A similar study of the powder was made with different chemicals and the results are presented in the Table.3.

The fluorescence properties are found to be a valuable aid in the identification of the powdered drug. Many substances, both plant and animal origin exhibit fluorescence when exposed to ultra violet radiation. It is characteristic both qualitatively and quantitatively. They

are useful for drug analysis. Since, the solvent and the pH are capable of modifying the fluorescence of many substance, the powder is treated with different chemicals and then observed under ultraviolet light (Kokoshi et al., 1958 and Chase and Pratt 1949). A similar study was made and the powder emits green and brownish green fluorescence predominantly (Table 4).

Most biochemicals formed in an organism are the result of the metabolic pathways. These biochemicals are formed as intermediary products. These chemicals can alter the activity of the drug. In the present study, the protein content of Acalypha indica Linn. was found to be markedly greater than that of the Hedyotis puberula Linn; where as the sugar content of both the plants was more or less equal and lesser than protein content. (Table 5, 6)

The mineral composition in a drug is most important for enzymatic activities concerned with metabolism of the organism. Hence the physical constant is determined to find out the insolubility of the ash, thereby to determine the geochemical content. The total ash content was found to be 11 % in Acalypha indica Linn. and 8.5 % in Hedyotis puberula Linn (Table 7).

The active principles in a drug is most important in curing the diseases and the supreme task is to link the drugs according to the constituents to which the activity is due, in order to construct a pharmacochemical method that

leads to pharmacognosical study (Pasquale, 1984). In the present study it was found that phenols, tannins aldehydes, triterpenoids, sterols, oils, flavaonoids, and suberin have been found predominant apart from other constituents (Table 8).

By considering the present findings and that of earlier reports (Aulakh et al., 1989 ; Rastogi and Mehrotra, 1990 ; and Dymock, 1891) these two plants may be given for asthma treatment. Gnapathi et al. ., (1979) used Acalypha indica in medicine for treating asthma. The drug in 30 ml to 60 ml doses was given twice daily to 38 patients suffering form asthma. However its efficacy in this treatment may be confirmed by pharmacological studies.

Summary and Conclusion

SUMMARY AND CONCLUSION

The present pharmacognostic studies on the anti-asthmatic plants - Acalypha indica Linn. and Hedyotis puberula Linn. are used as a tool to elucidate and evaluate the drugs originating from it.

To study the external and internal characters of the drug, taxonomic and histologic parameters are used respectively.

Powder study of the above is made by using the parameters like -

- i) Powder analysis - Organoleptic and microscopic evaluation
- ii) Behaviour of the powder with different chemicals
- iii) Reaction of the powder with chemical reagents under UV light
- iv) Biochemical estimation of total proteins and total soluble sugars.
- v) Determination of ash content and its solubility
- vi) Phytochemical screening for active ingredients.

If the above parameters are used in the identification of the drug, one can check its adulterants or substitution from the present study and previous findings the plants may be given for asthma after finding its lethal dose and toxicity.

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