CHAPTER - 2

PHARMACOGNOSTICAL STUDIES
2.1 INTRODUCTION

In early civilisation food and medicine were linked and many plants were eaten for their therapeutic properties. The knowledge, resources and rights of ethnic groups have been continuously ignored. In India, ethnomedico-botanical surveys and inventories receive little attention and detailed information and documentation on the uses of medicinal plants in indigenous communities are lacking. This disinterest in the existing knowledge and wanton neglect by authorities and public slowly result in the loss of this knowledge. It is high time that effective measures and programmes are adopted to save this information before it is lost for ever.

The past decade has witnessed a tremendous resurgence in the interest and use of medicinal plant products, especially in North America. Surveys of plant medicinal usage by the American public have shown an increase from just about 3% of the population in 1991 to over 37% in 1998. The past decade has also witnessed intense interest in "nutraceuticals" (or "functional foods") in which phytochemical constituents can have long term health promoting or medicinal qualities. In contrast many medicinal plants exert specific medicinal actions without serving a nutritional role in the human diet and may be used in response to specific health problems over short or long term intervals.

The increasing use of herbs and phytomedicines by more and more consumers and the growing interest in these products by licensed health care
professionals raise questions about their appropriate therapeutic uses\textsuperscript{3}. For many of the medicinal plants of current interest, a primary focus of research to date has been in the areas of phytochemistry, pharmacognosy and horticulture.

### 2.1.1 Importance of Pharmacognosy

Hundred years back the term pharmacognosy was used to describe the comprehensive knowledge of medicines from plants, animals and minerals\textsuperscript{4} and was largely descriptive. Pharmacognosy had gone through various metamorphoses in seeking to evolve as a true experimental and investigative science and in this respect the last 50 years had witnessed a considerable transformation in the subject.

"Pharmacognosy may be defined as the branch of science which deals with botanical, physico-chemical and economical features of the crude drugs of animal and plant origin"\textsuperscript{5,6}. Pharmacognosy's continued and increased legitimacy depended on its ability to integrate standardization methods, elucidation of total chemical and pharmacological profiles and clinically proven treatments into the development of phytomedicines that could be used with confidence in medical regimens, as well as laying a much more scientific basis to the claims made for "health foods", "dietary supplements" and "complementary/alternative medicines".
In a large number of cases botanically different plants have now come
to be used for the same drug in different places and some times even in the
same locality. Identification of the actual plant is not easy. Sometimes two
plants are known by the same local vernacular name. When the plant is
being collected, it is very possible that another plant is being collected at the
same time and this will be mixed up with the plant which actually has the
activity. One example of this is manifested when one collects the plant,
**Boerhaavia diffusa** Linn. (Fam. **Nyctaginaceae**) which is used widely as a
“quality of life enhancer” in the traditional systems of medicine. However, both
**Boerhaavia diffusa** and the plant, **Trianthema portulacastrum** Linn. are
known as “punarnava” in Sanskrit and so both the plants may be collected
one for the other. Similarly **Trianthema decandra** Linn. and **Trianthema
portulacastrum** Linn. (Fam. **Ficoidaceae**) are commonly known as
“Vellaisharunai” and “Vattasharunal” respectively in Tamil. Both the plants are
called “Punarnavi” in Sanskrit. The vernacular name “Koranti” is used to
denote both **Salacia reticulata** Wight. (Fam. **Celestraceae**) and **Barleria
buxifolia** Linn. (Fam. **Acanthaceae**). Hence there is every possibility of
adulteration of one species with another because of the confusion in
vernacular names. “Bhumyamalaki” is the vernacular name in Sanskrit and
Hindi assigned to represent four herbaceous species of **Phyllanthus** namely
**Phyllanthus amarus** Schum. and Thonn., **Phyllanthus fraternus** Webster.,
**Phyllanthus urinaria** L. and **Phyllanthus virgatus** Forst. They are used for
treating jaundice. The similarity in appearance coupled with the common vernacular name create confusions in identifying a particular species.

A second area of concern is that plant collectors may substitute cheaper material that looks the same, for the actual plant material which has the therapeutic effect and this may be described as a case of adulteration and cheating. For example, the morphological features of the roots of *Vinca rosea* (Fam. Apocyanaceae) and *Prosopis julifera* (Fam. Leguminosae) are similar. Hence the roots of the *Vinca rosea* which are used for curing cancer is adulterated with the roots of *Prosopis julifera* which do not cure cancer. Another example which is very well known, concerns the bark of *Caesalpina sappen*. When this plant is collected, bark from other plants such as *Gluta travancorea* and *Toona cilita* are substituted for the bark of *Caesalpina sappen*. Other examples with commonly used medicinal plants in India are substitution of the bark of *Saraca indica* by the bark of *Trema orientalis* and substitution of the plant, *Holarrhena antidysenterica* by the plant *Wrightia tinctoria*.

Hence a systematic determination of pharmacognostical characters such as

i) anatomical studies of the fresh root, stem and leaf

ii) Fluorescence analysis
iii) Physico-chemical characters such as ash values, loss of weight on drying, residue on ignition and extractive values will be very much useful in the correct identification and standardisation of the crude drug.

2.1.2 The need for standardization

When a medicine is administered to a patient, the doctor who prescribes the medicine needs to be assured that the medicine contains the correct amount of the right substances as only then would the medicine induce its therapeutic effect. It is very important that a system of standardization is to be established for every plant medicine in the market because the scope for variation in the phytochemical content and therefore in its therapeutic effect. Plant material may vary in its phytochemical content according to different places of collection, with different times in a year for collection, with collection at the same time and place but in different years and with different environmental factor surrounding the cultivation of a particular medicinal plant.

As long as herbal medicines were grown locally (in a kitchen garden) and used fresh whenever needed, it may not have been necessary to carry out extensive procedures of standardization. Herbal medicines were now being prepared in factories and pharmaceutical houses and packed for use all over the country and also for export. Quality control had therefore to be carried out with their products and this could only be carried out if standards
had been laid down to ensure quality. These standards had to be developed for different plants and for the final products and the whole issue of standardization of herbal remedies and medicinal plants came to the fore. The biological activity of a plant depends not only on the use of the proper plant and its contents but also on the presence of the required quality and nature of secondary metabolites in the raw drug. The availability of these secondary metabolites depends on environmental and other factors such as time and season of collection, stage of plant growth, storage, drying procedure and geographical variation. Unless clear-cut standards are laid down for the evaluation of the final product, these factors could certainly modify and affect the efficiency of the product and lead to failures in therapy.

The Evaluation or Standardisation of a crude drug involves a number of methods\(^\text{10}\) that may be classified as:

a) Organoleptic methods

b) Pharmacognostic methods

c) Phytochemical methods

d) Pharmacologic methods

Organoleptic refers to the evaluation by means of the macroscopic appearance of the drug, its odour, taste and the “feel” of the drug to touch. For example the four common Ocimum species viz. Ocimum sanctum (Krishna Tulasi), Ocimum gratissimum Linn. (Rama Tulasi), Ocimum basilicum Linn.
(Tirnutupatchai) and *Ocimum canum* Sims. (Nay Tulasi) can be very easily differentiated by looking into the size and colour of the leaves and also by odour. Pharmacognosy is an applied science which deals with botanical, physico-chemical and economical features of crude drugs. Phytochemistry is concerned with the enormous variety of organic substances that are accumulated by plants and deals with the chemical structures of these substances, their synthesis, turnover and metabolism, their natural contribution and their biological function. Qualitative and quantitative estimation of the phytochemical constituents will be very much useful in the standardisation of crude drugs. Pharmacologic activity of certain drugs can also be applied for the evaluation and standardisation of crude drugs. Pharmacological screening consists of a specified set of procedures to which a series of compounds or crude drugs can be subjected.

2.2 PAST WORK ON MEDICINAL PLANTS OF OUR RESEARCH INTEREST

Sushruta, as early as 1000 B.C., classified medicines in two groups: one which promotes vigour and vitality in healthy persons and the other which destroys the diseases of sick persons. He listed several hundred medicinal plants many of which find a useful place even today. Charaka (600 B.C.) described 700 medicinal plants for the treatment of various diseases. With the realisation that indigenous medicines should form an indispensable part of health care, several organisations like the CSIR, ICMR, ICAR, many
Universities and National Laboratories have been working vigorously to evaluate clinically the usefulness of local medicinal plants. Rastogi and Dhawan (1982) in their excellent review have reported that out of 1973 plants extensively screened and assayed for medicinal properties at the Central Drug Research Institute, Lucknow, 411 were found to exhibit biological activity. About 45 plants used in traditional Indian medicine have also found to be potential in treating diabetes mellitus. Extracts of these plants are reported to possess a variety of actions relating to the antidiabetic properties such as reducing insulin requirements by enhancing endogenous insulin availability, improving vitiated blood glucose.

2.2.1 Medicinal importance of *Dichrostachys cinerea* (Linn.) Wt. and Arn.

*Dichrostachys cinerea* (Linn.) Wt. and Arn. syn. *Caillaea cinerea* Macb. (Family – *Mimosaceae*) is commonly known as "Viravriksha" in Sanskrit and "Vidattalai" in Tamil. It is one of the important indigenous drugs used in a number of diseases in Ayurveda. It is a thorny shrub or a small tree, often with a gnarled trunk, occurring in the dry scrub forests and arid hills of north-western, central and southern India. The root is astringent and is used in rheumatism, urinary calculi, renal troubles and in the diseases of vagina and uterus. Tender shoots of the plant are bruised and applied to the eyes in the case of ophthalmia.
2.2.2 Medicinal importance of Hemidesmus indicus R.Br.

*Hemidesmus indicus* R.Br., (Family - *Asclepiadaceae*) is a twining wiry shrub with polymorphous leaves, varying in shape from elliptic or almost orbicular and obtuse to long linear and narrow and occurring over the greater part of India, from the upper Gangetic plain eastwards to Assam and throughout central, western and southern India\(^{21}\). It is commonly known as "Nannari" in Tamil and "Ananta" in Sanskrit and is used as a substitute for "Sarsaparilla". It is popularly known as "Indian Sarsaparilla" and finds extensive application in the Indian system of medicine\(^{22}\). The root of *H. indicus* is used as tonic, alterative, diaphoretic, diuretic and blood purifier. It is employed in nutritional disorders, syphilis, chronic rheumatism, gravel and other urinary diseases and skin affections\(^{23}\). The root in combination with other drugs is prescribed in snake bite and scorpion—sting but the root is not an antidote to either snake—venom or scorpion—venom\(^{24}\). Root powered and mixed with cow’s milk is given with much benefit in cases of scanty and high coloured urine and in those of gravel and strangury\(^{25}\).

2.2.3 Medicinal importance of Parmelia perlata Ach.

*Parmelia perlata* Ach. (family - *Parmeliaceae*) is a lichen known as "Kalpasi" in Tamil and "Shitashiva" in Sanskrit, spreads itself upon trees like oak and pine\(^{26}\). In Ayurvedic system of medicine\(^{27}\) it is used as fragrant, vulnerant and antipyretic. It is also useful in diseases of the blood and the heart, biliousness, bronchitis, scabies, leprosy, enlarged spleen, burning
sensations, bleeding piles, thirst, vomiting, asthma etc. In Yunani system of medicine it is used as an astringent, laxative, tonic, alterative, carminative and aphrodisiac. It is also useful in inflammations, stomach disorders and vesicular calculus. When burnt, the smoke relieves headache.

2.2.4 Medicinal importance of *Sida acuta* Burm. F.

*Sida acuta* Burm. F. (syn. *S. carpinifolia* Mast. (Family – *Malvaceae*) is an erect, perennial undershrub or shrub, distributed throughout the hotter parts of India and Nepal. It is commonly known as “Vattatiruppi” in Tamil and “Bala” in Sanskrit. This species is not only important as a medicine, but also yields a good fibre. The root is bitter and said to possess astringent, cooling, tonic, stomachic, diaphoretic and antipyretic properties. It is useful in nervous and urinary diseases, disorders of blood and bile and in chronic bowel complaints. Fresh juice of the root is applied to wounds and ulcers. In the form of an electuary, the root is employed as a vermifuge. A strong decoction of it is given in mild cases of debility. Leaves are considered to possess demulcent and diuretic properties and are used in rheumatic affections. A decoction of leaves and roots is credited with emollient and tonic properties and is used in the treatment of haemorrhoids and impotence.
2.2.5 Medicinal importance of *Sida cordata* (Burm. F.) Borssum

*Borssum*

*Sida cordata* (Burm.F.) Borssum syn. *S. veronicaefolia* Lam. and *S. humilis* Cav. (Family-*Malvaceae*) is a very variable, more or less hairy herb, often procumbent and sometimes rooting at the nodes is distributed throughout the hotter parts of India and Nepal up to an altitude of 1500 m. It is known as “Nagabala” and “Bhumibala” in Sanskrit and “Palampasi” in Tamil. The herb is considered to possess cooling, astringent and tonic properties and is used in fever and urinary complaints\(^{34}\). Oral administration of a decoction of the herb is reported to prevent the swelling of joints due to arthritis in experimental animals. Root bark is said to be useful in the treatment of leucorrhoea, gonorrhoea and micturition\(^{35}\).

2.2.6 Past work on the medicinal plants of present investigation

A complete review of the work done on all the plants of our research interest has been presented.

2.2.6.1 Past work on *Dichrostachys cinerea* (Linn.) Wt. and Arn.

A number of compounds have been isolated from *D. cinerea*. Makkar and Becker\(^{36}\) have isolated tannins from the leaves of *D. cinerea*. The n-hexane extract of aerial parts of *D. cinerea* showed 100% inhibition to
the growth of *Escherichia coli* and *Pseudomonas aeruginosa*\(^3\). Chloroform, methanol and aqueous extracts of *D. cinerea* fruits and leaves exhibited antibacterial activity\(^3\). The ethanolic extract of the plant was found to possess cycloxygenase inhibitory activity\(^3\). Proanthocyanidin from *D. cinerea* was shown to possess fungitoxic activity against *Rhizoctonia solani*\(^4\). Methanolic extract of the root of *D. cinerea* was found to possess significant decrease in the mortality percentage in venom induced mice\(^4\). Tannins isolated from *D. cinerea* exhibited remarkable bacteria toxic activity\(^4\).

### 2.2.6.2 Past work on *Hemidesmus indicus* R.Br.

As medicine, “Indian Sarsaparilla” holds a reputed place in all systems of medicine in India. Hydrodistilled essential oil obtained from *H. indicus* exhibited marked anti-bacterial activity\(^4\) against both gram-negative and gram-positive organisms, even at the concentration of 0.2%. An aqueous extract of *H. indicus* roots exhibited bacteriostatic activity in mice infected with *Mycobacterium leprae*. The paramethoxy salicylic aldehyde in the crude extract seems to be the active principle.\(^4\) Chloroform and ethanol extracts of *H. indicus* root showed antifungal activity against *Aspergillus niger*\(^4,4\). Oral treatment with the ethanol extract of the root significantly prevented rifampicin and isoniazid–induced hepatotoxicity in rats. The activity has been attributed to a free radical scavenging activity of the cumarinolignoids present in the extract\(^4\). Effect of cell culture extract of *H. indicus* (CCH) on normal
and hyper cholesterolemic rats were studied by Bopanna et al. Rats receiving CCH have prevented hypercholesterolemia. The methanolic extract of *H. indicus* root significantly neutralized the viper venom–induced lethality and hemorrhagic activity in albino rats and mouse. A Pure compound 2-hydroxy-4-methoxy benzoic acid isolated from the methanolic extract effectively neutralized viper venom–induced changed in serum phosphatase and transaminase activity in male albino rats. *Hemidesmus indicus* has also shown to have significant activity against pharmacological and physiological disorders.

2.2.6.3 Past work on *Parmelia perlata* Ach.

The use of this lichen in the form of a poultice, placed over the renal and lumbar regions to produce diuresis, is noticed in the pharmacopoeia of India. Efficacy of Speman, a herbal formulation comprising of *Parmelia perlata* as one of the ingredients was evaluated in 50 male patients with idiopathic infertility by Mukherjee *et al.* The treatment with Speman improved sperm morphology and sperm density. The chemical components (lichen substances) and colour reactions of 20 type specimens of *Parmelia* are described by Mason. Evaluation of air pollution using *Parmelia* as an indicator was performed by Takahashi *et al.*
2.2.6.4 Past work on *Sida acuta* Burm. F.

Of the different species of *Sida, S.carpinifolia* and *S. cordifolia* are most used in western India. *Sida acuta* is an ingredient in Siddha formulations useful in rheumatism, facial paralysis, pulmonary tuberculosis, sciatica haemorrhage, spermatorrhoea, leucorrhoea and gonorrhoea\(^56\).

The major alkaloid of *S. acuta* was cryptolepine and this exhibited antimicrobial activity against *Proteus vulgaris\(^57\).* Anti microbial activity of alkaloids from the aerial parts of *S. acuta* against Gram–positive and Gram–negative bacteria have also been performed and the alkaloid extract displayed good antimicrobial activity against several test microorganisms. The powdered aerial parts of *S. acuta* showed significant oedema suppressant activity and the aqueous extract of the whole plant showed significant hepatoprotective activity against carbon tetrachloride, paracetamol and rifampicin induced hepatotoxicities\(^58\) in experimental albino rats. Antibacterial activities were observed by Sushil Kumar *et al*\(^59\) in the seeds of *S. acuta*. Ethanolic extract of the whole plant of *Sida acuta* showed partial neutralization effect against venom lethal effect\(^60\). Whole plant of *Sida acuta* was more active against *Plasmodium falciparum*. The IC\(_{50}\) value obtained confirmed that the antimalarial activity of *Sida acuta* was due to alkaloids\(^61\).
2.2.6.5 Past work on Sida cordata (Burm. F.) Borssum

The alcoholic extract of *S. veronicaefolia* has been shown to sensitize the uterus to oxytocin\(^62\). The water soluble fraction of the alcohol extract of the herb *S. veronicaefolia* appears to contain a spasmogenic principle or principles and the main site of action is a muscarinic site\(^63,64\). It suggests the possible dangers of the indiscriminate use of the herb as an enema and also orally as a general purgative by some pregnant women in Ghana\(^65\) because of its observed tonic effects on gastrointestinal smooth muscle. The herb is also used post partum to stimulate milk formation and release. The uterotonic activity\(^66-88\) has been investigated on various laboratory animals and an oxytocin–like activity has been reported.
2.3 PAST WORK ON PHARMACOGNOSY OF MEDICINAL PLANTS OF THE PRESENT INVESTIGATION

Ash value, acid insoluble ash, alcohol and water soluble extractives, crude fibre contents, fluorescence analysis, TLC pattern of petroleum ether extract and microscopical studies of the root of *Dichrostachys cinerea* (Linn.) Wt.& Am. collected from Jhalana Dungri forest of Rajasthan have been determined.

"Indian Sarsaparilla" is an important ingredient in more than 60 Ayurvedic preparations. The drug is often adulterated with the roots of *Ichnocarpus frutescens*, *Cryptolepis buchanani* and *Decalepis hamiltonii*. The macroscopical and microscopical characters, physical constants, fluorescence characters, chemical constituents and important uses of the roots of 4 different taxa viz. *Hemidesmus indicus*, *Ichnocarpus frutescens*, *Cryptolepis buchanani* and *Decalepis hamiltonii* used under the names "Sariva" have been compared by Nayar. Physico-chemical characters of "Jatyadi Ghrta" (a composite Ayurvedic drug containing several ingredients including *H. indicus*) have been determined in an attempt to fix the standard for the drug. The drug "Ushba" is identified as *Smilax ovalifolia*, *S. ornata* and *Hemidesmus indicus*. A comparative study of constituents of Sarsaparillas (*Hemidesmus indicus* and *Decalepis hamiltonii* sold in Indian bazars) was made by Rao et al. Clear differences were found in their chemical and anatomical characters. Karnick has carried
out the fluorescence studies\textsuperscript{75} of powdered plant samples of \textit{H. indicus}. Nair and co-workers have analysed\textsuperscript{76} the alcoholic extracts of \textit{H. indicus} and \textit{I. frutescens} by T.L.C and spectrometry and have come to the conclusion that \textit{I. frutescens} cannot be used as a substitute for \textit{H. indicus} in drugs. Using thin layer chromatography the constitution of the diethyl ether extracts of 11 species of lichens endemic in the Philippines was studied by Sevilla Santos and co-workers\textsuperscript{77}. Sama and co-workers have determined the pharmacognostical characters of \textit{Sida rhomboidea} Roxb\textsuperscript{78}. Incineration of the seeds of \textit{S. acuta} gave 14.5% ash, containing Ca, Mg, K and Na as basic radicals and carbonate, sulphate and chloride as acid radicals\textsuperscript{79}. Pharmacognostical and phytochemical evaluation of \textit{Sida cordifolia} Linn. root was performed by Balakrishnan \textit{et al.}\textsuperscript{80}. No detailed information is available on the pharmacognostic studies of \textit{Sida acuta}, \textit{Sida cordata} and \textit{Parmelia perlata}.

2.4 SCOPE OF THE PRESENT WORK

Identification of plants with botanical verification is essential as contamination due to misidentification of plant species or parts is common. Substandard source materials or finished products will yield therapeutically less effective drugs; hence the quality control of herbal drugs has to be dealt from cultivation of the plant to the finished product. Pharmacognosists work in establishing standards whereby the quality of commercial plant material can be maintained. To standardise or to evaluate a crude drug means to identify it
and to determine its quality and purity. Generally, the evaluation or standardization of a drug involves a) organoleptic, b) microscopic, c) biologic, d) chemical and e) physical methods. A systematic survey of the literature reveals that Pharmacognostic studies have already been performed on two medicinal plants of present investigation viz, *Dichrostachys cinerea*\(^6\) and *Hemidesmus indicus*\(^7,70-76\) and no such studies have been performed for *Parmelia perlata*, *Sida acuta* and *Sida cordata*. Hence the objective of the present investigation is to perform a systematic pharmacognostical determination such as

a) synonym and regional names and distribution

b) anatomical studies of the fresh root, stem and leaf

c) fluorescence analyses

d) loss of weight on drying

e) total ash

f) acid–insoluble ash

g) water–soluble ash

h) residue on ignition and

i) extractive values

on *Parmelia perlata*, *Sida acuta* and *Sida cordata*
2.5 RESULTS

2.5.1 Parmelia perlata Ach.

*Parmelia perlata* is found on trees, old plants, walls and on rocks on the Himalayas, Punjab, Persia etc.

2.5.1.1 Macroscopic characters of thallus

The plant body is thin, membranous and foliaceous in nature (Fig. 2.1). It is classified under the morphological category of 'Foliose-lichen'. It is loosely attached to the substratum, especially on the trunk bark of the perennial trees. The upper (adaxial) surface is greyish brown or greyish green; the lower surface (abaxial side) is dark due to the substratum material adhering to the thallus. Minute outgrowths are often seen on the lower surface as well as margins of the thallus. The thallus is attached to the substratum by these outgrowths. The thallus is highly sinuous and wavy, especially the margins are undulate.

2.5.1.2 Microscopic characters of thallus

The cross-sectional view of the thallus is dorsiventral with distinct adaxial and abaxial sides (Fig. 2.1.1.). The thallus is undulate, but the surface is smooth. The thallus consists of four distinct zones:

1. Adaxial (upper) cortical zone
2. Adaxial algal (phycobiont) zone
3. Middle (mycobiont) fungal zone
4. Abaxial (lower) cortical zone
Fig. 2.1. *Parmelia perlata* Ach. (photographic) showing its macroscopic characters.
Fig. 2.1.1. Cross-sectional view of the thallus of *Parmelia perlata* (photographic) (1) showing adaxial and abaxial sides and (2) a portion enlarged.

(AdS - Adaxial side; AbS - Abaxial side; Phy - Phycobiont; UCo - Upper cortex; LCo - Lower cortex and My - Mycelium)
The adaxial (upper) cortical zone is variable in thickness from 10-20 μm. It consists of compact, thick walled parenchyma cells; this zone is called pseudoparenchymatous. This zone is highly compressed, heavily gelatinized hyphae (Fig. 2.1.1.(1)).

Beneath outer cortex, lies the algal zone where the phycobiont or the algal partner occurs loosely entangled by the fungal mycelium (Fig. 2.1.1.(2)). The phycobiont is unicellular, spherical cells of varying sizes. The algal zone is also not sharply defined in its boundary; it is up to 20 μm in width. The algal cells render the thallus greyish-green colour when the thallus is fresh.

The middle zone is the broadest region where the fungal mycelium is interwoven into a loose network. The mycelium consists of delicate, branched hyphae. No reproductive bodies are seen in the mycelial zone (Fig. 2.1.1).

The lower cortex is darker and more compact than the upper cortex (Fig. 2.1.1.(2).). The fungal hyphae are strongly cemented with each other, become thick-walled forming pseudoparenchymatous structure. The lower cortex is about 20 μm thick.

In paradermal (surface) section of the lichen thallus, the phycobiont (algal component) appears uniformly distributed and are free from each other (Fig. 2.1.2. (1)). The fungal hyphae entangle the algal cells and form physiological relationships. The paradermal section through the middle zone
Fig. 2.1.2. Paradermal section of the thallus of *Parmelia perlata* (photographic) showing (1) the phycobiont and (2) the fungal hyphae.

(Phy – Phycobiont and My - Mycelium)
of the thallus shows the fungal hyphae, which are loosely spread and oriented parallel to the surface of the thallus (Fig. 2.1.2.(2)).

In transverse section of the thallus, are seen several cylindrical, thread like outgrowths arising from the lower surface. These outgrowths are called rhizine. The rhizine is uniformly cylindrical and measures about 80 µm thick. The rhizine consists of outer cortex and central mycelial zone; no algal component is evident (Fig. 2.1.3.).

The terminal part of the rhizine penetrates into the substratum and form wide patch of structure consisting of both the lichen material and the substratum. The rhizine is formed by downward growth of the lower cortex and middle fungal mycelium in the form of cylindrical root. It helps to fix the thallus into the substratum (Fig. 2.1.3.(3)).

The reproductive body of the thallus is cleistothecium. It is a minute closed ball-like body, often formed in the substratum. It consists of compact outer zone of mycelium and a central cavity. These reproductive cells, conidia are found in the central cavity. The conidium is an elongated cylindrical body with 6 or more cells arranged one below the other (Fig. 2.1.4). The conidia are thick-walled and brown in colour. A mature conidium is 40 µm long 10 µm thick.
Fig. 2.1.3. Details of transverse section of the thallus of *Parmelia perlata* (photographic) showing (1) the thallus, (2) sector enlarged and (3) rhizine enlarged.

(Th - Thallus; Rh - Rhizine; Sub –substratum and My- Mycelium)
Fig. 2.1.4. Details of the reproductive body of the thallus of *Parmelia perlata* (photographic) showing (1) cleistothecium and (2) conidium.

(CO - Conidium; CI - Cleistothecium and Sub - Substratum)
2.5.2 *Sida acuta* Burm. F.

*Sida acuta* Burm. F. (syn. *Sida carpinifolia* Mast.) is a weed of waste places, very common in open grounds and road sides. It is distributed throughout the hotter parts of India and Nepal.

2.5.2.1 Macroscopic characters of *Sida acuta* Burm. F.

It is an erect, perennial undershrub or shrub, 1.5 m. high, much branched; branches slender, terete, minutely stellately hairy. Leaves 2.5–6.3 cm. long, lanceolate, with rounded base sharply serrate, glabrous on both sides; petioles 0.6 mm. Long, shorter than the stipules. Pedicels 1-2 in each axil, shorter or longer than the petiole, joined about the middle. Calyx 6-8 mm. long; lobes triangular, acute. Corolla nearly twice as long as the calyx, yellow, flowers in the leaf axils; solitary or in small clusters. Fruit 5-6 mm. diam.; carpels 5-9, puberulous, not pubescent, strongly reticulated, toothed on the dorsal margins; awns 2, nearly linear, about one third the length of the carpel. Seeds are smooth and black. The roots are thin, long, cylindrical, very rough and contorted (Fig. 2.2.).

2.5.2.2 Microscopic Characters of *Sida acuta* Burm. F.

2.5.2.2.1 Leaf

Midrib is prominent; it projects adaxially into a short hump and on the abaxial side it is broad and hemispherical (Fig. 2.2.1.a). The adaxial hump
Fig. 2.2. *Sida acuta* Burm. F. (photographic) showing its macroscopic characters.
Fig. 2.2.1. Transverse section of the leaf of *Sida acuta* (photographic) showing (a) midrib, (b) lamina with mucilage, (c) lamina with glandular trichome, (d) glandular trichome, (e) stellate type of trichome as seen under polarized light, (f) lamina showing calcium oxalate crystals under polarized light, (g) paradermal section showing stomata and (h) paradermal section showing venations.

(GTr - Glandular trichome; AdH - Adaxial hump; La - Lamina; X - Xylem; Ph - Phloem; MR - Midrib; Mu - Mucilage; MEP - Mucilaginous epidermis; PM - Palisade mesophyll; SM - Spongy mesophyll; MC - Mucilaginous cells; AdE - Adaxial epidermis)
includes a few collenchyma cells; the palisade tissue extends up to the shoulders of the hump. The abaxial part has circular parenchyma cells. The vascular strand is single, top-shaped and collateral. Neither bundle sheath or sclerenchymatous elements are associated with the bundle. The midrib is 450 μm thick.

2.5.2.2.1 Lamina

The lamina is dorsiventral. The adaxial epidermis has mucilaginous cells, some of which break liberating the mucilage. The abaxial epidermis is thin and stomatiferous. The palisade tissue consists of about five layers of short narrow palisade cells, the spongy mesophyll is about three-layered and the cells are lobed. Some of the palisade cells are mucilaginous and liberate the mucilage contents (Fig. 2.2.1.b).

2.5.2.2.1.2 Trichomes

The leaves and tender branches bear two types of trichomes (Fig. 2.2.1.c). One is the glandular type and the other is nonglandular, covering type of stellate type. The glandular trichomes are multicellular and curious in shape; they have a short, one-celled stalk and a wide spherical body which is gradually extended into uniseriate tapering terminal part; at the summit of the tapering part is a globular cell; these flask-shaped glandular trichomes are less frequent (Fig. 2.2.1.d). The stellate trichomes are more abundant, especially on the lower surface of the lamina. The stellate
trichomes are sessile and have unicellular, needle-like branches which radiate in all directions from the center (Fig. 2.2.1.e).

Calcium oxalate crystals are very frequent in the midrib and lamina. They are of druse-type. They appear as glittering bodies when viewed under the polarized light microscope (Fig. 2.2.1.f).

2.5.2.2.1.3 Stomata

The stomata are predominantly anisocytic; three subsidiary cells of unequal size encircle a stoma (Fig. 2.2.1.g). The anticlinal walls of the epidermal cells are wavy and thin; cuticular markings are not evident.

2.5.2.2.1.4 Venation pattern

In the paradermal sections, the vein-islets appear distinct. The shape and size of the islets are variable. Each islet has invariably, a single unbranched vein-terminations. Mucilaginous masses are seen in surface section (Fig. 2.2.1.h).

2.5.2.2.2 Petiole

In cross-sectional view, the petiole has dorsiventral symmetry; the outline is undulate (Fig. 2.2.2.a). A distinct epidermal layer is followed by a single chlorenchymatous layer and thin walled, compact parenchyma cells. The chlorenchyma cells tend to shrink forming a thin line. The vascular tissues occur in horizontally-flattened circle. The circle is wavy forming lateral
Fig. 2.2.2. Transverse section of the petiole of *Sida acuta* (photographic) showing (a) petiole entire and (b) petiole entire showing the crystals.

(AdS - Adaxial side; Ep - Epidermis; GT - Ground tissue; Chl - Chlorenchyma; Scl - Sclerenchyma; Ph - Phloem; X - Xylem and Cr - Crystals)

Fig. 2.2.3. Transverse section of *Sida acuta* (photographic) Showing (a) stem and (b) sector enlarged.

(Col - Collenchyma; Co - Cortex; Ph - Phloem; X - Xylem; W - Wing; Pi - Pith and Ep - Epidermis)
lobes. Xylem has radial lines of vessels and fibres. Phloem surrounds the xylem and thick zone of sclerenchyma cells encircles the vascular cylinder. When the petiole is viewed under the polarized microscope, fairly large druses are seen in the outer and central ground tissues and in phloem zone (Fig. 2.2.2.b.).

2.5.2.2.3 Stem

The stem is four-angled in sectional view with four broad, blunt ridges (Fig. 2.2.3.a). A thin epidermal layer of cubical cells ensheaths the stem; the ridges have collenchyma cells and the narrow cortex has tangentially oblong parenchyma cells (Fig. 2.2.3.b). The vascular cylinder is also quadrangular in outline; it is a continuous thick cylinder consisting of sparsely distributed radial lines of vessels and thick-walled lignified fibres (Fig. 2.2.3.b). Pith is wide, homogeneous and parenchymatous; the cells are thin-walled and compact. Crystals are not evident in the stem.

2.5.2.2.4 Root

A thick root measuring 3 mm in diameter was studied. The root consists of a thick, fissured bark of 500 μm thick. The bark is differentiated into a thin periderm and a broad zone of secondary phloem. The periderm consists of about eight layers of narrowly oblong, thin-walled, suberised phellem cells. The secondary phloem consists of dilated phloem rays, tangential blocks of phloem fibres and narrow radial zones of sieve elements (Fig. 2.2.4.b.). Secondary xylem is a solid, dense cylinder of even circumference.
Fig. 2.2.4. Transverse section of *Sida acuta* (photographic) showing (a) old root and (b) a sector enlarged.

(Pe - Periderm; Sph - Secondary phloem; SX - Secondary xylem; PrX - Primary xylem; PhF - Phloem fibre; XF - Xylem fibre; PhR - Phloem ray; XR - Xylem ray and V - Vessel)
Secondary xylem consists of wide, circular/oval-shaped vessels, thick-walled libriform fibres and wide xylem rays. The vessels are either solitary or in tangential multiples.

2.5.3 *Sida cordata* (Burm. F.) Borssum

*Sida cordata* (syn. *Melochia cordata* Burm. F., *Sida veronicaefolia* Lam., *Sida humilis* Cav.) is distributed throughout the hotter parts of India and Nepal up to an altitude of 1,500 m.

It is a straggling wayside shrub that sometimes mats with sandy soil and is found very often growing in shady places. It is found mainly in the southern areas of Ghana, especially in the Ashanti low plateau region about 30 m above sea level.

2.5.3.1 Macroscopic characters of *Sida cordata*

The plant is nearly a shrub with horizontal branches; branches prostrate or trailing, sometimes rooting, more or less hairy; plant surface stellate tomentose. Leaves 1-2.5 cm. long, cordate, ovate, margins serrate, Lower side stellate tomentose; Petioles 1-2.2 cm. long. Pedicels 2 cm. long, slender, axillary, solitary or twin, joined a little above the middle. Calyx 4 mm. long, five-lobed, triangular; petals-five, yellow, slightly exceeding the calyx; stamens-united into a hairy column; ovary-5 celled; Fruit – schizocarp with five mericarps; seeds brown, glabrous (Fig. 2.3).
Fig. 2.3. *Sida cordata* (Burm. F.) Borssum (photographic) showing its macroscopic characters.
2.5.3.2 Microscopic Characters of *Sida cordata*

2.5.3.2.1 Leaf

Midrib prominently projecting as a pyramid on the adaxial side and as broad hemisphere on the abaxial side (Fig. 2.3.1.a). The adaxial hump consists of collenchymatous cells. The palisade tissue extends up to the adaxial part of the midrib but not excurrent across the midrib. The abaxial midrib has compact parenchyma and some of the cells disintegrate producing mucilaginous substance. The vascular bundle is single, broad, flat and collateral. Large druses of calcium oxalate are located in the ground cells, especially in the phloem cells (Fig. 2.3.1.b.).

2.5.3.2.1.1 Lamina

The lamina (Fig. 2.3.1.c) has more mucilaginous secreting cells. The adaxial epidermal cells are larger and thin walled. The outer periclinal walls have wide papillate extensions. The abaxial epidermis is thin and possess rectangular to circular cells. The mesophyll tissue consists of a single short, broad palisade cells and small lobed spongy mesophyll parenchyma cells with wide air-chambers.

2.5.3.2.1.2 Trichome

The leaves and tender branches bear stellate trichomes (Fig. 2.3.1.d.). The stellate trichomes are sessile and have unicellular, needle-like branches which radiate in all directions from the center.
Fig. 2.3.1. Transverse section of the leaf of *Sida cordata* (photographic) showing (a) leaf midrib, (b) leaf midrib under polarized light (c) lamina, (d) trichome, (e) trichome full view, (f) stomata, (g) petiole, and (h) petiole under polarized light.

(Tr - Trichome; AdH - Adaxial hump; La - Lamina; Mu - Mucilage; VB - Vascular bundle; MR - Midrib; Cr - Crystal; AdE - Adaxial epidermis; PM - Palisade mesophyll; SM - Spongy mesophyll; AbE - Abaxial epidermis; St - Stomata; Sc - Subsidary cells; Ep - Epidermis; MB - Median bundle; LB - Lateral bundle; AdB - Adaxial bundle; AdS - Adaxial side; Chl - Chlorenchyma)
2.5.3.2.1.3 Stomata

The stomata (Fig. 2.3.1.f) occur on the lower epidermis; the stomata are mostly anisocytic with three unequal subsidiary cells. The guard cells are mostly circular, rarely elliptic. The anticlinal walls of the epidermal cells are straight and thin cuticular; striations not evident.

2.5.3.2.2 Petiole

The petiole (Fig. 2.3.1.g) is circular and even with adaxial flattened side. The epidermal layer is thin, but distinct with circular, thick-walled cells. The sub-epidermal layer of cells are chlorenchymatous and rest of the ground tissue is parenchymatous and compact. Some of the ground cells are mucilaginous-secreting cells. The vascular system consists of a central larger median bundle, two smaller lateral bundles and smallest adaxial bundle. All the bundles are collateral with abaxial sclerenchymatous bundle cap. Large druses of calcium oxalate crystals are seen, scattered in the ground cells and in the phloem zones (Fig. 2.3.1.h).

2.5.3.2.3 Stem

2.5.3.2.3.1 Young stem

The young stem (Fig. 2.3.2.a) has primary structure of vascular tissues. The stem has a thin epidermal layer, broad cortex and wide pith. The stele is a closed thin cylinder. The outer cortex is chlorenchymatous and major portion of the cortex is parenchymatous. The inner boundary of the cortex is marked by discontinuous cylinder of perivascular fibres. The stele consists of
Fig. 2.3.2. Transverse section of *Sida cordata* (photographic) showing (a) young stem, (b) old stem, (c) young stem sector enlarged under polarised light and (d) old stem sector enlarged.

(Ep - Epidermis; Co - Cortex; Ph - Phloem; X - Xylem; Mu - Mucilage; Sph - Secondary phloem; Sx - Secondary xylem; Cr - Crystal; Pi - Pith; Fi - Fibre and DR - Dilated rays)
wedges of collateral bundles. Mucilaginous masses are seen in the pith region.

2.5.3.2.3.2 Old stem

The old stem (Fig. 2.3.2.b.) shows some extent of secondary growth. The epidermis remains intact. The cortex is broad and parenchymatous; some of the cortical cells have undergone shrinkage forming a thin circular line. Secondary phloem is broad with wide dilated rays (Fig. 2.3.2.d). Secondary xylem is a thick cylinder comprising of radial lines of narrow circular vessels and thin walled fibres. Pith is parenchymatous and contains copious amount of mucilage (Fig. 2.3.2.b). When the stem is viewed under polarized light, large druses are seen in the cortical cells (Fig. 2.3.2.c).

2.5.3.2.4 Root

Young root shows a narrow zone of periderm, wide zone of secondary phloem and wide solid cylinder of secondary xylem (Fig. 2.3.3.a). The periderm consists of four or five layers of thin-walled tabular phellem cells. Secondary phloem consists of wide, slightly dilated rays and tangential blocks of fibres. Sieve elements occur in between the fibre blocks. Secondary xylem consists of wide circular or ovate, thick-walled vessels and thin-walled libriform fibres (Fig. 2.3.3.a). The vessels are mostly solitary or in short radial chains. When the root section is viewed under polarized microscope, the entire cortical cells are seen filled with large druses (Fig. 2.3.3.c)
Fig. 2.3.3. Tranverse section of *Sida cordata* (photographic) showing (a) young root sector enlarged, (b) old root sector enlarged, (c) old root sector enlarged under polarised light and (d) old root secondary xylem enlarged.

(Pe - Periderm; PhR - Phloem ray; V - Vessels; PhF - Phloem fibre;
In old and thick root, the secondary xylem shows distinct growth rings, especially in the central zone (Fig. 2.3.3.b). The secondary xylem has more of vessel multiples than of solitary vessels (Fig. 2.3.3.d). Vessel frequency is increased and vessel diameter is diminished. The secondary phloem has more number of circles of phloem fibres; the total radial width of the bark is also increased.

2.5.4 Fluorescence analysis

The powdered plant body of *Parmelia perlata*, the powdered root of *Sida acuta* and the powdered aerial part of *Sida cordata* and their extracts in various solvents were examined under ordinary light and also under ultra-violet light (365 nm). These powders were also treated with various chemical reagents and the changes in colour were recorded in Tables 2.1.–2.3. These fluorescence characters were determined according to the methods of Chase and Pratt81.

2.5.5 Quantitative determination

The percentage of loss of weight on drying, total ash, acid-insoluble ash, water-soluble ash and the residue on ignition of the above said plants were obtained by employing methods of analysis as described in Pharmacopoeia82 of India. The percentage of extractive values in petroleum ether (40° – 60°C), benzene, chloroform, methanol and water extracts of *Parmelia perlata, Sida acuta* and *Sida cordata* were also determined and the results are presented in Tables 2.4.–2.6.
### Table 2.1

**Fluorescence characters of thallus of *Parmelia perlata* and it’s extracts in various solvents**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatments</th>
<th>Under ordinary light</th>
<th>Under UV light (365nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Powder as such</td>
<td>Greenish brown</td>
<td>Dark brown</td>
</tr>
<tr>
<td>2</td>
<td>Powder + IN NaOH (aqueous)</td>
<td>Dark brown</td>
<td>Dark brown at the centre and violet at the edge</td>
</tr>
<tr>
<td>3</td>
<td>Powder + IN NaOH (ethanolic)</td>
<td>Brown at the centre and greenish-yellow at the edge</td>
<td>Brown at the centre and violet at the edge.</td>
</tr>
<tr>
<td>4</td>
<td>Powder + IN HCl</td>
<td>Light brown</td>
<td>Dark brown</td>
</tr>
<tr>
<td>5</td>
<td>Powder + 1:1 H$_2$SO$_4$</td>
<td>Brown</td>
<td>Brown at the centre and violet at the edge</td>
</tr>
<tr>
<td>6</td>
<td>Powder + 1:1 HNO$_3$</td>
<td>Brown</td>
<td>Brown at the centre and violet at the edge</td>
</tr>
<tr>
<td>7</td>
<td>Extracts:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) Petroleum ether (40° - 60°C)</td>
<td>Pale yellow</td>
<td>Greenish-yellow</td>
</tr>
<tr>
<td></td>
<td>b) Benzene</td>
<td>Yellow</td>
<td>Greenish-yellow</td>
</tr>
<tr>
<td></td>
<td>c) Chloroform</td>
<td>Pale green</td>
<td>Bright greenish-yellow</td>
</tr>
<tr>
<td></td>
<td>d) Methanol</td>
<td>Brown</td>
<td>Brown at the centre and greenish yellow at the edge</td>
</tr>
<tr>
<td></td>
<td>e) Water</td>
<td>Brown</td>
<td>Greenish-yellow</td>
</tr>
</tbody>
</table>
Table 2.2

Fluorescence characters of the roots of *Sida acuta* and their extracts in various solvents

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatments</th>
<th>Under ordinary light</th>
<th>Under UV light (365 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Powder as such</td>
<td>Flesh colour</td>
<td>Light brown</td>
</tr>
<tr>
<td>2</td>
<td>Powder + IN NaOH (aqueous)</td>
<td>Pale yellow</td>
<td>Dark brown</td>
</tr>
<tr>
<td>3</td>
<td>Powder + IN NaOH (ethanolic)</td>
<td>Pale yellow</td>
<td>Dark brown</td>
</tr>
<tr>
<td>4</td>
<td>Powder + IN HCl</td>
<td>Flesh colour</td>
<td>Light brown</td>
</tr>
<tr>
<td>5</td>
<td>Powder + 1:1 H₂SO₄</td>
<td>Brownish yellow</td>
<td>Dark brown</td>
</tr>
<tr>
<td>6</td>
<td>Powder + 1:1 HNO₃</td>
<td>Orange</td>
<td>Dark violet</td>
</tr>
<tr>
<td>7</td>
<td>Extracts:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) Petroleum ether (40°–60°C)</td>
<td>Dull brown</td>
<td>Dull brown</td>
</tr>
<tr>
<td></td>
<td>b) Benzene</td>
<td>Pale yellow</td>
<td>Light violet</td>
</tr>
<tr>
<td></td>
<td>c) Chloroform</td>
<td>Yellowish–brown</td>
<td>Dark violet</td>
</tr>
<tr>
<td></td>
<td>d) Methanol</td>
<td>Brown</td>
<td>Dark violet</td>
</tr>
<tr>
<td></td>
<td>e) Water</td>
<td>Dark brown</td>
<td>Dark violet</td>
</tr>
</tbody>
</table>
Table 2.3

Fluorescence characters of the aerial parts of *Sida cordata* and their extracts in various solvents

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatments</th>
<th>Under ordinary light</th>
<th>Under UV light (365 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Powder as such</td>
<td>Green</td>
<td>Dark brown</td>
</tr>
<tr>
<td>2</td>
<td>Powder + IN NaOH (aqueous)</td>
<td>Greenish–yellow</td>
<td>Dark brown</td>
</tr>
<tr>
<td>3</td>
<td>Powder + IN NaOH (ethanolic)</td>
<td>Greenish–yellow</td>
<td>Dark brown</td>
</tr>
<tr>
<td>4</td>
<td>Powder + IN HCl</td>
<td>Green</td>
<td>Dark brown</td>
</tr>
<tr>
<td>5</td>
<td>Powder + 1:1 H₂SO₄</td>
<td>Dark green</td>
<td>Dark brown</td>
</tr>
<tr>
<td>6</td>
<td>Powder + 1:1 HNO₃</td>
<td>Light brown</td>
<td>Dark violet</td>
</tr>
<tr>
<td>7</td>
<td>Extracts:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) Petroleum ether (40°–60°C)</td>
<td>White</td>
<td>Violet</td>
</tr>
<tr>
<td></td>
<td>b) Benzene</td>
<td>Yellowish–brown</td>
<td>Dark violet</td>
</tr>
<tr>
<td></td>
<td>c) Chloroform</td>
<td>Greenish–brown</td>
<td>Dark violet</td>
</tr>
<tr>
<td></td>
<td>d) Methanol</td>
<td>Greenish–brown</td>
<td>Dark violet</td>
</tr>
<tr>
<td></td>
<td>e) Water</td>
<td>Reddish–brown</td>
<td>Dark violet</td>
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Table 2.4

Physico–chemical characters of the thallus of *Parmelia perlata*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Particulars</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>1</td>
<td>Loss of weight on drying</td>
<td>78.13</td>
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<tr>
<td>2</td>
<td>Total ash</td>
<td>5.70</td>
</tr>
<tr>
<td>3</td>
<td>Acid insoluble ash</td>
<td>2.24</td>
</tr>
<tr>
<td>4</td>
<td>Water soluble ash</td>
<td>0.42</td>
</tr>
<tr>
<td>5</td>
<td>Residue on ignition</td>
<td>5.26</td>
</tr>
<tr>
<td>6</td>
<td>Extractive values</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) Petroleum ether (40°–60° C)</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>b) Benzene</td>
<td>3.56</td>
</tr>
<tr>
<td></td>
<td>c) Chloroform</td>
<td>5.32</td>
</tr>
<tr>
<td></td>
<td>d) Methanol</td>
<td>10.12</td>
</tr>
<tr>
<td></td>
<td>e) Water</td>
<td>5.28</td>
</tr>
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Table 2.5

Physico–chemical characters of the roots of *Sida acuta*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Particulars</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Loss of weight on drying</td>
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<tr>
<td>2</td>
<td>Total ash</td>
<td>4.23</td>
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<tr>
<td>3</td>
<td>Acid insoluble ash</td>
<td>0.51</td>
</tr>
<tr>
<td>4</td>
<td>Water soluble ash</td>
<td>1.35</td>
</tr>
<tr>
<td>5</td>
<td>Residue on ignition</td>
<td>3.56</td>
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<tr>
<td>6</td>
<td>Extractive values</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) Petroleum ether (40°–60°C)</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>b) Benzene</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>c) Chloroform</td>
<td>0.76</td>
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<tr>
<td></td>
<td>d) Methanol</td>
<td>3.84</td>
</tr>
<tr>
<td></td>
<td>e) Water</td>
<td>4.80</td>
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Table 2.6

Physico–chemical characters of the aerial parts of *Sida cordata*

<table>
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<th>S.No.</th>
<th>Particulars</th>
<th>Percentage</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Loss of weight on drying</td>
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<tr>
<td>2</td>
<td>Total ash</td>
<td>13.54</td>
</tr>
<tr>
<td>3</td>
<td>Acid insoluble ash</td>
<td>2.31</td>
</tr>
<tr>
<td>4</td>
<td>Water soluble ash</td>
<td>4.34</td>
</tr>
<tr>
<td>5</td>
<td>Residue on ignition</td>
<td>12.23</td>
</tr>
<tr>
<td>6</td>
<td>Extractive values</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) Petroleum ether (40°–60° C)</td>
<td>0.53</td>
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<tr>
<td></td>
<td>b) Benzene</td>
<td>0.80</td>
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<tr>
<td></td>
<td>c) Chloroform</td>
<td>1.00</td>
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<tr>
<td></td>
<td>d) Methanol</td>
<td>5.16</td>
</tr>
<tr>
<td></td>
<td>e) Water</td>
<td>16.40</td>
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2.6 DISCUSSION

Out of three plants of our research interest, the two plants *S. acuta* and *S. cordata* belong to *Malvaceae* family and third plant *Parmelia perlata* belongs to *Parmeliaceae*. Pharmacognostical studies have already been performed on two medicinal plants of present investigation viz. *Dichrostachys cinerea* (Fig. 2.4.) and *Hemidesmus indicus* (Fig. 2.5.). Before any botanical drug is used it is essential that it must be identified correctly as the desired species. Botanical classification depends almost entirely on morphological characteristics, both macroscopical and microscopical. Confirmation of identity, purity and quality are the three parameters used in drug evaluation. Such an evaluation can be done by examining characteristics under the organoleptic evaluation, microscopic evaluation, physical evaluation, chemical evaluation and biological evaluation.

2.6.1 Macroscopic characters

The thallus of *Parmelia perlata* is thin, membraneous and foliaceous in nature. The upper surface is greyish–brown or greyish–green. The lower surface has minute overgrowths and with the help of these overgrowths the thallus is attached to the substratum. The thallus is highly sinuous and wavy and the margins are undulate.

Leaves of *Sida acuta* are lanceolate serrate with rounded base and glabrous on both sides. Petioles are shorter than the stipules. Calyx is 6-8 mm
Fig. 2.4. *Dichrostachys cinerea*

Fig. 2.5. *Hemidesmus indicus*
long with triangular and acute lobes. Corolla is yellow in colour. Flowers are solitary or seen as small clusters and arise in the leaf axils. Carpels are 6-9 and each carpel has 2 awns.

Plant surface of *Sida cordata* is stellate tomentose. Leaves are cordate ovate with serrate margins and the lower sides are stellate tomentose. Flowers are seen in lax racemes, petals are yellow in colour and are five in number. The calyx is five lobed and triangular. Stamens are united into a hairy column. Fruit is schizocarp with five mericarps. These macroscopic characters can be used as a diagnostic tool for the identification of the plant.

2.6.2 Microscopic Characters

2.6.2.1 Thallus

The cross-sectional view of the thallus of *Parmelia perlata* is dorsiventral with distinct adaxial and abaxial sides. The adaxial (upper) cortical zone is highly compressed and consists of compact, thick-walled parenchyma cells and heavily gelatinized hyphae. The phycobiont is unicellular and spherical in size. In the middle zone the fungal mycelium is interwoven into a loose network and this zone does not contain any reproductive bodies. The lower cortex is darker and more compact than upper cortex.
In paradermal section of the lichen thallus, the phycobionts are uniformly distributed and are free from each other. The middle zone of the thallus shows loosely spread fungal hyphae which are oriented parallel to the surface of the thallus.

Transverse section of the thallus shows several cylindrical, thread like out growths (Rhizine) arising from the lower surface. The terminal part of the rhizine penetrates into the substratum and helps to fix the thallus into the substratum.

The reproductive body of the thallus is a closed ball-like body and consists of compact outer zone of mycelium and a central cavity. In the central cavity the reproductive cells (conidia) are found.

2.6.2.2 Leaves

Leaves of both *Sida acuta* and *Sida cordata* have prominent midribs projecting adaxially into short humps. The adaxial humps consist of collenchyma cells and the abaxial parts have paraenchyma cells in both the cases. In the case of *Sida cordata* some of the parenchyma cells disintegrate producing mucilaginous substance where as in *Sida acuta*, cells do not disintegrate. Vascular strands are single and collateral in both the cases.

2.6.2.2.1 Lamina

The spongy mesophyll is about three-layered and the cells are lobed in *Sida acuta*. In the case of *Sida cordata* the mesophyll tissue consists of a
single short, broad palisade cells and small-lobed spongy mesophyll parenchyma cells with wide air-chambers.

2.6.2.2.2 Trichomes

Three types of trichomes were recognised by wallis. They are 1) covering trichomes 2) glandular trichomes and 3) hydathodes and other special types. In *Sida acuta* two types of trichomes are recognized where as in *Sida cordata* only one type of trichomes are recognized. In *Sida cordata* they are the covering type of stellate type. In *Sida acuta* they are glandular and non glandular, covering type of stellate type. The glandular trichomes are flask-shaped and are less frequent. The stellate trichomes are more abundant, especially on the lower surface of the lamina. The stellate trichomes are sessile and have unicellular, needle-like branches which radiate in all directions from the center. These types of stellate trichomes are seen in hamamelis and altheas leaves. Unicellular glandular trichomes are not common and most glandular trichomes are pluricellular. In *Sida acuta* also the glandular trichomes are multicellular and they have short, one-celled stalk. In *Digitalis purpurea* a few such trichomes have a unicellular stalk. In *Sida cordata* glandular trichomes are not recognized.

2.6.2.2.3 Stomata

Stomata are epidermal structure possessing great diagnostic value. A stomata consists of two similar cells, the guard-cells, placed with their long axes parallel and having a small intercellular space, the porus, between them.
The leaves of ordinary land plants possess stomata which vary in their
distribution. They may be present in exceptional cases on the upper surface
only, as in *Ammophila arundinacea*, the Marram grass and on the floating
leaves of plants such as the water-lily. In many coriaceous leaves, such as
cherrylaurel, mate, coca and rosemary, they are present on the lower surface
only. In a few instances stomata occur in about equal numbers on the upper
and lower surface as in Senna and Mistletoe.

In both *Sida acuta* and *Sida cordata* stomata are present on the
lower surface only. Four types of stomata may be distinguished by the
characters of the guard-cells. They are 1) The moss type 2) The
gymnospermous type 3) The gramineous type and 4) The dicotyledonous
type. Amongst dicotyledons, four well-marked types of stoma occur. These
are distinguished by the form and arrangement of the surrounding cells, more
especially of the subsidiary cells as seen in the mature leaf. They are i) the
anomocytic (irregular-celled) type, ii) the paracytic (parallel-celled) type, iii) the
diacytic (cross-celled) type and iv) the anisocytic (unequal-celled) type. In
both *Sida acuta* and *Sida cordata* anisocytic type of stomata occur
predominantly where three subsidiary cells of unequal size encircle a stoma.
Such a type of stomata is recognised in belladonna, stramonium, henbane and
many plants of the family *Solanaceae*. The anticlinal walls of the epidermal
cells are wavy in *Sida acuta* but they are straight in *Sida cordata*. Hence the
two *Sida* species can be very well distinguished using these microscopic
characters.
2.6.2.3 Petiole

In cross-sectional view, the petiole of *Sida acuta* has dorsiventral symmetry and the outline is undulate but the petiole of *Sida cordata* is circular and even with adaxial flattened side. The vascular tissues occur in horizontally flattened circle and the circle is wavy forming lateral lobes in *Sida acuta*. In *Sida cordata* the vascular system consists of a central larger median bundle, two smaller lateral bundles and smallest adaxial bundle.

2.6.2.4 Stem

In *Sida acuta* the stem is four–angled in sectional view with four broad, blunt ridges (Fig. 2.2.3.a.). The epidermal layer consists of cubical cells, the ridges have collenchyma cells and the narrow cortex has tangentially oblong parenchyma cells. In *Sida cordata* the cortex is broad and parenchymatous and some of the cortical cells have undergone shrinkage forming a thin circular line. Pith is parenchymatous in both the cases and in *Sida cordata* copious amount of mucilage is present in the pith. The vascular cylinder in *Sida acuta* has sparsely distributed radial lines of vessels and thick-walled lignified fibres where as *Sida cordata* consists of radial lines of narrow circular vessels and thin walled fibres (Fig. 2.3.2.).

2.6.2.5 Root

Roots of both *Sida acuta* and *Sida cordata* show a narrow zone of periderm, a broad zone of secondary phloem and a wide solid cylinder of
secondary xylem. The periderm in *Sida acuta* consists of about 8–layers of narrowly oblong, thin-walled, suberised phellem cells but the periderm in *Sida cordata* consists of four or five layers of thin-walled tabular phellem cells. The secondary phloem in both the cases consist of dilated phloem rays, tangential blocks of phloem fibres and sieve elements. In both *Sida acuta* and *Sida cordata* the secondary xylem vessels are wide, circular/oval shaped. They are either solitary or in tangential multiples in *Sida acuta* and are mostly solitary or seen in short radial chains in *Sida cordata*. The libriform fibres are thick-walled in *Sida acuta* but thin-walled in *Sida cordata*. In *Sida cordata* the old and thick root shows distinct growth rings, especially in the central zone.

### 2.6.3 Fluorescence characters

Several drugs such as starch, hydrastis, calumba, viburnum, etc., show a marked fluorescence in ultra–violet light. For a few drugs the fluorescence is of diagnostic importance, especially for *Lonchocarpus nicou* D.C and the two varieties of *Derris* roots, viz. *Derris elliptica* (Roxb.) Benth and *D. malaccensis* Prain. and also for Chinese Rhubarb and Rhapontic Rhubarb.

The powdered thallus of *Parmelia perlata* shows dark brown fluorescence under UV light (365 nm) when viewed as such and on treatment with IN HCl. The powder shows brown fluorescence at the centre and violet at the edge under UV light (365 nm) on treatment with IN NaOH (aqueous or
ethanolic), 1:1H₂SO₄ and 1:1HNO₃. The petroleum ether(40°–60°C), benzene, chloroform and water extracts of the powder show greenish–yellow fluorescence when viewed under UV light (365 nm). The methanol extract shows brown fluorescence at the centre and greenish–yellow at the edge.

The root powder of *Sida acuta* shows light brown fluorescence under UV light (365 nm) when viewed as such and on treatment with 1N HCl. The powder shows dark brown fluorescence under UV light (365 nm) on treatment with 1N NaOH (aqueous or ethanolic) and 1:1 H₂SO₄ and dark violet fluorescence on treatment with 1:1 HNO₃. All the four extracts of *S. acuta* except petroleum ether extract (40°–60°C) show a violet fluorescence.

The root powder of *Sida cordata* shows brown fluorescence under UV light (365 nm) on treatment with 1N HCl, 1:1 H₂SO₄, 1N NaOH and viewed as such whereas a violet fluorescence is observed for all the five extracts and also when the powder is treated with 1:1 HNO₃. These fluorescence characters can be used as a diagnostic tool for the correct identification of the plants.

### 2.6.4 Physico–chemical characters

The percentage of loss of weight on drying, total ash, acid-insoluble ash and residue on ignition were maximum in *Sida cordata* (Table-2.6.) followed by *Parmelia perlata* and *Sida acuta* where as water soluble ash
was maximum in *Sida cordata* followed by *Sida acuta* and *Parmelia perlata*. Although total ash values are of little significance in the drug evaluation because of wide range of variations observed in different samples of the same drug but still very high ash values are indicative of contamination, adulteration or carelessness in preparing the drug for the market. Determination of acid-insoluble ash is of significance as the higher content of acid insoluble ash indicates possibility of sand being mixed with the drug. Pharmacopoeial limit for acid insoluble ash vary from 0.5 per cent (agar) to 12 per cent (hyoscyamus). Drugs like hyoscyamus with glandular trichomes have a capacity of retaining clay and thus the acid-insoluble ash value is higher in such cases. Water soluble ash is used to detect the presence of material exhausted by water and is used more especially for tea leaves and ginger rhizomes.

Presence of moisture in a crude drug can lead to its deterioration due to either activation of certain enzymes or growth of microbes. The moisture present in drugs depends largely upon the amount of moisture in the atmosphere. In crude drugs some times the active chemical constituents cannot be determined and thus the water, alcohol or ether soluble extractive values are determined for evaluation of such drugs.

Loss of weight on drying is maximum in *Sida cordata* (84.80%) followed by *Parmelia perlata* (78.13%) and *Sida acuta* (64.28%) (Table 2.4-2.6.).
The total ash value of *Parmelia perlata* is 5.70%. The solubility of ash in water is by 0.42% and insolubility in acid is by 2.24%. The extractive value of *P. perlata* is minimum (0.96%) in petroleum ether and maximum (10.12%) in methanol (Table 2.4).

The total ash value of *Sida acuta* is 4.23% (Table 2.5.). The solubility of ash in water is by 1.35% and insolubility in acid is by 0.51%. The extractive value of *S. acuta* is minimum (0.32%) in petroleum ether and maximum (4.80%) in water. The extractive values in benzene and chloroform are the same for *S. acuta*.

The total ash value of *Sida cordata* is 13.54% (Table 2.6.). The solubility of ash in water is by 4.34% and insolubility in acid is by 2.31%. The extractive value of *S. cordata* is minimum (0.53%) in petroleum ether and maximum (16.4%) in water.

The extractive values of methanol and water are generally high when compared with other extractive values of less polar solvents. Among the three plants tested *Sida cordata* produced the maximum ash content 13.54%. This may be due to the presence of large druses of calcium oxalate in the ground cells, especially in the phloem cells (Fig. 2.3.1.b). The extractive values of the root of *Sida acuta* were low and hence large amount of the crude drug may be used to get the desired pharmacological effect or to extract and isolate the required amount of a chemical constituent. The moisture content of *S. acuta* root is not too high indicating a little amount of mucilage or starch and hence less chances of microbial degradation.
2.7 CONCLUSION

Although many valuable drugs in current widespread use originate from plant sources or exist as chemically modified forms of naturally occurring phytochemicals, there is no reason to believe that a drug for every disease exists in nature. There is, however, every reason to require that all plant products used as drugs be evaluated for safety and efficacy. The popularity of natural drugs all over the world in recent years is an indication of significant contributions of pharmacognosy in modern medicine.
2.8 EXPERIMENTAL

2.8.1 Collection of plants

*Parmelia perlata* was collected from Thantrikudi, Dindigul District of Tamil Nadu and *Sida acuta* and *Sida cordata* were collected respectively from Tirunelveli and Shenkotta of Tirunelveli Dististrict of Tamil Nadu in the month of september. The plants were identified by Dr. V. Chelladurai, Research officer (Botany), Survey of Medicinal and Aromatic plants Unit-Siddha, CCRAS, Palayamkottai, Tirunelveli District, Tamil Nadu, India and voucher specimens were deposited at Department of Chemistry, Manonmaniam Sundaranar University, Tirunelveli District, Tamil Nadu, India [*Parmelia perlata* (MSU 053), *Sida acuta* (MSU 054) and *Sida cordata* (MSU 055)].

Care was taken to select healthy plants and for normal organs. The required samples of different organs were cut and removed from the plants and fixed in Farmalin Acetic Acid Alcohol (FAA) (consists of farmalin – 5 ml, acetic acid – 5ml and 70 % ethyl alcohol – 90 ml). After 24 h of fixing, the specimens were dehydrated with graded series of Tertiary Butyl Alcohol (TBA) as per the schedule given by Sass. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point $58^0$–$60^0$C) until TBA solution attained supersaturation. The plant materials were cast into paraffin blocks.
2.8.2 Sectioning

The Paraffin embedded specimens were sectioned with the help of a rotary microtome. The thickness of the section was 10 to 12 μm. Dewaxing of the section was done by a customary procedure. The sections were stained with toluidine blue as per the method published by O'Brien et al. Since toluidine blue is a polychromatic stain, the staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. Wherever necessary sections were also stained with safranin and fast green and IKI (for starch).

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid were prepared. Glycerine mounted temporary preparations were made for macerated/clearded materials.

2.8.3 Photomicrographs

Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Labphot 2 microscopic unit. For normal observations bright field was used, for the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent
property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard anatomy books.

2.8.4 Fluorescence analysis

The powders of the medicinally important parts of the various medicinal plants studied in the present investigation and their extracts in various solvents were examined under ordinary light and UV light (365 nm.) These powders were also treated with 1N NaOH (aqueous), 1N NaOH (ethanolic), 1N HCl, 1:1 H₂SO₄ and 1:1 HNO₃ and changes in colour were recorded. The results are presented in Tables 2.1.–2.3.

2.8.5 Quantitative determination

The percentage of loss of weight on drying, total ash, acid-insoluble ash, water-soluble ash and residue on ignition were obtained by employing standard method of analysis described in pharmacopoeia of India. The results are presented in Tables 2.4.–2.6.

2.8.5.1 Determination of loss of weight on drying

A known quantity of fresh medicinally important parts of various medicinal plants studied in the present investigation were weighed separately and allowed to dry under shade until a constant weight was obtained.
The loss of weight on drying was calculated. The percentage of loss of weight on drying for various samples are presented in Tables 2.4.–2.6.

2.8.5.2 Determination of total ash

5 g of air-dried powdered sample was taken in a previously weighed nickel crucible and ignited carefully, not exceeding dull red heat until the ash was free from carbon. The crucible was cooled and weighed. The percentage of ash with reference to the air-dried sample was calculated. The percentage of total ash for various samples are presented in Tables 2.4.–2.6.

2.8.5.3 Determination of acid-insoluble ash

A known weight of ash (about 200 mg) was boiled with 25 ml of 4N hydrochloric acid. The insoluble matter was collected in a previously weighed sintered crucible, washed with hot water, dried to constant weight and weighed. The percentage of acid-insoluble ash with reference to the air-dried sample was calculated. The percentage of acid-insoluble ash determined for various samples are presented in Tables 2.4.–2.6.

2.8.5.4 Determination of water-soluble ash

A known weight of ash (about 200 mg) was boiled with 25 ml of distilled water. The insoluble matter was collected in a previously weighed sintered crucible, washed with hot water, dried to constant weight and weighed. The percentage of water-soluble ash with reference to the air-dried sample
was calculated. The percentage of water–soluble ash values of various samples are presented in Tables 2.4. – 2.6.

2.8.5.5 Determination of residue on ignition

5 g of air-dried, powdered sample was taken in a previously weighed nickel crucible and ignited carefully, not exceeding dull red heat until the ash was free from carbon. Then the ash was strongly ignited and weighed. The percentage of ignited ash with reference to the air-dried sample was calculated. The percentage of residue on ignition for various samples are presented in Tables 2.4.–2.6.

2.8.5.6 Determination of extractive values

The extractive values of medicinally important parts of the different medicinal plants subjected to the present investigation in petroleum ether (40°–60°C), benzene, chloroform, methanol and water were determined by employing the methods of analysis described in Pharmacopoeia of India. About 5 g of air-dried sample was taken in a stoppered flask. 100 ml of the solvent were added. Shaken well, and allowed to stand for 24 hours with occasional shaking. Then the content was filtered. 50 ml of the filtrate were pipetted out into a clean, previously weighed china dish and evaporated on a water bath. Finally it was dried at 105°C, cooled and weighed. The percentage of solvent soluble extractive with reference to the air-dried sample was calculated. The percentage of extractive value in various solvents are presented in Tables 2.4. –2.6.
2.9 REFERENCES


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