Chapter 6

Summary & conclusions
SUMMARY AND CONCLUSIONS

Plants have also been used as a common source of drugs in traditional systems of medicine. In a survey done by WHO it has been estimated that about 80% inhabitants of the world depend chiefly on traditional medicine for their primary health care needs and it can safely be presumed that a major part of traditional therapy involves the use of plant extracts or their active principles. In the developed countries too, plant derived drugs are important. In USA, for example, 25% of all prescriptions dispensed from community pharmacies, contain plant extracts or active principles prepared from the plants. It is mainly during the last hundred years that some of the active ingredients present in the herbal products have been isolated and introduced into "modern" medicine. It was also noted that there are at least 119 distinct chemical substances derived from the plants and are considered as safe drugs. Demand of medicinal plants are consistently increasing through India to cater the needs of pharmaceuticals and allied industries. Due to lack of detailed descriptions, it is practically impossible to decide the identity of the parts of medicinal plants. Therefore, an urgent need of proper characterization and standardization of crude drug samples is being felt necessary to encourage and to make effective the indigenous systems of medicine. In the present study different parts of medicinal plants viz: Acacia leucophloea, Calliandra haematocephala, Ehretia aspera, Leucaena leucocephala, Trianthema portulacastrum and Mallotus philippensis have been studied, characterized and standardized.

Acacia leucophloea (flowers):

(i) Detailed macroscopical and microscopical studies of the flower of A. leucophloea (Safed kikar) have been performed. The odour of the flower is sweet -scented and the colour is pale yellow. The taste is astringent. The detailed morphological studies of the peduncle, sepal, petal, stamen and gynoeccium have been carried out. The nature and arrangement of cells, their measurements and the
presence of different cell inclusions, e.g. calcium oxalate crystals and tannins have been noted. The other important diagnostic characters of the flower have been recorded for correct identification.

(ii) Physico-chemical values of the flower powder show the loss in weight on drying at 105°C (6.03%), the solid contents of the plant (55.24%), crude fibres (25.95%), water soluble matters (30.08%) and alcohol soluble matters (10.3%). The pH values of 1% and 10% aqueous solutions have been noted 7.23 and 7.18 respectively.

(iii) The percentage of total ash, water soluble ash, acid soluble and sulphated ash have been found 8.50%, 3.60%, 1.0% and 0.30% respectively.

(iv) The different inorganic elements of the ash have been detected i.e. phosphorus (0.30%), iron (0.15%), zinc (0.40%), cadmium (not detected), potassium (0.90%), calcium (0.37%), magnesium (0.23%), sodium (0.80%) and aluminum (0.07%) have been found in the ash obtained from the flowers.

(v) Extractive values of the flowers in different solvents such as hexane, pet. ether, benzene, chloroform, acetone, methanol and water are found to be 2.9%, 0.22%, 0.38%, 0.46%, 2.37%, 7.08% and 8.39% respectively.

(vi) The total percentage of alkaloid is found to be 0.03%.

(vii) The thin layer chromatography technique shows four maximum spots in acetone extract. The $R_f$ values are found to be 0.18, 0.36, 0.49 and 0.72 respectively.

(viii) Qualitative studies have shown the presence of alkaloids, tannins, carbohydrates, saponins and flavonoids in the methanol and aqueous extracts.

(ix) Fluorescence analysis of the powder is also conducted and the colours of the powder in Ultra-Violet light have been observed as
pinkish brown when treated with 1N NaOH in water and brown when mounted in nitrocellulose.

(x) Detailed chemical studies have further confirmed that the main organic constituent in the part studied is sucrose and its yield has been found 594 mg out of 250 gm of shade dried flowers. The compound has been found in colourless, rhomboic crystals, m.p. 186-188°C and these crystals have been found sweetish in taste.

*Calliandra haematocephala* (flower):

A detailed morphological and anatomical studies of the flower of selected species have been described with their physico-chemical constants, qualitative and quantitative elemental analysis, thin layer chromatography and phytochemical constituents.

(i) Transverse section of the peduncle shows 3-5 layers of hexagonal, thick walled sclerenchymatous cells below the cortical zone. Pericycle contains a continuous and composite ring of sclerenchyma. The medullary rays are 1 or 2 celled thick, which are made up of elongated to rectangular cells.

(ii) Stomata of paracytic type occur on peduncle's bracts and petals.

(iii) Pollen grains have been observed in the form of pollinia.

(iv) The nature and arrangement of the cells, their measurements and the presence of cell inclusions eg. calcium oxalate crystals and tannins have been noted in different cell types. The other important diagnostic characters of flowers have been presented including the types of trichomes.

(v) The maximum extract is obtained in methanol which is 8.00% and lowest in petroleum ether (60-80°C) which is 0.20%. The loss in weight on drying at 105°C (10-69%), solid contents of the part (61.20%), crude fibres (27.72%), water soluble matters (27.52%) and alcohol soluble matter (9.60%). The pH values of 1% and 10% aqueous solutions have been noted as 7.21 and 7.16.
(vi) The percentage of total ash, water soluble ash, acid soluble ash and sulphated ash are found 5.56%, 1.57%, 0.82% and 0.02% respectively.

(vii) The inorganic elements of the ash have been detected i.e. phosphorus (0.33%), iron (0.14%), zinc (0.35%), potassium (0.95%), cadmium (0.40%), magnesium (0.20%), sodium (0.87%) and aluminum (0.09%).

(viii) Steroids, glycosides, carbohydrates, saponins, terpenes and alkaloids have given positive tests in the extracts of acetone and methanol.

(ix) The thin-layer chromatographic technique has shown six maximum spots in hexane extract. The Rf values are found to be 0.27, 0.45, 0.54, 0.63, 0.81 and 0.94 respectively.

(x) The colour of the drug as such is found reddish brown under ultra violet light and brown when treated with 1N HCl and mounted in nitrocellulose.

(xi) Detailed chemical examinations have revealed that the principal organic constituent (in the flower) is octacosane and its yield has been found 23 mg out of 150 gm shade dried flowers. The compound has been found as colourless, having m.p. 60-62°C.

*Ehretia aspera* (leaf):

The macroscopical, microscopical and phytochemical studies on the leaf of *E. aspera* have been performed.

(i) Dorsiventral structure has been observed in the species investigated. The epidermis is single layered with thin cuticle, cells are papilllose in the lower epidermis. Reticulate venation is found in the cleared leaf.

(ii) Single layered palisade tissue is present below the upper epidermis. Each cell of palisade is elongated, unarmed and compactly arranged.
(iii) Anomocytic type stomata occur on abaxial as well as adaxial surfaces of the leaf.
(iv) Long covering trichomes occur on the abaxial surface of the leaf.
(v) Cluster crystals of calcium oxalate are present below the palisade cells among parenchymatous cells.
(vi) Tanniferous sacs are recorded in the cortical region of the petiole around the vascular bundles in the species investigated.
(vii) The palisade ratio, vein islet number and vein termination numbers are 6-10, 10-18 and 9-15 respectively.
(viii) The percentage of the extractives is obtained as: hexane (10.12%), petroleum ether (5.92%), benzene (4.63%), chloroform (15.10%), acetone (13.95%), methanol (16.35%) and water (18.32%).
(ix) The loss in weight on drying at 105°C (85.3%), solid contents (55.3%), water soluble matters (63.1%), alcohol soluble matters (36.9%) and crude fibres (49.5%) are noted as standards of the drug. The pH values in 1% and 10% aqueous solutions have been recorded 6.95 and 6.90 respectively.
(x) The percentage of total ash, water soluble ash, acid insoluble ash and sulphated ash have been found 10.67%, 2.57%, 1.06% and 0.51% respectively.
(xi) Many inorganic elements of the ash have been detected i.e. phosphorus (0.41%), iron (0.19%), zinc (0.09%), potassium (2.35%), cadmium (<0.005 ppm), calcium (1.25%), magnesium (0.29%), sodium (2.95%) and aluminum (0.50%).
(xii) The thin layer chromatography has shown seven maximum spots in methanol extract. The Rf values are found to be 0.29, 0.33, 0.38, 0.45, 0.49, 0.67 and 0.81 respectively.
(xiii) Terpenes, flavonoids, tannins, alkaloids, glycosides, carbohydrates, saponins and resins have given positive tests in the methanol extract of the leaves.
(xiv) Fluorescence analysis of the leaf powder shows brown colour in Ultra Violet light and chocolate colour when treated with 1N NaOH in MeOH and mounted in nitrocellulose.

(xv) The detailed phytochemical studies have further confirmed that the main organic constituent of the leaves is β-amyrin and its yield has been found 20 mg out of 75 gm shade dried material. The compound has creamish colour and the m. p. of the crystals is 196-198°C.

**Leucaena leucocephala** (leaf):

A detail morphological and anatomical structures of the leaf have been described with their extractive values, physico-chemical constants, qualitative and quantitative elemental analysis of the ash and other organic constituents.

(i) The leaf is dorsiventral. The epidermal cells are short, hemispherical, sub-papillose particularly on the lower surface.

(ii) Only non-glandular trichomes occur on the margins of the leaflet. These are unicellular, elongated, unbranched and pointed.

(iii) Stomata of paracytic type occur on the abaxial as well as adaxial surfaces of the leaf.

(iv) The palisade tissue is represented by one to two layers of elongated radial cells. The spongy parenchymatous cells have been found polygonal in shape having little intercellular spaces among them.

(v) Solitary rhombohedral crystals are found entangled in spongy cells of the lamina.

(vi) The brown tanniniferous material has been observed in the cortical region of the midrib. Stone cells are also found below the upper epidermis.

(vii) The percentage of the extractive values is obtained as: hexane (5.07%), petroleum ether (16.51%), benzene (4.55%), chloroform 12.55%), acetone (14.35%), methanol (17.01%) and water (13.91%).
(viii) Physico-chemical values of the leaf powder show the loss in weight on drying at 105°C (91.4%), solid contents (49.9%), crude fibres (37.39%), water soluble matters (41.16%) and alcohol soluble matters (27.64%). The pH values in 1% and 10% of the aqueous solutions have been recorded 6.93 and 6.58 respectively.

(ix) The percentage of the total ash, water soluble ash, acid insoluble ash and sulphated ash are found to be 11.27%, 4.87%, 1.02% and 0.53% respectively.

(x) The qualitative and quantitative inorganic elements of the ash have been analyzed i.e. phosphorus (0.34%), iron (0.15%), zinc (0.19%), cadmium (not detected), potassium (1.52%), calcium (0.39%), magnesium (0.39%), sodium (2.13%) and aluminum 0.50%.

(xi) The percentage of the total alkaloids is recorded 0.05%.

(xii) The thin layer chromatography has given six maximum spots in chloroform extract. The Rf values are found to be 0.08, 0.18, 0.45, 0.59, 0.68 and 0.84 respectively.

(xiii) Tannins, steroids, carbohydrates, resins and saponins have given positive tests in methanol and water extracts.

(xiv) The compound is eluted from petroleum ether (60-80°C). On TLC examination gives single spot. The m.p. is recorded 178-180°C and yield 10 mg.

In NMR spectrum it is recorded, that it is an impure compound and hence it could not be characterized.

_Trianthema portulacastrum_ (leaf, stem and root):

The present morphological, anatomical, pharmacognostical and chemical examinations have revealed some diagnostic features of various parts of the plant:

(i) The epidermal cells of the leaf are composed of large bladder-like water storage cells of various shapes and sizes. The cuticle is smooth. Deposition of small crystals in the epidermal cells is characteristic.
(ii) There are no trichomes on leaf and stem surfaces.

(iii) Stomata of anomocytic type are found on abaxial and adaxial surfaces of the leaf. The stomatal index is 18. The frequency of its occurrence is 32/sq mm.

(iv) The crystals of inorganic salts are found abundantly in the blade and are distributed in a linear fashion throughout the lamina.

(v) Cleared leaf shows anastomosing venation with well developed areolae. The palisade ratio is 4 and vein islet number varies from 3-5.

(vi) Transverse section of the petiole shows large cortical region and these cells are found mostly spherical in shape in 6 to 7 layers. Cortex is well differentiated into collenchyma and parenchyma.

(vii) Pericycle is devoid of sclerenchyma in the stem. The pith cells are more or less hexagonal in shape.

(viii) Anamolous secondary growth are observed in the stem and root.

(ix) The complete zone or ring of secondary vascular tissue consisting of a narrow zone of phloem and broader zone of xylem is observed in the root. The secondary vascular tissue is found well developed with large vessels.

(x) The xylem vessels have bordered pits and have simple perforations on their lateral walls. The length of the vessel (in the root) is from 65.7-135.2 μm and the width is from 15.7-45.8 μm.

(xi) The maximum extracts of leaf, stem and root have been obtained in methanol which are 2.10%, 2.15% and 2.06 while minimum extracts in benzene are 0.46%, 0.21% and 0.04% respectively.

(xii) The percentage of total ash, water soluble ash, acid insoluble ash and sulphated ash of the leaf, stem and root have been found to be 2.6%, 7.5% and 10.5%; 9.0%, 8.2% and 7.7%; 0.6%, 0.08% and 1.0%; 0.39%, .73% and 0.51% respectively.
The loss in weight on drying at 105°C of leaf, stem and root are: 7.9%, 6.2% and 5.5% respectively. The percentage of total alkaloids of leaf, stem and root are recorded 0.02%, 0.03% and 0.5% respectively.

The maximum quantity of potassium is detected: 1.23% in leaf, 1.35% in stem and 1.63% in root.

Alkaloids, tannins, glycoside, saponins and terpenes are the main organic constituents which are present in the leaf, stem and root of the methenolic extracts.

Detailed chemical studies have further confirmed that the main organic constituents in the root is β-sitosterol (compound TP-1). Its yield has been found 30 mg out of 500 gm shade dried plant material. The compound has been found in white needle shaped crystals, m.p. 140°C.

Compound TP-2 is characterized as stearic acid from the root.

Compound TP-3 is confirmed as palmitic acid from the root.

Compound TP-4 is identified as inorganic salt such as potassium nitrate (KNO₃) from the root.

*Mallotus philippensis* (powdery covering of ripe fruit):

The macroscopical and microscopical examinations have revealed some important characteristic features of the powder (unorganized drug) which are presented below:

(i) Kamala is a beautiful purplish red or brick red powder.

(ii) The brick red, fine granular powdery substance has no specific taste or odour.

(iii) The fine, reddish powder consists of no cellular structure except the characteristic globular glands and trichomes.

(iv) The glands are externally lined by a delicate yellowish thin membrane and densely filled with a dark reddish resinous
substances which are secreted by a number of simple club shaped cells radiating from a common point.

(v) The broad ends of glands are generally found facing outwards.

(vi) Stellate trichomes are thick walled, lignified, curved, unicellular and arranged in small radiating groups.

(vii) It is insoluble in cold water and only slightly soluble in boiling water, but it is freely soluble in alkalis, alcohol and others, forming a deep red solution.

(viii) The percentage of the extractives is found in different solvents such as hexane (11.37%), petroleum ether (5.31%), chloroform (13.7%), acetone (12.21%), methanol (3.12%) and distilled water (4.51%).

(ix) Loss in weight on drying 105°C is found 4.31% and solid contents 90.85%.

(x) The percentage of total ash, water soluble ash, acid insoluble ash and sulphated ash were found to be 53.51%, 0.17%, 45.72% and 20.55% respectively. Qualitative elemental analysis of the ash has confirmed various elements: sodium, calcium, iron and aluminum.

(xi) Tannins, resins and glycosides have given positive tests in the methanol extract of the part studied.

(xii) Thin layer chromatographic technique shows eight maximum spots in petroleum ether (60-80°C).

(xiii) The colour of the powder shows coffee colour under ultra violet light when treated with 1N NaOH in methanol.

(xiv) Detailed phytochemical studies have further confirmed that the main organic compound in the powder studied is rotterlin. The compound has been found in dark brown rhombic crystals, m.p. 203-05°C.