CHAPTER - 5

PHYTOCHEMICAL STUDIES
5.1 INTRODUCTION

Phytoconstituents are the chemical substances of organic nature which are formed in plants through the activity of their individual cells. The process by which the plants are able to convert the simple chemical substances into complex organic compounds with the help of enzymes is known as biosynthesis\(^1\). The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. The combinations of secondary products in a particular plant are often taxonomically distinct\(^2\). Primary products such as carbohydrates, lipids, heme, chlorophyll and nucleic acids, which are common to all plants are involved in the primary metabolic processes of building and maintaining plant cells\(^3\). Although plant secondary products have historically been defined as chemicals that do not appear to have a vital biochemical role in the process of building and maintaining plant cells, recent research has shown a pivotal role of these chemicals in the ecophysiology of plants. Accordingly, secondary products have both a defensive role against herbivory, pathogen attack and inter-plant competition and an attractant role toward beneficial organisms such as pollinators or symbionts\(^4\). Plant secondary products also have protective actions in relation to abiotic stresses such as those associated with changes in temperature, water status, light levels, UV exposure and mineral nutrients\(^3\). Furthermore, recent work has indicated potential roles of secondary products at the cellular level as plant growth regulators, modulators of gene expression and in signal transduction.
Although secondary products can have a variety of functions in plants, it is likely that their ecological function may have some bearing on potential medicinal effects for humans. For example, secondary products involved in plant defense through cytotoxicity toward microbial pathogens could prove useful as antimicrobial medicines in humans. Likewise, secondary products involved in defense against herbivores through neurotoxin activity could have beneficial effects in humans (i.e. as antidepressants, sedatives, muscle relaxants, or anesthetics) through their action on the central nervous system. Some plant secondary products may exert their action by resembling endogenous metabolites, ligands, hormones, signal transduction molecules, or neurotransmitters and thus have beneficial medicinal effects on humans due to similarities in their potential target sites.

For pharmaceuticals ranging from digitalis to vincristine the ethno-botanical approach to drug discovery has proved successful. The advent of high-throughput, mechanism-based in-vitro bioassays coupled with candidate plants derived from painstaking ethno-pharmacological research has resulted in the discovery of new pharmaceuticals such as prostratin (1), a drug candidate for treatment of human immunodeficiency virus, as well as a variety of novel antiinflammatory compounds.
5.2 PAST WORK ON MIMOSACEAE

The occurrence and distribution of rare amino acids in 104 species and 40 genera of the *Mimosaceae* have been studied from a comparative phytochemical viewpoint\(^5\). The results reveal characteristic distribution patterns of non-protein amino acids which obviously reflect specific features of genera of this family.

5.2.1 Past work on *Dichrostachys cinerea*

Total phenolics in leaves, pods and seeds of *Dichrostachys cinerea* were investigated by Ernst *et al*\(^6\). The concentration of phenolic substances was high in young leaves, in immature fruits and seeds and low in mature leaves and pods. Krishnamoorthy and Seshadri\(^7\) identified cyanidin (2) and quercetin (3) in *D. cinerea*. Krishna and Tara\(^8\) have isolated n-octacosanol (4), β-amyrin (5), friedelan-3-one (6), friedelan-3β-ol (7) and β-sitosterol (8) from the root of *D. cinerea*. The chemical constituents of *D. cinerea* leaves were studied by Khattab\(^9\). The coumarin fraction of *D. cinerea* leaves was
found to contain imperatorin (9), marmesin (10), and aesculetin (11), whereas stigmasterol (12), and γ-sitosterol (13) were isolated from the unsaponifiable fraction.

\[ \text{(2)} \]

\[ \text{(3)} \]

\[ \text{(4)} \]

\[ \text{(5)} \quad R = H \\
\text{(28)} \quad R = \text{CH}_3\text{CO} \]
Solvent extraction of aerial parts of *D. cinerea* and fractionation led to the characterization$^{10}$ of n-octacosanol (4), β-sitosterol (8), friedelin (14), epifriedelinol (7), and α-amyrin (15).
5.3 PAST WORK ON ASCLEPIADACEAE

The distribution of flavonoid glycosides in some south Indian plants belonging to the family *Asclepiadaceae* was studied by Sankara and Nair\textsuperscript{11}. These plants contained significant amount of quercetin (3) and traces of kaempferol (16). Flavone (17) was absent in all the plants examined. While examining the possibility of using renewable plant materials as sources of hydrocarbons, laticiferous plants belonging to the family *Asclepiadaceae* were evaluated by Bhatia \textit{et al}\textsuperscript{12}. Biocrude can be obtained from laticiferous species by tapping, preserving and coagulating latex, or by successive extraction of dried plant biomass with hexane and methanol to obtain biocrude and polar extracts respectively\textsuperscript{13}. 

\[ (15) \]
5.3.1 Past work on *Hemidesmus indicus* R. Br.

Prakash *et al*\(^4\) have isolated two new pregnane glycosides designated as indicine (18) and hemidine (19) from the dried stem of *Hemidesmus indicus*. Chemical and spectroscopic evidence is consistent with the structures, calogenin-3-0-beta-D-digitoxopyranoside (18) and calogenin-3-O-beta-D-boivino pyranoside (19) respectively. Three new pregnane oligoglycosides medidesmine (20), hemisine (21), and desmisine (22) were isolated from the dried stem of *Hemidesmus indicus*\(^5\). On the basis of chemical and spectroscopic evidence, the structure of pregnane oligoglycosides were established and they were identified as sarcostin-3-alpha-D-glucopyranosyl (1-4) -O-beta-D-digitoxopyranosyl (1→4) -O-beta-D-oleandro pyranoside (20), calogenin-3-O-beta-D-cymaropyranosyl (1→4) -O-[3-O-methyl] beta-D-glucopyranosyl (1→4) -O-beta-D-glucopyranosyl (1→4) -O-beta-D-cymaropyranoside (21) and calogenin- 3-O-beta-D-xylopyranose (1→4)-O-beta-D-
digitoxopyranosyl \((1\rightarrow4)\)-\(\beta\)-D-xylopyranosyl \((1\rightarrow4)\)-\(\beta\)-D-digitoxopyranoside (22) respectively.

\[
(18) \quad R^1 = R^2, \quad R^4 = H \\
(19) \quad R^1 = R^3, \quad R^4 = H
\]

\[
(20) \quad R_1 = R_2 = R_3 = R_4 = OH, \quad R_5 = R_6 \\
(21) \quad R_1 = OH, \quad R_2 = R_3 = R_4 = H, \quad R_5 = R_7 \\
(22) \quad R_1 = OH, \quad R_2 = R_3 = R_4 = H, \quad R_5 = R_8
\]

Sigler \textit{et al}\textsuperscript{16} have also isolated two novel pregnane glycosides designated as denicunine (23) and heminine (24) from the dried stem of \textit{H. indicus}. By chemical transformations and spectroscopic evidences, they
were identified as calogenin 3-O-3-O-methyl-β-D-fucopyranosyl-(1→4)-O-β-D-oleandro pyranoside (23) and calogenin 3-O-β-D-cymaro pyranosyl- (1→4)-O-β-D digitoxo pyranoside (24) respectively.

Rutin (25) was isolated from the leaves of *Hemidesmus indicus* by Sankara and Nair. Padhy *et al*. have isolated triterpenoids from the roots of
*H. indicus*. The petroleum ether extract of *H. indicus* roots on repeated chromatographic separation yielded hexatriacontane (26), lupeol octacosanoate (27), β-amyrin acetate (28), lupeol acetate (29), α-amyrin (15), lupeol (30), β-amyrin (5) and β-sitosterol (8). A new pregnane ester diglycoside named desinine (31) was isolated from the dried twigs of *H. indicus*. On the basis of chemical and spectroscopic evidences, its structure has been established as drevogenin -B-3-0-β-D- oleandropyranosyl- (1-4)- β-D-oleandropyranoside (31).
(27) $R = \text{CH}_3(\text{CH}_2)_{26}\text{COO}$

(29) $R = \text{CH}_3\text{COO}$

(30) $R = \text{H}$
Hemidesminine (32), a new coumarinolignoid isolated from *H. indicus* has been assigned the structure based on UV, IR, NMR and mass spectral data\(^{19}\). Das *et al.\(^{20}\)* have isolated two more new coumarinolignoids hemidesmin-1 (33) and hemidesmin-2 (34) from the roots of *H. indicus*. Their structures were detected from UV, IR, PMR and mass spectral data.

\[
\begin{align*}
(33) & \quad R = \text{Me}, \; R^1 = \text{OMe} \\
(34) & \quad R = R^1 = \text{H}
\end{align*}
\]

### 5.4 PAST WORK ON PARMELIACEAE

The chemical components of lichen substances and colour reactions of 20 type specimens of *Parmelia* have been listed by Mason\(^{21}\). A study of the morphology and lichen chemistry of the North American members of the *Parmelia quercina* complex has lead to the recognition of 5 species. *P. quercina* (California), *P. coronata* (Mexico), *P. confoederata* (Southeastern United States), *P. galbina* (Eastern North America) and
P. livida (Eastern and South Central United States). All the five species produce the depside atranorin (35). P. quercina and P. confoederata also produce the depside lecanoric acid (36) and the closely related P. coronata produces gyrophoric acid (37), chemically similar to lecanoric acid (36)\textsuperscript{22}.

\[
\begin{align*}
\text{CH}_3 & \quad \ldots \quad \text{R} \\
\text{R'} & \quad \ldots \quad \text{OH} \\
\text{CH}_3 & \quad \ldots \quad \text{COOM''}
\end{align*}
\]

(35) $R = \text{CHO}$, $R' = M'' = \text{CH}_3$, $M = M' = \text{H}$

(38) $R = R' = M = M' = \text{CH}_3$, $M'' = \text{H}$

\[
\begin{align*}
\text{CH}_3 & \quad \ldots \quad \text{OH} \\
\text{HO} & \quad \ldots \quad \text{COOH} \\
\text{CH}_3 & \quad \ldots \quad \text{COOH}
\end{align*}
\]

(36)

\[
\begin{align*}
\text{CH}_3 & \quad \ldots \quad \text{OH} \\
\text{HO} & \quad \ldots \quad \text{COO} \\
\text{CH}_3 & \quad \ldots \quad \text{COOH}
\end{align*}
\]

(37)
Lichen substances were identified by Kurokawa\textsuperscript{23} by crystal tests and paper and thin-layer chromatography in \textit{Parmelia} species. \textit{P. mesogenes} contained atranorin (35) and diffractaic acid (38). \textit{P. insueta} contained atranorin (35), diffractaic acid (38) and protocetraric acid (39). \textit{P. ferax} and \textit{P. gerlachi} both contained usnic acid (40) and physodalic acid (41). \textit{P. psoromifera} produced usnic acid (40) and psoromic acid (42).

\begin{center}
\begin{tikzpicture}
\begin{scope}
\node at (0,0) (a) {CH$_3$};
\node at (1,0) (b) {CH$_2$OM};
\node at (1,-1) (c) {CHO};
\node at (2,-1) (d) {COOH};
\node at (2,1) (e) {OH};
\node at (1,1) (f) {CO};
\node at (0,1) (g) {O};
\node at (0,2) (h) {OH};
\node at (0,1.5) (i) {m=H};
\path (a) edge (b);
\path (b) edge (c);
\path (c) edge (d);
\path (d) edge (e);
\path (e) edge (f);
\path (f) edge (g);
\path (g) edge (h);
\path (h) edge (i);
\end{scope}
\node at (3,0) (j) {CHO};
\node at (4,0) (k) {CH$_3$};
\node at (3,-1) (l) {HO};
\node at (4,-1) (m) {CO(CH$_3$)$_2$};
\node at (3,1) (n) {HO};
\node at (3,-0.5) (o) {H$_3$C};
\node at (4,1) (p) {COCH$_3$};
\node at (4,0) (q) {OH};
\node at (3,0.5) (r) {O};
\node at (3,1.5) (s) {m=COCH$_3$};
\path (j) edge (k);
\path (k) edge (l);
\path (l) edge (m);
\path (m) edge (n);
\path (n) edge (o);
\path (o) edge (p);
\path (p) edge (q);
\path (q) edge (r);
\path (r) edge (s);
\end{tikzpicture}
\end{center}

Chemical examination of some lichens of \textit{Parmelia} has been performed by Murthy and Venkateswarlu\textsuperscript{24}. Atranorin (35) and salazinic acid (43) were found in \textit{P. nepalensis}. Atranorin (35), salazinic acid (43) and
lecanoric acid (36) in *P. cristifera* and in *P. reticulata* and atranorin (35), salazinic acid (43), lecanoric acid (36) and norstictic acid (44) in *P. tinctorum*.

![Chemical structure of lecanoric acid](image)

(42)

![Chemical structure of salazinic acid](image)

(43) \( R = \text{CH}_2\text{OH}, \ M = \text{H} \)

(44) \( R = \text{CH}_3, \ M = \text{H} \)

(48) \( R = \text{M = CH}_3 \)

The content of free amino acid in 28 kinds of domestic lichens has been determined by an amino acid auto analyzer and total N by the semi micro-Kjeldahl method by Fujikawa and Hirai\textsuperscript{25}. Using thinlayer chromatographic studies of the constituents of the Ether-Methanol extract of
11 species of lichens endemic in the Philippines have been performed by Sevilla et al.\textsuperscript{26} Chemical investigations of Indian lichens have been performed by Malik et al\textsuperscript{27}. Silva and Carrazzoni have isolated graianic acid (45) from \textit{Parmelia} species and identified it by its UV and IR spectra\textsuperscript{28}.

Ergosterol (46) and tocopherol (47) were quantitatively detected in dried and milled samples of Swedish lichens by Dasilva and Englund\textsuperscript{29}. 

\begin{figure}
\centering
\includegraphics[width=\textwidth]{chemical Structures}
\caption{(45)}
\end{figure}
Six *Parmelia* species in Pakistan were analyzed by colour and microcrystal tests. *P. flaventior* produced the cortical substance usnic acid (40) in addition to atranorin (35). The remaining species *P. borreri*, *P. reddenda*, *P. rudecta*, *P. stictica* and *P. subrudecta* produced only atranorin (35) in the cortex. Diagnostic medulary substances obtained were lecanoric acid (36) and gyrophoric acid (37) and various fatty acids.

Elemental composition of two epiphytic lichens, *Usnea* and *Parmelia* were examined by Bosserman and Hagner. The application of microbore, reversed phase columns in the separation of lichen metabolites, pyridine derivatives and pyrimidine bases, each with different operating conditions is described by Lochmueller *et al*.

The protein composition of lichens of genus, *Parmelia* was determined by Blyum and Brun. Considerable variability was noted within species with regard to the season and the growth substrate.

Investigations on carotenoids in lichens from Brazil was performed by Czeczuga and Xavier. The carotenoid content of lichens from Greenland was performed by Czeczuga and Alstrup. The composition and content of
these carotenoids were not correlated with major ecological differences. Column and thinlayer chromatography revealed the presence of carotenoids in the thalli of 10 lichen species from various Italian environments

From the shade-dried South Indian lichens, Parmelia praesorediosa and P. reticulata atranorin (35), lecanoric acid (36) and stictic acid (48) have been isolated by Ramesh and Ali.

5.4.1 Past work on Parmelia perlata Ach.

Usnic acid (40) and atranorin (35) percentages were determined in Parmelia perlata by Reyes et al. The amount of lichen substances atranorin (35), chloroatranorin (49) and salazinic acid (43) in P. perlata have been determined by Siegfried and Gerhardt. The chloroform soluble fraction of diethyl ether extract of P. perlata gave atranorin (35) and chloroatranorin (49) and the chloroform insoluble fraction of the same gave salazinic acid (43). Tarique et al have isolated a new lanost-2-en type triterpene, named parmelanostene (50) and a new labdane type diterpenoid, named permelabdone (51). These compounds were also found to have antibacterial potential against Staphylococcus aureus and Escherecia coli bacterial strains.
5.5 PAST WORK ON SIDA

The roots of various *Sida* spp. Including *S. rhombifolia*, *S. veronicaefolia*, *S. glutinosa*, *S. chinensis* and *S. cordifolia* contain steroids, alkaloids and fatty oils. The alkaloid content for the various spp. ran about 0.053%, a little higher for *S. acuta* (0.066%) and *S. glutinosa* (0.064%). Seeds of *S. acuta* contained 0.260% total alkaloids. Six *Sida* species viz. *S. acuta*, *S. cordifolia*, *S. racemosa*, *S. rhombifolia* and *S. rhomboidea* and an un identified species were screened for the pharmocologically active alkaloids, cryptolepine (52), ephedrine and vasicine (53). All the species contained alkaloids. Three types of alkaloidal constituents viz., \(\beta\)-phenethylamines, quinazolines and carboxylated tryptamines in addition to choline (54) and betaine (55) have been isolated from roots and aerial parts of *S. acuta*, *S. humilis*, *S. rhombifolia* and *S. spinosa* and characterized. The favourable combinations of sympathomimetic amines and vasicinone (56) present in these species probably account for their major therapeutic uses. The bio production of the quinazoline alkaloids is probably a characteristic feature of this genus. Thus the genus *Sida* possesses great potential for development of various formulations on modern parameters.

The phytoecdysteroid profiles in seeds of 11 species of *Sida* are examined by Dinan et al., Among 11 species of *Sida* examined, seed
extracts of *S. acuta* and *S. rhombifolia* were found to contain significant amounts of ecdysteroids, seed extracts of *S. filicaulis* contained only moderate levels, while the remaining species showed no detectable levels of ecdysteroids.

\[
R_1 = H, R_2 = \text{H}_2 \\
(52)
\]

\[
R_1 = H, R_2 = \text{O} \\
(53)
\]

\[
(54)
\]

\[
(55)
\]

5.5.1 Past work on *Sida acuta* Burm. F.

Khaleque *et al.* have isolated oxalic acid and \(\beta\)-sitosterol (8) from the petroleum ether extract of *S. acuta* roots. Sterol composition of *Sida acuta*
with particular emphasis on cholesterol (57) occurrence has been performed by Chauhan and Shukla\textsuperscript{48}. From the roots of \textit{S. acuta} \(\alpha\)-amyrin (15), starch, ecdysterone (58) and the alkaloids ephedrine and cryptolepine (52) have been isolated by Rao \textit{et al}\textsuperscript{49}. Chemical analysis of the whole plant led to the isolation of \(\alpha\)-amyrin (15), \(\beta\)-sitosterol (8) and its glucoside, n-octacosanol (4), dimethyl terephthalate (59) and four alkaloids\textsuperscript{50}. Extraction of air-dried and powdered aerial parts of \textit{S. acuta} yielded fractions containing normal and branched chain alkanes, pristane (60), phytane (61), hexatriacontane (26), nonacosane (62), cholesterol (57), campesterol (63), stigmasterol (12), \(\beta\)-sitosterol (8) and stigmast-7-enol (64)\textsuperscript{51}.

Seed oils of \textit{S. acuta} contained sterculic acid (65) and malvalic acid (66)\textsuperscript{52}. Pandit \textit{et al}\textsuperscript{53}, isolated an insect molting hormone from \textit{S. acuta} and identified it as ecdysterone (58).
5.5.2 Past work on *Sida cordata* (Burm. F.) Borssum

Long chain n–alkanes, alcohols and phytosterols were isolated and identified in various fractions of *S. cordata*\(^5\). Alcohols with even number of carbon atoms were predominant in various fractions. The root of *S. cordata* contains steroids, alkaloids and fatty oils\(^4\).

5.6 OBJECTIVES OF THE PRESENT WORK

A recent thorough literature survey revealed that various parts of *Dichrostachys cinerea, Hemidesmus indicus, Parmelia perlata, Sida acuta* and *Sida cordata* have been used to cure many ailments (see chapter2). Amino acids are reported to possess calculi dissolving ability and flavonoids and sugars are reported to possess diuretic activity. So it is worthwhile to perform qualitative and quantitative analysis of various phytochemical constituents of the said five medicinal plants. The main objectives of the present work are:
1. To study the preliminary phytochemistry of medicinally important parts of *Dichrostachys cinerea, Hemidesmus indicus, Parmelia perlata, Sida acuta* and *Sida cordata*.

2. To study the TLC and paper chromatographic behaviour of the various solvent extracts of the above said plants.

3. To determine quantitatively the sugars, amino acids and flavonoids of medicinally important parts of *D. cinerea, H. indicus, P. perlata, Sida acuta* and *Sida cordata*.

4. To identify various amino acids present in the medicinally important parts of *D. cinerea, H. indicus, P. perlata, Sida acuta* and *S. cordata* by performing paper chromatography.
5.7 RESULTS

5.7.1 Preliminary phytochemical screening

1000 g of air-dried medicinally important parts of *Dichrostachys cinerea*, *Hemidesmus indicus*, *Parmelia perlata*, *Sida acuta* and *Sida cordata* are individually and successively treated with petroleum ether (40°–60° C), benzene, chloroform, methanol and water. The different extracts have been tested for steroids, triterpenoids, reducing sugars, alkaloids, phenolic compounds, saponins, xanthoproteins, tannins, flavonoids and aromatic acids. The various phytochemical tests performed are presented in Table 5.1. and the results are presented in Tables 5.2. to 5.6.

Preliminary phytochemical screening of the various extracts of the root of *D. cinerea* showed some significant results and are presented in Table 5.2. Petroleum ether and benzene extracts of *D. cinerea* contain steroids. Methanolic and aqueous extracts of *D. cinerea* show the presence of reducing sugars, saponins, tannins, flavonoids and amino acids. Chloroform extract of *D. cinerea* shows the presence of reducing sugars and phenolic compounds. Alkaloids are present in methanolic extract of *D. cinerea*. Petroleum ether (40°–60°C) and benzene extracts of *H. indicus* show the presence of steroids, triterpenoids and phenolic compounds (Table 5.3). Chloroform extract of *H. indicus* shows the presence of phenolic compounds. Methanolic and aqueous extracts of *H. indicus* show the presence of reducing sugars, saponins, tannins, flavonoids and aminoacids. The results
Table 5.1

Preliminary phytochemical screening tests.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Experiments</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Test solution + a few drops of CHCl₃ + 3 – 4 drops of acetic anhydride and one drop of conc H₂SO₄</td>
<td>Purple colour changing to blue or green</td>
<td>Presence of steroids</td>
</tr>
<tr>
<td>2.</td>
<td>Test solution + piece of tin + 3 drops of thionyl chloride</td>
<td>Violet or purple colour</td>
<td>Presence of Triterpenoids</td>
</tr>
<tr>
<td>3.</td>
<td>Test solution + 2 ml of Fehling’s reagent and 3 ml of water and boil</td>
<td>Red or orange colour</td>
<td>Presence of reducing sugars</td>
</tr>
<tr>
<td>4.</td>
<td>Test solution + very small quantity of anthrone + few drops of conc H₂SO₄ and heat</td>
<td>Green to purple colour</td>
<td>Presence of reducing sugars</td>
</tr>
<tr>
<td>5.</td>
<td>Test solution taken with 2 N HCl. Aqueous layer formed, decanted and to which are added one or two drops of Mayer’s reagent</td>
<td>White turbidity or precipitate</td>
<td>Presence of alkaloids</td>
</tr>
<tr>
<td>6.</td>
<td>Test solution in alcohol + one drop of neutral ferric chloride</td>
<td>Intense colour</td>
<td>Presence of phenolic compounds</td>
</tr>
<tr>
<td>7.</td>
<td>Test solution in alcohol + a bit of magnesium and one or two drops of conc HCl</td>
<td>Red or orange colour</td>
<td>Presence of flavonoids</td>
</tr>
<tr>
<td>8.</td>
<td>Test solution in alcohol + Ehrlich reagent and few drops of conc HCl + excess ammonia</td>
<td>Pink colour</td>
<td>Presence of catechins</td>
</tr>
<tr>
<td>9.</td>
<td>Test solution + H₂O and shaken well</td>
<td>Foamy lather</td>
<td>Presence of saponins</td>
</tr>
<tr>
<td>10.</td>
<td>Test solution + conc HNO₃ acid + excess ammonia</td>
<td>Reddish – orange precipitate</td>
<td>Presence of xanthoproteins</td>
</tr>
<tr>
<td>11.</td>
<td>Test solution + H₂O and lead acetate solution</td>
<td>White precipitate</td>
<td>Presence of tannins</td>
</tr>
<tr>
<td>12.</td>
<td>Test solution + 1% Ninhydrin in alcohol</td>
<td>Blue or violet colour</td>
<td>Presence of amino acids</td>
</tr>
<tr>
<td>13.</td>
<td>Test solution + Magnesium acetate solution</td>
<td>Pink colour</td>
<td>Presence of anthroquinons</td>
</tr>
<tr>
<td>14.</td>
<td>Test solution + NaHCO₃</td>
<td>Brisk effervescence</td>
<td>Presence of aromatic acids</td>
</tr>
</tbody>
</table>

Mayer’s reagent: 5 g of KI and 1.4 g of Mercuric chloride in 100 ml of distilled water
Ehrlich reagent: Para – dimethylamino benzaldehyde.
Table 5.2

Preliminary phytochemical screening of the root of *Dichrostachys cinerea*

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Steroids</th>
<th>Triterpenoids</th>
<th>Reducing sugars</th>
<th>Alkaloids</th>
<th>Phenolic compounds</th>
<th>Saponins</th>
<th>Xantho Proteins</th>
<th>Tannins</th>
<th>Flavonoids</th>
<th>Anthra quinones</th>
<th>Aromatic acids</th>
<th>Amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether (40°–60° C)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Benzene</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chloroform</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>Methanol</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>-</td>
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<tr>
<td>Water</td>
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<td>+</td>
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<td>+</td>
</tr>
</tbody>
</table>

+ Positive  - negative
Table 5.3

Preliminary phytochemical screening of the root of *Hemidesmus indicus*

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Steroids</th>
<th>Triterpenoids</th>
<th>Reducing sugars</th>
<th>Alkaloids</th>
<th>Phenolic compounds</th>
<th>Saponins</th>
<th>Xantho Proteins</th>
<th>Tannins</th>
<th>Flavonoids</th>
<th>Anthra quinones</th>
<th>Aromatic acids</th>
<th>Amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether (40º–60º C)</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Benzene</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Chloroform</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Methanol</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<td>−</td>
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<td>−</td>
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<td>−</td>
<td>−</td>
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<td>+</td>
</tr>
</tbody>
</table>

+ Positive  - negative
### Table 5.4

**preliminary phytochemical screening of Parmelia perlata**

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Steroids</th>
<th>Triterpenoids</th>
<th>Reducing sugars</th>
<th>Alkaloids</th>
<th>Phenolic compounds</th>
<th>Saponins</th>
<th>Xantho Proens</th>
<th>Tannins</th>
<th>Flavonoids</th>
<th>Anthra quinones</th>
<th>Aromatic acids</th>
<th>Amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether (40°–60° C)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Benzene</td>
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<tr>
<td>Chloroform</td>
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</tbody>
</table>

+ Positive  - negative
# Table 5.5

Preliminary phytochemical screening of the root of *Sida acuta*

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Steroids</th>
<th>Triterpenoids</th>
<th>Reducing sugars</th>
<th>Alkaloids</th>
<th>Phenolic compounds</th>
<th>Saponins</th>
<th>Xantho Protops</th>
<th>Tannins</th>
<th>Flavonoids</th>
<th>Anthra quinones</th>
<th>Aromatic acids</th>
<th>Amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether (40°– 60° C)</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Benzene</td>
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<td>+</td>
<td>–</td>
<td>+</td>
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<td>+</td>
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<td>–</td>
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</tr>
<tr>
<td>Chloroform</td>
<td>–</td>
<td>–</td>
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<td>Methanol</td>
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<td>–</td>
<td>+</td>
<td>+</td>
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<td>–</td>
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<td>Water</td>
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<td>+</td>
</tr>
</tbody>
</table>

+ Positive  - negative
<table>
<thead>
<tr>
<th>Extracts</th>
<th>Steroids</th>
<th>Triterpenoids</th>
<th>Reducing sugars</th>
<th>Alkaloids</th>
<th>Phenolic compounds</th>
<th>Saponins</th>
<th>Xantho</th>
<th>Proteins</th>
<th>Tannins</th>
<th>Flavonoids</th>
<th>Anthra</th>
<th>quinones</th>
<th>Aromatic</th>
<th>acids</th>
<th>Amino acids</th>
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</thead>
<tbody>
<tr>
<td>Petroleum ether (40(^\circ)–60(^\circ) C)</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Chloroform</td>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

+ Positive  - negative
of preliminary phytochemical analysis of various extracts of *P. perlata* are presented in Table 5.4. Petroleum ether (40°–60° C) extract shows the presence of triterpenoids, phenolic compounds, saponins and aromatic acids. Benzene, chloroform, methanol and water extracts of *P. perlata* show the presence of phenolic compounds, saponins, tannins and aromatic acids. Steroids and triterpenoids are present in petroleum ether and benzene extracts of *S. acuta*. Alkaloids are present in all the extracts of *S. acuta* except aqueous extract. Chloroform, methanol and water extracts show the presence of saponins and tannins. Amino acids and flavonoids are present only in methanolic and aqueous extracts. Benzene and chloroform extracts of *S. cordata* show the presence of steroids. Petroleum ether, benzene and chloroform extracts of *S. cordata* show the presence of alkaloids. Reducing sugars and flavonoids are present in chloroform, methanol and aqueous extracts of *S. cordata*. Methanolic and aqueous extracts show the presence of amino acids.

5.7.2 Determination of sugars, amino acids and flavonoids.

5.7.2.1 Sugars

Sugars present in the medicinally important parts of *Dichrostachys cinerea*, *Hemidesmus indicus*, *Parmelia perlata*, *Sida acuta* and *Sida cordata* were quantitatively determined and the results are presented in Table 5.7. From the table it is very clear that *Parmelia perlata* contains maximum amount of sugars followed by *Dichrostachys cinerea*,
Table 5.7
Sugars, total free amino acids, L-proline and flavonoids contents of medicinally important parts of the five medicinal plants.

<table>
<thead>
<tr>
<th>Medicinal Plant</th>
<th>Sugars (mg/g d.w.)</th>
<th>Total free amino acids (mg/g d.w.)</th>
<th>L-Proline (μg/g d.w.)</th>
<th>Flavonoids (A/g d.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dichrochachys cinerea</em> (root)</td>
<td>6.50</td>
<td>0.604</td>
<td>18.3</td>
<td>2.96</td>
</tr>
<tr>
<td><em>Hemidesmus indicus</em> (root)</td>
<td>5.93</td>
<td>0.702</td>
<td>24.1</td>
<td>4.19</td>
</tr>
<tr>
<td><em>Parmelia perlata</em> (thallus)</td>
<td>9.85</td>
<td>0.671</td>
<td>13.4</td>
<td>4.22</td>
</tr>
<tr>
<td><em>Sida acuta</em> (root)</td>
<td>5.56</td>
<td>0.095</td>
<td>6.07</td>
<td>2.62</td>
</tr>
<tr>
<td><em>Sida cordata</em> (aerial parts)</td>
<td>6.03</td>
<td>0.590</td>
<td>14.3</td>
<td>4.93</td>
</tr>
</tbody>
</table>
*Sida cordata* and *Hemidesmus indicus*. *Sida acuta* has the lowest amount of sugars.

5.7.2.2 Total free amino acids

The total free amino acid contents of medicinally important parts of *Dichrostachys cinerea, Hemidesmus indicus, Parmelia perlata, Sida acuta* and *Sida cordata* are presented in Table 5.7. Total free amino acid contents are higher in *Hemidesmus indicus* followed by *Parmelia perlata* and *Dichrostachys cinerea*. The lowest amount of total free amino acids are present in *Sida acuta*.

5.7.2.3 L-Proline

Proline content of medicinally important parts of *Dichrostachys cinerea, Hemidesmus indicus, Parmelia perlata, Sida acuta* and *Sida cordata* are presented in Table 5.7. It is found that *Hemidesmus indicus* has got the maximum proline content and *Sida acuta* has got the minimum proline content.

5.7.2.4 Flavonoids

Flavonoid contents of medicinally important parts of *Dichrostachys cinerea, Hemidesmus indicus, Parmelia perlata, Sida acuta* and *Sida cordata* are also presented in Table 5.7. Of the five plants studied, *Sida cordata* possesses the maximum amount of flavonoids and *Sida acuta* possesses the lowest amount of flavonoids.
5.7.3 Chromatographic studies

5.7.3.1 Thin layer chromatography

Thin layer chromatographic studies have been performed for the petroleum ether (40°–60° C), benzene, chloroform and methanol extracts of the medicinally important parts of *Dichrostachys cinerea, Hemidesmus indicus, Parmelia perlata, Sida acuta* and *Sida cordata*. The TLC plates have been viewed through ultraviolet fluorescence viewing cabinet (365 nm) before keeping in an iodine chamber and the $R_f$ values of the fluorescing spots have been measured. Different solvent systems are found to be effective to get the maximum number of spots for the various extracts. $R_f$ values of the various spots obtained when the chromatogram was developed in an iodine chamber are presented in Tables 5.8–5.12.

5.7.3.2 Identification of amino acids

The amino acids present in the methanolic and aqueous extracts of medicinally, important parts of *Dichrostachys cinerea, Hemidesmus indicus, Parmelia perlata, Sida acuta* and *Sida cordata* have been identified by paper chromatographic technique by comparison with the standard aminoacids based on their $R_f$ values. The concentrated methanolic and aqueous extracts were subjected to ascending paper chromatography and the papers were developed in n-butanol : acetic acid : water (12:3:5; V/V/V). 0.2% ninhydrin in acetone was used as a spraying reagent. The $R_f$ values of spots have been measured and the results are presented in Tables
Table 5.8
Thinlayer chromatographic behaviour of the root of *Dichrostachys cinerea*

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Solvent system used</th>
<th>R_f value of the spots under UV light</th>
<th>R_f value of the spots under iodine chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether (40°-60° C) extracts</td>
<td>Petroleum ether (40° - 60° C) : Benzene (1:3)</td>
<td>0.05 (greenish yellow)</td>
<td>0.05*, 0.36*, 0.85*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.83 (greenish yellow)</td>
<td></td>
</tr>
<tr>
<td>Benzene extract</td>
<td>Benzene : Chloroform (1:3)</td>
<td>0.04 (bluish green)</td>
<td>0.04*, 0.23*, 0.72*</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>Chloroform (100%)</td>
<td>0.34 (pale blue)</td>
<td>0.03*, 0.42*, 0.62*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.62 (bluish green)</td>
<td></td>
</tr>
<tr>
<td>Methanol extract</td>
<td>Chloroform: methanol (3:7)</td>
<td>------</td>
<td>0.34*, 0.52*, 0.59*, 0.79*</td>
</tr>
</tbody>
</table>

* More intense; **Moderately intense; o-less intense
<table>
<thead>
<tr>
<th>Extracts</th>
<th>Solvent system used</th>
<th>( R_f ) value of the spots under UV light</th>
<th>( R_f ) value of the spots under iodine chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether (40°– 60° C) extract</td>
<td>Benzene:Chloroform (1:1)</td>
<td>---</td>
<td>0.11 *, 0.23 *, 0.67 *</td>
</tr>
<tr>
<td>Benzene extract</td>
<td>Benzene:Chloroform (1:3)</td>
<td>0.32 (greenish yellow) 0.84 (greenish yellow)</td>
<td>0.02 *, 0.64 *, 0.79 *</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>Chloroform:Methanol (4:1)</td>
<td>0.44(pale blue) 0.62(violet)</td>
<td>0.13 *, 0.43 *, 0.47 *, 0.69 *</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>Chloroform:Methanol (3:2)</td>
<td>-----</td>
<td>0.03 *, 0.24 *, 0.42 *, 0.62 °</td>
</tr>
</tbody>
</table>

* -More intense; * -Moderately intense; ° -Less intense.
### Table 5.10

**Thinlayer chromatographic behaviour of the thallus of *Parmelia perlata***

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Solvent system used</th>
<th>$R_f$ value of the spots under UV.light</th>
<th>$R_f$ value of the spots under iodine chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether (40°–60° C) extract</td>
<td>Petroleum ether (40 °– 60°C):</td>
<td>0.07 (greenish yellow)</td>
<td>0.13°, 0.42 °, 0.97 *</td>
</tr>
<tr>
<td></td>
<td>Benzene (1:2)</td>
<td>0.97 (greenish yellow)</td>
<td></td>
</tr>
<tr>
<td>Benzene extract</td>
<td>Benzene:Chloroform (1:1)</td>
<td>0.09 (bluish green)</td>
<td>0.09 *, 0.29 *, 0.43 *</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.23 bluish green</td>
<td></td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>Chloroform (100%)</td>
<td>0.04 (greenish yellow)</td>
<td>0.04 *, 0.15 °, 0.31 <em>, 0.66</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.15 (greenish yellow)</td>
<td>0.99 *</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.31 (violet)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.66 (greenish yellow)</td>
<td></td>
</tr>
<tr>
<td>Methanol extract</td>
<td>Chloroform (100%)</td>
<td>0.04 (violet)</td>
<td>0.04 *, 0.17 *, 0.41 *, 0.71 *</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.66 (greenish yellow)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.71 (greenish yellow)</td>
<td></td>
</tr>
</tbody>
</table>

*- More intense;  * - Moderately intense; ° - Less intense
Table 5.11

Thin layer chromatographic behaviour of the root of *Sida acuta*

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Solvent system used</th>
<th>$R_f$ value of the spots under UV light</th>
<th>$R_f$ value of the spots under iodine chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether (40°–60°C) extract</td>
<td>Petroleum ether (40 °–60 ° C): Benzene (1:2)</td>
<td>0.02 (pale blue) 0.98(greenish yellow)</td>
<td>0.02 *, 0.11 *, 0.27 *, 0.38 *</td>
</tr>
<tr>
<td>Benzene extract</td>
<td>Benzene : Chloroform (1:3)</td>
<td>0.03 (pale blue) 0.17(greenish blue)</td>
<td>0.03 *, 0.17 *, 0.98 *</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>Chloroform (100%)</td>
<td>0.02 (blue) 0.06 (greenish yellow) 0.09 (purple) 0.98 (violet)</td>
<td>0.02 *, 0.08 °, 0.16 *, 0.96 *</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>chloroform: Methanol (1:3)</td>
<td>------</td>
<td>0.07*, 0.22*, 0.95*</td>
</tr>
</tbody>
</table>

*More intense : * - Moderately intense; ° - less intense.
Table 5.12

Thin layer chromatographic behaviour of the aerial parts of *Sida cordata*

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Solvent system used</th>
<th>( R_f ) value of the spots under UV light</th>
<th>( R_f ) value of the spots under iodine chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether (40°-60°C) extract</td>
<td>petroleum ether (40°-60°C) : Benzene (1:2)</td>
<td>0.07 (greenish yellow) 0.98 (greenish yellow)</td>
<td>0.07 *, 0.11 *, 0.19 *</td>
</tr>
<tr>
<td>Benzene extract</td>
<td>Benzene : Chloroform (1:1)</td>
<td>0.06 (bluish green)</td>
<td>0.06 *, 0.14 °, 0.25 *</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>Chloroform (100%)</td>
<td>0.02 (violet) 0.08 (bluish green) 0.98 (pale blue)</td>
<td>0.02 *, 0.08 °, 0.16 *, 0.98 *</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>Chloroform (100%)</td>
<td>0.18 (pale blue)</td>
<td>0.16 *, 0.26 *</td>
</tr>
</tbody>
</table>

* More intense; * - Moderately intense; ° - less intense
5.13 and 5.14. In the present study it has been found that a maximum of six and eight amino acids out 17 amino acids tested are present respectively in the methanolic and aqueous extracts of *Dichrostachys cinerea*. *H indicus* is found to contain three amino acids in the methanolic extract and five amino acids in the aqueous extract. Five amino acids are found to be present in the aqueous extract of *P. perlata* and the methanolic extract of *P. perlata* did not give any positive result for the presence of amino acids. *S. acuta* is found to possess four amino acids in the methanolic extract and 3 amino acids in the aqueous extract. *S. cordata* has three and one amino acids respectively in the methanolic and aqueous extracts.
Table 5.13
Ascending paper chromatography of amino acids in the methanolic extract of the five medicinal plants

<table>
<thead>
<tr>
<th>Name of the Species</th>
<th>Phenylalanine</th>
<th>Ornithine</th>
<th>Leucine</th>
<th>Cystine</th>
<th>Lysine</th>
<th>Glycine</th>
<th>Aspartic acid</th>
<th>Proline</th>
<th>Tyrosine</th>
<th>Valine</th>
<th>Hydroxyproline</th>
<th>Alanine</th>
<th>Arginine</th>
<th>Serine</th>
<th>Glutamic acid</th>
<th>Tryptophan</th>
<th>Threonine</th>
</tr>
</thead>
<tbody>
<tr>
<td>R&lt;sub&gt;f&lt;/sub&gt; Value</td>
<td>.64</td>
<td>.09</td>
<td>.65</td>
<td>.06</td>
<td>.10</td>
<td>.14</td>
<td>.12</td>
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<td>.22</td>
<td>.24</td>
<td>.11</td>
<td>.16</td>
<td>.18</td>
<td>.46</td>
<td>.23</td>
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<td>Dichrostachys cinerea</td>
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<tr>
<td>Parmelia perlata</td>
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<tr>
<td>Sida cordata</td>
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</tbody>
</table>

+positive; -negative
Table 5.14

Ascending paper chromatography of amino acids in the aqueous extract of the five medicinal plants

<table>
<thead>
<tr>
<th>Name of the Species</th>
<th>Phenylalanine</th>
<th>Ornithine</th>
<th>Leucine</th>
<th>Cystine</th>
<th>Lysine</th>
<th>Glycine</th>
<th>Aspartic acid</th>
<th>Proline</th>
<th>Tyrosine</th>
<th>Valine</th>
<th>Hydroxyproline</th>
<th>Alanine</th>
<th>Arginine</th>
<th>Serine</th>
<th>Glutamic acid</th>
<th>Tryptophan</th>
<th>Threonine</th>
</tr>
</thead>
<tbody>
<tr>
<td>R&lt;sub&gt;f&lt;/sub&gt; value</td>
<td>.64</td>
<td>.09</td>
<td>.65</td>
<td>.06</td>
<td>.10</td>
<td>.14</td>
<td>.28</td>
<td>.31</td>
<td>.44</td>
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<td>.24</td>
<td>.11</td>
<td>.16</td>
<td>.18</td>
<td>.46</td>
<td>.23</td>
<td></td>
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<tr>
<td><strong>Dichrostachys cinerea</strong></td>
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<tr>
<td><strong>Hemidesmus indicus</strong></td>
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<td><strong>Parmelia perlata</strong></td>
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<tr>
<td><strong>Sida acuta</strong></td>
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<tr>
<td><strong>Sida cordata</strong></td>
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</tbody>
</table>

+positive; -negative
5.8 DISCUSSION

The search for new drugs of plant origin has yielded fruitful result in the past. Isolated phytoconstituents have been found to be useful in therapeutics and in pharmaceutical formulations. Pharmaceutical importance/therapeutic value of medicinal plants is due to specific constituents/combinations of secondary metabolites present in them. The main group of phytoconstituents of therapeutic significance can be classified as: carbohydrates, glycosides, tannins and phenolic compounds, lipids, volatile oils, resins and resin combinations and alkaloids.

Carbohydrates have many uses in pharmacy. Carbohydrates can be conveniently classified into three main groups: the mono- and oligosaccharides (true sugars); the polysaccharides (nonsugars); and the derived carbohydrates (gums, mucilages and pectin). Glycosides are the substances which on hydrolysis yield one or more sugars along with a non-sugar compound. The sugar component is known as the glycone and the non-sugar component is called the aglycone or genin. When the sugar part is glucose the substance may be called as glucoside or glycoside.

Glycosides are widely distributed in nature and are the constituents of many therapeutically active drugs. Cardiac glycosides have a strong, direct action on the heart, helping to support its strength and rate of contraction when it is failing. Cardiac glycosides are also significantly diuretic. They help to transfer fluids from the tissues and circulatory system to the urinary tract, thereby lowering blood pressure. Certain
sugars like glucose and sucrose are also found to be diuretic. The present study revealed that all the five plants contain considerable amount of sugars which may be responsible for their diuretic activity.

Amino acids, monomers of peptides and proteins have extensive industrial applications. In the food industry, amino acids are used alone or in combination to enhance food quality and for flavour. Since proteins are continually being synthesized and broken down in the living organisms, all the amino acids are present in varying concentrations in tissues and in the body fluids such as the blood. They also serve as neurotransmitters. Various amino acids are also involved in the biosynthesis of pyrimidine and purine bases of DNA and RNA. Amino acids are reported to possess calculi dissolving ability. L-lysine monohydrochloride is found to possess diuretic activity. The fungus *Polyporus umbellatus* is reported to possess strong diuretic action. The major components isolated from it are proteins (which are composed of amino acids) and ergosterol. In the present investigation total free amino acids are found to be maximum in *H. indicus* followed by *P. perlata* and *D. cinerea*. The lowest amount of total free amino acids are present in *S. acuta*. It has also been found that *H. indicus* has got the maximum proline content and *S. acuta* has got the minimum proline content. Hence these amino acids could have been responsible for the diuretic activity.

Flavonoids are the largest group of naturally occurring phenolic compounds, which occur in different plant parts both in free state and as
glycosides. The presence of these flavonoids is responsible for the various colours and combinations of colours exhibited by bark, leaves, flowers, fruits and seeds of plants. The flavonoids also exhibit various biological activities in mammals, the most important one being the antioxidant activity. They are anti-inflammatory and are especially useful in maintaining healthy circulation. Flavonoids are reported to possess diuretic activity in rats. Of the five plants studied, S. cordata has the maximum amount of flavonoids followed by P. perlata and H. indicus. S. acuta has the minimum amount of flavonoids. The flavonoids present in these medicinal plants may be responsible for the diuretic activity.
5.9 EXPERIMENTAL

5.9.1 Preliminary phytochemical screening

1000 g each of the medicinally important parts of *Dichrostachys cinerea*, *Hemidesmus indicus*, *Parmelia perlata*, *Sida acuta* and *Sida cordata* were individually and successively extracted with petroleum either (40°–60° C), benzene, chloroform, methanol and water in a soxhlet apparatus. The solvents were removed by reduced pressure distillation. The different extracts were tested for steroids, reducing sugars, triterpenoids, alkaloids, phenolic compounds, flavonoids, saponins, tannins, anthraquinons and aromatic acids. The various phytochemical tests were performed according to the method of Brinda et al. and are presented in Table 5.1 and the results are presented in Tables 5.2 – 5.6.

5.9.2 Determination of Sugars, amino acids and flavonoids

5.9.2.1 Sugars

For the estimation of sugars, the procedure of Nelson was followed.

Principle:

The colorimetric method is based on the determination of cuprous oxide produced by heating sugar with alkaline copper reagents and with arsenomolybdate reagent. The blue colour developed was read at 620 nm using Perkin Elmer (Lambda – 25 Model).
Reagents:

(i) 80% Ethanol

(ii) 50% Hydrochloric acid

(iii) 0.2 N Sodium hydroxide

(iv) Arsenomolybdate reagent

2.5 g ammonium molybdate was dissolved in 45 ml water, followed by 2.5 ml sulphuric acid and mixed well. Then 0.3 g disodiumhydrogenarsenate was dissolved in 25 ml water. Both the solutions were mixed well and incubated at 37°C for 24 – 48 h.

(v) Low Alkalinity copper reagent

Copper reagent - A

2.5 g Anhydrous sodium carbonate, 2 g sodium bicarbonate, 2.5 g sodium potassium tartrate and 20 g anhydrous sodium sulphate were dissolved in 80 ml of water and made up to 100 ml.

Copper reagent – B

15 g Copper sulphate was dissolved in a small volume of distilled water and then one drop of sulphuric acid was added and made upto 100 ml.

96 ml of reagent A and 4 ml of reagent B were mixed before use.
Procedure:

200 mg each of dried sample was boiled with 15 ml of 80% ethanol for 5 min. It was centrifuged at 3000 rpm for 10 minutes. The supernatant was made up to known volume with 80% ethanol and was used to assay the reducing sugar. From this extract 5 ml was taken and added with 1 ml of 50% hydrochloric acid and followed by a drop of 0.2 N sodium hydroxide. This was the source for total sugar. From these sources 0.01 ml of the extract was taken separately and added with 1 ml of low alkalinity copper reagent and boiled for 10 minutes vigorously and allowed to cool. After cooling 1 ml of arsenomolybdate reagent was added and made up to 5 ml with distilled water. It was allowed to stand for 15 minutes and the OD was read at 620nm.

5.9.2.2 Total free amino acids

For the estimation of total free amino acids, the procedure of Moore and Stein\textsuperscript{64} was followed.

Principle:

The amino group of all amino acids gives a characteristic pink coloured complex when interacts with ninhydrin. It shows condensation with ninhydrin to form an enol which gives a characteristic pink colour. The absorbance of pink coloured complex can be measured at 560 nm using Perkin Elmer Lambda-25 Model.
Reagents:

a) 0.2 M Acetic acid – Sodium acetate buffer (pH – 5.5)

b) Ninhydrin reagent for estimation: 2 g of ninhydrin was dissolved in 25 ml of methyl cellosolve and 25 ml of acetate buffer (pH 5.5)

c) 80% Ethanol and 50% ethanol.

Procedure:

200 mg each of dried sample was homogenised with 10 ml of 80% ethanol in a mortar and pestle. The extract was centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and made upto 10 ml with 80% ethanol. 1 ml of the extract was added with 3 ml of distilled water and 1 ml of ninhydrin reagent. It was boiled for 1 h in a water bath at 70°C to 80°C till the solution get the colour. Then it was allowed to cool. To this, 1 ml of 50% ethanol was added and the absorbance was read at 560 nm against the reagent blank. The colour intensity at 560 nm was measured in a spectrophotometer using Perkin Elmer (Lambda –25 Model). The amount of free amino acid is expressed as glycine equivalents per gram dry weight.

5.9.2.3 L – Proline

Free proline from the sample was selectively extracted in aqueous sulphosalicylic acid following the method of Bates et al.
Principle:

Proline concentration was measured using the conventional ninhydrin method employed to estimate the amino acids. The absorbance of the pink coloured complex formed by the reaction of the amino group of proline with ninhydrin could be measured at 520 nm.

Reagents:

a) Acid ninhydrin: 1.25 g of ninhydrin was dissolved in a warm mixture of 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid.

b) Glacial acetic acid.

c) 3% (w/v) sulphosalicylic acid (SSA).

d) Toluene

Procedure:

500 mg each of the sample was homogenised in 10 ml of 3% aqueous SSA and the homogenate was filtered through Whatman No. 2 filter paper. The extraction procedure was repeated and the filtrates were pooled. 2 ml of the filtrate was reacted with 2 ml each of ninhydrin and glacial acetic acid in a test tube for 1 h at 100°C, in a water bath and the reaction was terminated in an ice bath. To these contents 4 ml of sulphur free toluene was added and the tubes were agitated vigorously for 15–20 sec. The chromatophore containing toluene was aspirated from the aqueous phase and warmed to room temperature. The absorbance of this proline extract was measured at
520 nm using spectrophotometer. A standard graph using authentic proline was also prepared. The proline content in the sample is expressed in μg of proline per gram of dry weight of the sample.

### 5.9.2.4 Flavonoids

Flavonoid content was estimated spectrocolorimetrically following the method of Mirecki and Teramura\(^\text{66}\).  

100 mg of dried sample was separately placed in 80% acidified methanol, [methanol : water : hydrochloric acid (80 : 20 : 1)] for 12 h at 4°C in darkness to extract the flavonoids. The methanolic extract was used to read the absorbance at 315 nm using Perkin Elmer (Lambda – 25 Model) UV – Vis Spectrophotometer and was expressed as A/g dry weight of the sample.

### 5.9.3 Chromatographic studies

#### 5.9.3.1 Thin layer chromatography

The petroleum ether (40\(^\circ\)–60\(^\circ\)C), benzene, chloroform and methanol extracts of the medicinally important parts of *D. cinerea, H. indicus, P. perlata, S. acuta* and *S. cordata* were subjected to thin layer chromatographic analyses. "Silica gel –G for TLC" was sprayed in thin layers on glass plates by preparing semi–solid slurry. The plates were dried until they were free from moisture and activated in an air oven at 110\(^\circ\)C for about 3 h. Different solvent systems were employed for various extracts of the samples. The plates were viewed under UV light (365 nm) using a UV–visible
viewing cabinet. The fluorescence spots were located (if any) and R$_f$ values were measured. The plates were then developed in an iodine chamber and the R$_f$ values of the spots were calculated. The solvent system employed and the R$_f$ values obtained are presented in Tables 5.8–5.12.

5.9.3.2 Paper chromatography

The concentrated methanolic and aqueous extracts of medicinally important parts of *D. cinerea*, *H. indicus*, *P. perlata*, *S. acuta* and *S. cordata* were subjected to paper chromatographic studies. Whatmann No.1 filter paper was used for this purpose, n-butanol : acetic acid : water mixture (12 : 3 : 5, v / v / v) was used as the solvent system. 0.2% ninhydrin in acetone was used as a spraying reagent. Amino acids have been identified by comparison with standard amino acids based on their R$_f$ values. The results are presented in Tables 5.13 and 5.14.
5.10 REFERENCES


