CHAPTER 4

PHYTOCHEMICAL STUDIES
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4.1. INTRODUCTION

Medicinal plants, since times immemorial, have been used virtually in all cultures as a source of medicine. For thousands of years natural products have been playing an important role throughout the world in treating and preventing human diseases. Historical experiences with plants as therapeutic tools have helped to introduce single chemical entities in modern medicine. Several well known species including licorice (*Glycyrrhiza glabra*), myrrh (*Commiphora* species) and poppy capsule latex (*Papaver somniferum*) were referred to in the first known written record on clay tablets from Mesopotamia in 2600 BC and these plants are still in use for the treatment of various diseases as ingredients of official drugs or herbal preparations used in systems of various traditional medicine (Newmann *et al.*, 2000). Fabricant and Farnsworth (2001) pointed out that the uses of 80% of 122 plant derived drugs were related to their original ethnopharmacological purposes. Some of the prominent commercial plant derived medicinal compounds include: colchicine (1), betulinic acid (2), camptothecin (3), irenotecan, topotecan (4), delta-9-tetrahydrocannabinol, beta apochoine, lapochol, teniposide (5), etoposide (6), podophyllotoxin (7), vinblastine (8), vincristine (9), vindestine, vinorelbine, docetaxel, paclitaxel (10), tubocurarine, pilocarpine and scopolamine (Patwardhan *et al.*, 2004). Recent successes in the field of plant derived compounds include the Chinese antimalarial drug, artemisinin (11) and the Indian ayurvedic drug, forskolin (12).
(1) \( R_1 = H; R_2 = H; R_3 = H \)

(2) \( R_1 = H; R_2 = \text{CH}_2\text{-NMe}_2; R_3 = \text{OH} \)

(3) \( R_1 = H; R_2 = H; R_3 = H \)

(4) \( R_1 = H; R_2 = \text{CH}_2\text{-NMe}_2; R_3 = \text{OH} \)

(5) \( R = H; R' = \)

(6) \( R = H; R' = \)
(7) $R = \text{CH}_3$; $R' = \text{H}$

(8) $R = \text{CH}_3$; (9) $R = \text{CHO}$

(10)

(11)

(12)
Till now natural product compounds discovered from medicinal plants and their analogues thereof have provided numerous clinically useful drugs. This illustrates the potential value and relevance of plant derived secondary metabolites as viable compounds for modern drug development (Balandrin et al., 1993). An analysis of the origin of the drugs developed from 1981 to 2006 showed that natural product derived drugs comprised of 28% of all new chemical entities (NCEs) launched into the market. In addition 24% of these NCEs were synthetic or natural mimic compounds based on the study of pharmacophore related to natural products. This combined percentage (52% of all NCEs) suggests that the natural products are important sources for new drugs and are also good lead compounds suitable for further modification during drug development (Newmann and Cragg, 2007).

Chin et al. (2006) have described 23 new drugs derived from natural sources which have been launched in the market during 2000-2005. Of which five drugs have been derived from terrestrial plants viz. apomorphine (13) from *Papaver somniferum* to treat Parkinson’s disease, tiotropium bromide (14) a derivative of atropine from *Atropa belladonna* to treat chronic obstructive pulmonary disease, nitisinone (15) from *Callistemon citrinus* to treat hereditary tyrosinaemia type-1, galanthamine hydrobromide (16), an alkaloid from *Galanthus nivalis* for the treatment Alzheimer’s disease and arteether (17), an antimalarial drug developed from artemisinin, a sesquiterpene lactone isolated from *Artemisia annua*. 
Considerable research on pharmacognosy, chemistry, pharmacology and clinical therapeutics has been carried out on Ayurvedic medicinal plants. Numerous molecules have come out of Ayurvedic experiential base, examples include Rauwolfia alkaloids (18) for hypertension, Psoralens (19) in vitiligo, Holarrhena alkaloids in amoebiasis, Guggulsterones (Z-Guggulsterone (20), E-Guggulsterone (21) as hypolipidemic agents, **Mucuna pruriens** for Parkinson’s disease, Piperidines as bioavailability enhancers, Baccosides in mental retention, Picrosides (Picroside-I (22), Picroside-II (23), in hepatic protection, Phyllanthins as antivirals, Curcumines in inflammation, Withanolides and many other steroidal lactones and glycosides as immunomodulators (Patwardhan, 2005).
It is estimated that there are 250,000 to 500,000 species of higher plants on earth. But, only a relatively small percentage (5%-15%) has been systematically investigated for the presence of bioactive compounds (Taylor, 2000). Higher plants are still regarded as potential sources of new medicinal compounds. There are many approaches available for the selection of plants for drug discovery; however, the ethnobotanical approach to pharmaceutical lead drug discovery may significantly enhance the probability of identifying a potential drug molecule from medicinal plants.

Drug discovery from plants involves a multidisciplinary approach combining botanical, ethnobotanical, phytochemical and biological techniques. Drug discovery strategies based on natural products and traditional medicines are re-emerging as attractive options. It has been estimated that nearly 75% of about 120 biologically active plant derived substances used throughout the world were discovered by following up on leads from traditional medicine.
A recent thorough literature survey revealed that various parts of *Acalypha fruticosa* and *Dipteracanthus patulus* (syn. *Ruellia patula*) have been used traditionally to treat skin diseases (refer chapter 3) and reports on the pharmacological studies on *Acalypha fruticosa* have revealed its antidiarrhoeal (Mathad *et al.*, 1998), antioxidant, anti-inflammatory (Gupta *et al.*, 2003), anticancer (Mothana *et al.*, 2007), antiplasmodial (Alshawsh *et al.*, 2007) and cytotoxic properties (Mothana *et al.*, 2008 and Rajkumar *et al.*, 2010). However there are no reports on the detailed chemical investigation of this potent medicinal plant. Literature survey revealed that very little information is available on the phytochemical studies on *Dipteracanthus patulus* (Ahmad *et al.*, 1993 and Muthumani *et al.*, 2009). In the present study the aerial parts of *Acalypha fruticosa* and leaves of *Dipteracanthus patulus* (syn. *Ruellia patula*) were selected for phytochemical evaluation on the basis of their traditional uses in skin diseases in particular for healing wounds, in the study area.
4.2. REVIEW OF LITERATURE

4.2.1. Past work on the phytochemical studies on the species of Acalypha

The chemical investigation of Acalypha offered the isolation of different classes of natural products including flavonoids, tannins, sterols, diterpenes, triterpenes, amides and cyanogenic glycosides.

Nahrstedt et al. (1982) have isolated acalyphin (24), a cyanogenic glucoside from Acalypha indica.

\[
\text{(24) } R_1 = \text{CH}_3; R_2 = \text{OH}; R_3 = \text{H}; R_4 = \text{CN.}
\]

The stem extract of Acalypha macrostachya yielded two new friedolabdanes, 18-Hydroxy-7-oxo-15,16-epoxyfriedolabda-5,13(16),14-trien (25), 3α-Hydroxy-7-oxo-15,16-epoxyfriedolabda-5,13(16),14-trien (26) and a new curcumene derivative, 7,8-Dihydroxy-α-curcumene (27). A. diversifolia gave a known amide (28) (Siems et al., 1996). Acalyphidins M1, M2 and D and ellagitannins were isolated from Acalypha hispida (Amakura et al., 1999). Phytochemical studies on the powdered leaves of Acalypha racemosa revealed the presence of alkaloids, tannins, flavonoids and terpenes (Musa et al., 2000).
An activity directed fractionation of 50% aqueous ethanol extract of *Acalypha wilkesiana* and *Acalypha hispida* leaves resulted in the isolation of gallic acid (29), corilagin (30) and geraniin (31) as the compounds responsible for the antimicrobial activity. Quercetin 3-0- rutinoside (32) and kaempferol-3-0-rutinoside (33) were isolated from the inactive fraction of *A. hispida* (Adesina *et al.*, 2000).
Three new cycloartane-type triterpenes, 16α-hydroxymollic, 15α-hydroxymollic and 7β,16β-dihydroxy-1,23-dideoxyjessic acids were isolated from the aerial parts of *Acalypha communis* (Gutierrez-Lugo *et al.*, 2002). Anthocyanins acylated with gallic acid (Reiersen *et al.*, 2003) were reported from *Acalypha hispida*. Thymol (34), camphor (35) and γ-terpinene (36) were identified by GC-MS analysis of the essential oil obtained from the aerial parts of *Acalypha phleoides* (Adela *et al.*, 2004).

Four known kaempferol glycosides, mauritianin (37), clitorin (38), nocotiflorin (kaempferol-3-O-rutinoside) (33) and biorubin have been isolated
from the flowers and leaves of *Acalypha indica* (Nahrstedt *et al.*, 2006). A novel tetraterpene, acalyphaser A (39), was isolated from *Acalypha siamensis* (Kambara *et al.*, 2006). 15,16-Epoxylabda-13(16),14-dien-8alpha-ol, bicyclogermacrene, tau-muurolol, beta-caryophyllene and epi-bicyclosesquiphellandrene were identified from the essential oil from the fresh leaves of *Acalypha plicata* by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) (Meccia *et al.*, 2006).

Octocosonal, sitosterol (40), lindersine and kaempferol (41) were isolated from the whole plant of *Acalypha indica* (Anitha *et al.*, 2007). Preliminary phytochemical study revealed the presence of alkaloids, tannins,
saponins and cardenolides in *A. fimbriata*, *A. hispida*, *A. ornata*, *A. racemosa* and *A. wilkesiana* (Soladoye et al., 2008).

Wang *et al.* (2008) have isolated emodin (42), beta-sitosterol (40), loliolide (43), 2,6-dimethoxy-1,4-benzoquinone, nicotinic acid (44), protocatechuic acid (45), daucosterol (46), gallic acid (29), rutin (32) succinic acid (47) and brevifolin (48) from *A. australis*. 
Ogunwande et al. (2008) identified alpha-pinene, 1,8-cineole, phytol and delta-3-carene in the essential oil of *A. segetalis* by GC and GC/MS analysis. Seven cyanopyridone derivatives namely, acalyphin (24), epiacalyphin (49), noracalyphin (50), epinoracalyphin (51), acalyphin amide (52), epiacalyphin amide cycloside (53), ar-acylphidone (54) and one corresponding seco compound (seco-acalyphin) (55) have been isolated from the methanol extract of the inflorescences and leaves of *Acalypha indica* (Hungeling et al., 2009). 

(49) R₁ = CH₃; R₂=H; R₃=OH; R₄=CN

(50) R₁ = H; R₂=OH; R₃=H; R₄=CN.

(51) R₁ = H; R₂=H; R₃=OH; R₄=CN.

(52) R₁ = CH₃; R₂=OH; R₃=H; R₄=CONH₂.
4.2.2. Past work on the phytochemical studies on the species of *Ruellia*

Three flavonoid glycosides viz., apigenin-7-glucoside (56), luteolin-7-glucoside (57) and apigenin-7-β-glucuronide (58) were reported from buds and flowers of *Ruellia prostrata* syn. *Dipteracanthus prostratus* (Subramanian and Nair, 1972).
The leaves of *Ruellia tuberosa* contained traces of apigenin (59) and luteolin (60) while the flowers contained malvidin 3,5-diglucoside (61) and the flower buds contained the maximum proportion of flavonoids yielding 3% of apigenin-7-O-glucuronide (58), and the other flavones identified were apigenin-7-O-glucoside (56), apigenin-7-O-rutinoside and luteolin-7-O-glucoside (57) (Nair and Subramanian, 1974).
Johne et al. (1975) isolated tetramethylputrescine (62) from the roots and aerial parts of *Ruellia rosea*. Floral flavonoids of the species of *Ruellia* revealed that apigenin-7-glucuronide (58) and malvidin 3,5-diglcoside (61) were common in blue flowered species whereas isosalipurposide (63) and pelargonidin 3,5-diglcoside were shared by the red flowered ones (Bloom, 1976).
Banerjee (1984) reported the percentage composition of stigmasterol (65), \( \beta \)-sitosterol (40), 24-methyl cholesterol and cholesterol and brassicosterol in the sterol mixture isolated from the petroleum ether (60°-80°C) extract of *Ruellia prostrata* Poir. 2-O-alpha-D-galactopyranosyl glycerol hexaacetate (66) was isolated from the methanol extract of the whole plant of *Ruellia brittoniana* (Ahmad *et al.*, 1990).

Two lignan glycosides identified as 5,5'-dimethoxylariciresinol-9-Q-\( \beta \)-D glucopyranoside (rupaside) (67) and lyoniresinol-9-Q-\( \beta \)-D-glucopyranoside (68) along with ethyl-\( \alpha \)-D-galactopyranoside, \( \alpha \)-and \( \beta \)-D-glucose (69), (70), and \( \beta \)-D-fructose (71) were isolated from the methanol extract of the whole plant of *Ruellia patula* (Ahmad *et al.*, 1993).
(67) $\text{HOCH}_2\text{HjC}$

(68)

(69) $R=\text{OH}, R'=\text{H}$

(70) $R=\text{H}, R'=\text{OH}$

Alpha-ethyl galactose (72), $\beta$-sitosterol (40), apigenin-7-0-rutinoside (73), dimer of methyl 1-2,4-diene-hexandioate and paramethoxy-benzoic acid (74) were isolated from *Ruellia brittoniana* (Akhtar, 1993).
Misra et al., (1997) have isolated two new aliphatic compounds from *Ruellia tuberosa*. TLC and HPLC fingerprint analysis of the extracts of *Ruellia praetermissa* revealed the presence of lupeol (75) in hexane extract and flavonoids (apigenin (59) and luteolin (60) in methanol and aqueous extracts (Salah et al., 2001).

Singh et al. (2002) isolated 21-methylshammar-22-en-3β, 18,27-trio1 from the aerial parts of *Ruellia tuberosa*. 1,8-cineole and S(−)-camphor were reported from the aerial parts of *Ruellia menthoides* (Facundo et al., 2004).
Five flavonoids cirsimaritin (76), cirsimarin (77), cirsiol 4'-glucoside, sorbifolin and pedalitin (78) along with betulin (79), vanillic acid (80) and indole-3 carboxaldehyde (81) were isolated from the ethyl acetate extracts of *Ruellia tuberosa* (Lin *et al*., 2006).
Muthumani et al., (2009) isolated 7-Hydroxy-4-Methyl coumarin, stigmasterol (65), stigmasterol-6-en-3-beta-ol, campesterol (82) and dicoumarol (83) from the ethanolic extract of *Ruellia patula.*
4.2.3. Studies on mineral elements of medicinal plants

Reddy and Reddy (1997) analyzed a large number of medicinally important leafy samples for elemental concentrations such as Zn, Cu, Ni, Co, Pb, As, Se, K, Cr, Na, P, S, Fe, Ca, Mg, Mn and Fe. The concentrations of macro nutrients such as Ca, P, Mg, K, Fe and S ranged from 9.62 to 4174, 1.00–8.630, 3.53–35.50, 12.04–56.28, 0.111–3.845 g/kg and 1.124–5.843 mg/kg, of micro nutrients such as Cr, Co, Cu, Mn, Na and Zn ranged from 0.360–8.630, 0.050–3.470, 17.60–57.30, 10.5–81.6, 1.47–27.10 and 10.06–145.6 mg/kg whereas those of trace metals such as Pb, Cd, Ni, As and Se ranged from 1.19–16.30, 0.0036–0.453, 1.23–19.60, 0.12–7.360 and 0.654–3.50 mg/kg respectively. Pereira and Felcman (1998) analyzed the concentration of five minerals viz. silicon, manganese, iron, copper, and zinc in sixteen medicinal plants which were used in wound healing to study their possible role in the healing processes. The trace elements like copper, manganese, selenium, and zinc act as cofactors of antioxidant enzymes (Leung, 1998). Mineral contents of thirty-two plants used as condiments in Turkey were determined by Inductively Coupled Plasma Atomic Emission Spectrometry (Ozcan, 2004). All materials contained high amounts of Al, Ba, Ca, Fe, K, Mg, P and S. The highest levels of Ca, Fe, K, Mg and S were found in *Thymus vulgaris, Lavandula officinalis, Anethum graveolens, Ocimum basilicum* and *Sinapis alba* respectively.

Ajasa *et al.* (2004) estimated the concentration of selected trace metals (Fe, Mn, Cu, Pb and Zn) and macronutrients (Na, K, Mg and Ca) along with P, by atomic absorption spectrophotometer in some of the important herbal plants
of the southwest part of Nigeria viz. *Anacardium occidentale*, *Azadirachta indica*, *Butyrospermum paradoxum*, *Mangifera indica*, *Morinda lucida*, *Ocimum canum*, *Solanum erianthum*, *Solanum torvum*, *Zingiber officinale* and *Hyptis suaveolens*. The metal contents in the samples were found at different levels. The highest mean levels (ppm) of Zn (35.1±0.01) and Cu (24.4±0.01) were found in *Hyptis suaveolens* while those of Mn (685±0.02) and Ca (51 340±21) were found in *Morinda lucida*. The result also showed that *Ocimum canum* had the highest amounts of K (36 600±350), P (3700±35) and Fe (241±0.05). *Anacardium occidentale* had the highest concentration of Na (613±0.60) while *Azadirachta indica* had the highest mean concentrations of Pb (0.49±0.03) and Mg (5630±12). Indrayan *et al.* (2005) studied the concentration of Cr, K, Ca, Cu, Zn and Mn in medicinally valued seeds of *Nelumbo nucifera*, *Embelia ribes*, *Eugenia jambolana* and leaves of *Artocarpus heterophyllus*.

The elemental composition in the leaves of four traditional medicinal plants (*Murraya koenigii*, *Mentha piperita*, *Ocimum sanctum* and *Aegle marmelos*) widely used in the treatment of diabetes-related metabolic disorders has been studied using atomic absorption spectroscopy (Narendhirakannan *et al.*, 2005). Sarközi *et al.* (2005) studied the concentration of 24 elements (Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Ti, V, Zn) in Chelidonii herba preparations, crude drugs (herb and root of *Chelidonium majus*) in their aqueous solutions (infusion, decoction) and alcoholic extracts by ICP-AES. Mineral composition of *Acalypha wilkesiana* was investigated by Ikewuchi and Ikewuchi (2009).
Tesleem et al. (2009) estimated the mineral and phytochemical content in the leaves of *Talinum triangulare*. Nile and Khobragade (2009) estimated the percent concentration of various mineral elements like P, K, Na, Ca, Fe, Zn, N, Mg, Cu and Cr in *Tinospora cordifolia, Gymnema sylvestre* and *Tricholepis glaberrima*. Eight inorganic elements (Ca, P, Mn, Zn, Ni, Fe, K and Mg) have been detected in *Quercus infectoria, Cassia fistula, Tinospora cordifolia, Butea monosperma* and *Cedrela toona* by AAS (Vermani et al., 2010).
4.3. MATERIALS AND METHODS

4.3.1. Preparation of powder and extract

The aerial parts of *Acalypha fruticosa* were shade-dried and pulverized to a coarse powder in a mechanical grinder. The shade-dried powder (1 Kg) was successively extracted with various solvents such as petroleum ether (40°-60°C), chloroform, ethanol and water. The leaves of *Dipteracanthus patulus* were dried in shade and powdered. The leaf powder (1 Kg) was successively extracted with various solvents such as petroleum ether (40°-60°C), benzene, chloroform, methanol and water. The extracts were concentrated under reduced pressure in a rotary evaporator (Buchi, USA). The powder and extracts of the plants were used for phytochemical studies.

4.3.2. Qualitative phytochemical analysis

The qualitative phytochemical tests for steroids, reducing sugars, triterpenoids, alkaloids, phenolic compounds, flavonoids, saponins, tannins and anthraquinones were carried out on the concentrated extracts using the standard procedures to identify the constituents as described by Harborne (1973), Brinda et al. (1981) and Trease and Evans (1985). Table 4.1. explains the various phytochemical tests performed.

4.3.3. Quantitative estimation of phytochemicals

Quantitative estimation of phytochemicals like alkaloids, flavonoids, tannins, phenols, saponins and steroids were carried out in the powdered samples of *Acalypha fruticosa* and *Dipteracanthus patulus*. 
<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 precipitate.</td>
<td>Presence of Reducing sugars</td>
</tr>
<tr>
<td>4</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>5</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>6</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>7</td>
<td>Test solution + H₂O and shaken well</td>
<td>Foamy lather</td>
<td>Presence of Saponins</td>
</tr>
<tr>
<td>8</td>
<td>Test solution + H₂O + Lead acetate solution</td>
<td>White precipitate</td>
<td>Presence of Tannins</td>
</tr>
<tr>
<td>9</td>
<td>Test solution + Magnesium acetate solution</td>
<td>Pink colour</td>
<td>Presence of Anthraquinones</td>
</tr>
</tbody>
</table>
4.3.3.1. Estimation of alkaloids

Alkaloids were determined by using the method of Harborne (1973). 5 g of the powdered sample was taken in a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added. The beaker was covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried and weighed.

4.3.3.2. Estimation of flavonoids

Flavonoids were determined by the method of Boham and Kocipal-Abyazan (1974). 10 g of the powdered plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman No.42 filter paper. The filtrate was later transferred into a previously weighed china dish and evaporated to dryness over a water bath. The residue was the flavonoids, which was weighed to a constant weight.

4.3.3.3. Estimation of tannins

0.5 g of the powdered material was transferred to a 250 ml conical flask and 75 ml of water was added and heated for 30 minutes. It was centrifuged at 2000 rpm for 20 minutes. The supernatant was made up to a known volume. From this extract 1 ml was taken and was added with 5 ml of Folin-Denis
reagent, 10 ml of 35% sodium carbonate and diluted to 100 ml with water. It was shaken well and allowed to stand for 15 minutes and the absorbance was read at 700 nm in Systronics double beam UV-VIS -180 Model Spectrophotometer. Tannic acid was kept as standard. The tannin content of the samples was determined as tannic acid equivalents from the standard graph (Mahadevan and Sridhar, 1982).

4.3.3.3.1. Preparation of reagents

4.3.3.3.1.1. Folin-Denis reagent

100 g of sodium tungstate and 20 g of phosphomolybdic acid were dissolved in 750 ml distilled water and to this 50 ml of phosphoric acid were added. The mixture was refluxed for 24 h and made up to one litre with water.

4.3.3.3.1.2. Sodium carbonate solution

350 g of sodium carbonate was dissolved in one litre of water and heated to 70°-80°C. It was allowed to stand overnight and filtered through glass wool.

4.3.3.3.1.3. Standard tannic acid solution

100 mg of tannic acid was dissolved in 100 ml of distilled water.

4.3.3.3.1.4. Working standard solution

5 ml of the standard tannic acid solution is diluted to 100 ml with distilled water. 1 ml contained 50 µg tannic acid.

4.3.3.4. Estimation of phenols

The estimation of phenols was based on the methods of Mahadevan and Sridhar (1982). 250 g of the powdered sample was taken and homogenized
with the extraction mixture [n-butanol : acetic acid : water (7:1:2)]. The extract was centrifuged at 10000 rpm for 10 minutes. The supernatant was taken and made up to a known volume with extraction mixture. 1 ml of the extract was taken and 1 ml of Folin-Ciocalteau reagent, followed by 2 ml of 20% sodium carbonate was added. The contents were shaken well and heated in boiling water bath exactly for 1 minute and cooled in running water. The blue colour complex formed was diluted with 25 ml of distilled water. The absorbance of the complex was read at 630 nm in Systronics double beam UV-VIS -180 Model Spectrophotometer. Catechol was kept as standard.

4.3.3.5. Estimation of saponins

Saponins were determined using the method of Obadoni and Ochuko (2001). 20 g of the powdered sample was taken in a conical flask and 100 ml of 20% aqueous ethanol was added. The conical flask was heated over a hot water bath for 4 h with continuous stirring at about 55°C and filtered. The residue obtained was re-extracted with another 100 ml of 20% aqueous ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and to this 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered in a previously weighed china dish, while the ether layer was discarded. To the aqueous layer, 60 ml of n-butanol was added. The combined n-butanol extract was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the residue obtained (saponins) was weighed to a constant weight.
4.3.3.6. Estimation of steroids

Steroids were determined by the method described by Okeke and Elekwa (2003). One gram of the powdered sample was dispersed in 100 ml distilled water and homogenized in a laboratory blender. The homogenate were filtered and the filtrate was eluted with ammonium hydroxide solution (pH 9). 2 ml of the eluate were put in a test tube and mixed with 2 ml of chloroform. 3 ml of ice-cold acetic anhydride were added to the mixture in the flask and 2 drops of conc. H₂SO₄ were cautiously added. Standard cholesterol solution was prepared and treated as described above. The absorbance of standard and prepared sample was measured in Systronics double beam UV-VIS Spectrophotometer-180 Model at 420 nm.

4.3.4. Isolation and characterization of chemical compounds

4.3.4.1. Gas Chromatogram- Mass Spectral (GC-MS) analysis

The following were subjected to GC-MS analysis.

i) As the ethanolic extract of *Acalypha fruticosa* and the methanolic extract of *Dipteracanthus patulus* were pharmacologically active (exhibited wound healing activity) the compounds present in the ethanolic extract of *Acalypha fruticosa* (AFE) and the methanolic extract of *Dipteracanthus patulus* (DPM) were identified by Gas Chromatogram- Mass spectral analysis.

ii) The petroleum ether (40°-60°C) extract of *Acalypha fruticosa* on cooling yielded a white powdery substance. It was named as AFP. This isolated white mass obtained from the petroleum ether (40°-60°C) extract of *Acalypha fruticosa* (AFP) was also subjected to GC-MS analysis.
iii) The methanolic extract (20 g) of *Dipteracanthus patulus* was made into slurry with silica gel (BDH India Ltd; 60-120 mesh) and transferred on to a column of silica gel (BDH India Ltd; 60-120 mesh) built in petroleum ether (40°-60°C). The column was successively eluted with solvents of increasing polarity viz. 100% petroleum ether (40°-60°C), petroleum ether-benzene mixture, 100% benzene, benzene-chloroform mixture and 100% chloroform, chloroform-methanol mixture and 100% methanol. Fractions of 50 ml were collected at each time, distilled and the resulting residues were examined by TLC on silica gel plates. Fractions which revealed identical behaviour in TLC plates were pooled together. Out of the 400 fractions collected the fractions 282-294 eluted with chloroform: methanol (70:30) mixtures were pooled and named as **DPMF**. This **DPMF** fraction was also subjected to GC-MS analysis.

GC-MS analysis of the extracts was carried out on a GC-MS Clarus 500 Perkin Elmer system comprising a AOC- 20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30mm x 0.25mm ID x 1 μMdf, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 μl was employed (split ratio of 10:1); injector temperature 250°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5 °C / min to 280°C, ending with a 9 min
isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 550 Da.

Interpretation on mass spectra of GC-MS was conducted using the database of National Institute of Standards and Technology (NIST). The mass spectrum of the unknown component was compared with the mass spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

4.3.5. Estimation of elements

The ground plant samples were sieved with a 2 mm rubber sieve and 2 g of the plant samples were weighed and subjected to dry ashing in a well-cleaned porcelain crucible at 550°C in a muffle furnace. The resultant ash was dissolved in 5 ml of HNO$_3$: HCl: H$_2$O (1:2:3) mixture and heated gently on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 ml of deionized water was added and heated until a colourless solution was obtained. The mineral solution in each crucible was transferred into a 100 ml volumetric flask by filtration through Whatman No. 42 filter paper and the volume was made to the mark with deionized water. This solution was used for elemental analysis. Potassium, sodium, calcium, magnesium, sulphur, zinc, copper, iron, manganese, boron and molybdenum were estimated using Atomic Absorption Spectrophotometer (Solaar AA series, Atomic Absorption Spectrophotometer).
4.4. RESULTS

4.4.1. Qualitative analysis of phytochemicals

Table 4.2. shows the results of the qualitative analysis of phytochemicals of the various extracts of the aerial parts of *Acalypha fruticosa*. Petroleum ether (40°-60°C) extract showed the presence of saponins and sugars. Chloroform extract showed the presence of saponins, anthraquinones, alkaloids and sugars. Triterpenoids, steroids, saponins, tannins, phenols, anthraquinones and alkaloids were present in the ethanolic extract. Water extract showed the presence of terpenoids, steroids, saponins, tannins, phenols, flavonoids and sugars. The results from various extracts have indicated the presence of triterpenoids, steroids, saponins, tannins, phenols, alkaloids, flavonoids, anthraquinones and sugars in *Acalypha fruticosa*.

Results of the qualitative analysis of phytochemicals of the various extracts of the leaves of *Dipteracanthus patulus* are presented in Table 4.3. Petroleum ether (40°-60°C) extract revealed the presence of triterpenoids, steroids and tannins. Steroids, phenols, anthraquinones and tannins were present in benzene extract. Chloroform extract showed the presence of steroids, tannins, and sugars. Steroids, flavonoids, phenols, saponins, tannins and sugars were present in the methanol extract. Phenols, saponins, tannins and sugars were present in the water extract. The results of the qualitative analysis of phytochemicals of the various extracts indicated the presence of triterpenoids, steroids, flavonoids, phenols, saponins, anthraquinones, tannins and sugars.
Table 4.2. Qualitative phytochemical analysis of the extracts of the aerial parts of *Acalypha fruticosa*

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Phytochemicals</th>
<th>Petroleum ether (40°-60°C)</th>
<th>Chloroform</th>
<th>Ethanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Triterpenoids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Phenols</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Anthraquinones</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Sugars</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) – Present and (-) – Absent.
Table 4.3. Qualitative analysis of phytochemicals of the various extracts of the leaves of *Dipteracanthus patulus*

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Compound</th>
<th>Petroleum-ether (40°-60°C)</th>
<th>Benzene</th>
<th>Chloroform</th>
<th>Methanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Triterpenoids</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Phenols</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Anthraquinones</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Sugars</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) – Present and (-) – Absent.
4.4.2. Quantitative analysis of phytochemicals

Quantitative estimation of phytochemicals present in the powdered samples of *Acalypha fruticosa* and *Dipteracanthus patulus* was carried out and the results are presented in Table 4.4. and Table 4.5. respectively. Flavonoids were present in high amount when compared to alkaloids, tannins, phenols, saponins and steroids in both the plants. The quantity of tannins and phenols were higher in *Acalypha fruticosa* than in *Dipteracanthus patulus*.

4.4.3. Gas Chromatogram- Mass Spectral analysis (GC-MS)

4.4.3.1. GC-MS analysis of the ethanolic extract of *Acalypha fruticosa*

GC-MS chromatogram of the ethanolic extract of *Acalypha fruticosa* (AFE) is presented in Fig. 4.1. GC-MS chromatogram of the extract of *A. fruticosa* (Fig. 4.1.) showed three peaks indicating the presence of three compounds AFE-1 (RT 16.16), AFE-2 (RT 18.74) and AFE-3 (RT 24.70). The compound AFE-1 was identified as n-Hexadecanoic acid (84). Figure 4.2 shows comparison of mass spectra of AFE-1 with that of the standard compound. AFE-2 was identified as 9,12-Octadecadienoic acid (z, z) (85). Figure 4.3 presents the mass spectrum of 9,12-Octadecadienoic acid (z, z). AFE-3 was identified as 1,2-Benzenedicarboxylic acid diisoctyl ester (86). Figure 4.4 shows the comparison of mass spectra of AFE-3 with that of the standard compound. The chemical compounds identified in the ethanolic extract of *Acalypha fruticosa* are presented in Table 4.6., with their retention time (RT), molecular formula, molecular weight and peak area (%). The peak area (%) was more for 1,2-Benzenedicarboxylic acid diisoctyl ester.
Table 4.4. Quantitative estimation of phytochemicals in the aerial parts of *Acalypha fruticosa*.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the phytochemicals</th>
<th>Amount (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>0.36</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>1.19</td>
</tr>
<tr>
<td>3</td>
<td>Tannins</td>
<td>0.56</td>
</tr>
<tr>
<td>4</td>
<td>Phenols</td>
<td>0.30</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>0.06</td>
</tr>
<tr>
<td>6</td>
<td>Steroids</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Table 4.5. Quantitative estimation of phytochemicals in the leaves of *Dipteracanthus patulus*.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the phytochemicals</th>
<th>Amount (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>3.46</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>3.75</td>
</tr>
<tr>
<td>3</td>
<td>Tannins</td>
<td>0.23</td>
</tr>
<tr>
<td>4</td>
<td>Phenols</td>
<td>0.09</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>0.12</td>
</tr>
<tr>
<td>6</td>
<td>Steroids</td>
<td>0.10</td>
</tr>
</tbody>
</table>
Mass spectrum of the compound AFE-1 (RT 16.16)

Fig. 4.2. Mass spectrum of the compound AFE-1 and its comparison with that of NIST Library spectrum
Fig. 4.3. Mass spectrum of 9,12-Octadecadienoic acid (Z,Z)
Fig. 4.4. Mass spectrum of the compound AFE-3 and its comparison with that of NIST Library spectrum
Table 4.6. Chemical constituents of the ethanolic extract of the aerial parts of *Acalypha fruticosa* [GC-MS]

<table>
<thead>
<tr>
<th>Compound number</th>
<th>Retention Time</th>
<th>Name of the Compound</th>
<th>Molecular Formula</th>
<th>Molecular Weight</th>
<th>Peak Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFE-1</td>
<td>16.16</td>
<td>n-Hexadecanoic acid</td>
<td>C₁₆H₃₂O₂</td>
<td>256</td>
<td>5.45</td>
</tr>
<tr>
<td>AFE-2</td>
<td>18.74</td>
<td>9,12-Octadecadienoic acid (z,z)</td>
<td>C₁₈H₃₂O₂</td>
<td>280</td>
<td>3.33</td>
</tr>
<tr>
<td>AFE-3</td>
<td>24.70</td>
<td>1,2- Benzene dicarboxylic acid diisooctyl ester</td>
<td>C₂₄H₃₈O₄</td>
<td>390</td>
<td>91.23</td>
</tr>
</tbody>
</table>
4.4.3.1.1. GC-MS analysis of the fraction of petroleum ether (40°-60°C) extract of Acalypha fruticosa (AFP)

Petroleum ether (40°-60°C) extract of Acalypha fruticosa on cooling yielded a white powdery substance (AFP). GC-MS chromatogram of AFP (Fig. 4.5.) showed 8 peaks. Of which, two peaks (peak-6 and peak-8) were prominent and were designated as AFP-1 (RT 12.29) and AFP-2 (RT 13.85) respectively. When the mass spectra of these compounds were compared with those of the compiled data for known compounds (Fig. 4.6. and Fig. 4.7.), compound AFP-1 (RT 12.29) was found to be identical with α-D-glucopyranoside (87) and AFP-2 (RT 13.85) was identified as Eicosyltrichlorosilane (88).
Fig. 4.5. GC-MS chromatogram of the fraction of petroleum ether (40°-60°C) extract (AFP) of the aerial parts of *Acalypha fruticosa*.
Mass spectrum of compound AFP-1 (RT 12.29)

Fig. 4.6. Comparison of mass spectrum of compound AFP-1
Mass spectrum of compound AFP-2 (RT 13.850)

Fig. 4.7. Comparison of mass spectrum of compound AFP-2
4.4.3.2. GC-MS analysis of the methanolic extract of the leaves of *Dipteracanthus patulus*

GC-MS chromatogram of the methanolic extract of the leaves of *Dipteracanthus patulus* is presented in Fig. 4.8. GC-MS chromatogram of the methanolic extract of *Dipteracanthus patulus* (DPM) (Fig. 4.8.) showed 17 prominent peaks indicating the presence of 17 compounds (DPM-1 to DPM-17) in the extract. The chemical compounds identified in the methanolic extract of *Dipteracanthus patulus* are presented with their retention time (RT), molecular formula, molecular weight and peak area in Table 4.7.

The compounds identified were, Octane3,3-dimethyl- (89), 2,6-Dimethyl-6-trifluoroacetoxyoctane (90), Tetradecane (91), 4-Hydroxy-2-methylpyrrolidine-2-carboxylic acid (92), Hexadecane (93), Octadecane (94), 1,2-Benzenedicarboxylic acid Bis(2-methylpropyl) ester (95), Hexadecanoic acid methyl ester (96), Dibutyl phthalate (97), n-Hexadecanoic acid (84), Hexadecanoic acid ethyl ester (98), 9,12-Octadecadienoic acid (z,z) (85), 9,12,15-Octadecatrienoic acid methyl ester (z,z,z) (99), Linoleic acid ethyl ester (100), 9,12,15-Octadecatrienoic acid ethyl ester (z,z,z) (101), Octadecanoic acid ethyl ester (102) and 1,2- Benzenedicarboxylic acid diisoocetyl ester (86).
Fig. 4.8. GC-MS chromatogram of the methanolic extract of the leaves of *Dipteracanthus patulus*
Table 4.7. Chemical constituents of the methanolic extract of the leaves of *Dipteracanthus patulus* [GC-MS]

<table>
<thead>
<tr>
<th>Compound number</th>
<th>Retention Time</th>
<th>Name of the Compound</th>
<th>Molecular Formula</th>
<th>Molecular Weight</th>
<th>Peak Area(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPM-1</td>
<td>3.87</td>
<td>Octane 3,3-dimethyl</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;22&lt;/sub&gt;</td>
<td>142</td>
<td>3.06</td>
</tr>
<tr>
<td>DPM-2</td>
<td>4.78</td>
<td>2,6-Dimethyl-6-trifluoroacetoxoctane</td>
<td>C&lt;sub&gt;12&lt;/sub&gt;H&lt;sub&gt;21&lt;/sub&gt;F&lt;sub&gt;3&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>254</td>
<td>2.67</td>
</tr>
<tr>
<td>DPM-3</td>
<td>8.88</td>
<td>Tetradecane</td>
<td>C&lt;sub&gt;14&lt;/sub&gt;H&lt;sub&gt;30&lt;/sub&gt;</td>
<td>198</td>
<td>2.86</td>
</tr>
<tr>
<td>DPM-4</td>
<td>9.99</td>
<td>4-hydroxy-2 methylpyrrolidine-2-carboxylic acid</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;11&lt;/sub&gt;NO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>145</td>
<td>7.94</td>
</tr>
<tr>
<td>DPM-5</td>
<td>11.30</td>
<td>Hexadecane</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;H&lt;sub&gt;34&lt;/sub&gt;S</td>
<td>226</td>
<td>6.68</td>
</tr>
<tr>
<td>DPM-6</td>
<td>13.84</td>
<td>Octadecane</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;38&lt;/sub&gt;</td>
<td>254</td>
<td>4.93</td>
</tr>
<tr>
<td>DPM-7</td>
<td>14.67</td>
<td>1-2-Benzenedicarboxylic acid Bis (2-methylpropyl) ester</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;H&lt;sub&gt;22&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;</td>
<td>278</td>
<td>8.02</td>
</tr>
<tr>
<td>DPM-8</td>
<td>15.50</td>
<td>Hexadecanoic acid methyl ester</td>
<td>C&lt;sub&gt;17&lt;/sub&gt;H&lt;sub&gt;34&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>270</td>
<td>3.21</td>
</tr>
<tr>
<td>DPM-9</td>
<td>16.00</td>
<td>Dibutyl phthalate</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;H&lt;sub&gt;22&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;</td>
<td>278</td>
<td>7.75</td>
</tr>
<tr>
<td>DPM-10</td>
<td>16.16</td>
<td>n-Hexadecanoic acid</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;32&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>256</td>
<td>13.41</td>
</tr>
<tr>
<td>DPM-11</td>
<td>16.47</td>
<td>Hexadecanoic acid ethyl ester</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;36&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>284</td>
<td>6.49</td>
</tr>
<tr>
<td>DPM-12</td>
<td>18.75</td>
<td>9,12-Octadecadienoic acid(z,z)</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;32&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>280</td>
<td>4.24</td>
</tr>
<tr>
<td>DPM-13</td>
<td>18.85</td>
<td>9,12,15-Octadecatrienic acid methyl ester (z,z,z)</td>
<td>C&lt;sub&gt;19&lt;/sub&gt;H&lt;sub&gt;32&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>292</td>
<td>9.66</td>
</tr>
<tr>
<td>DPM-14</td>
<td>19.01</td>
<td>Linoleic acid ethyl ester</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;36&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>308</td>
<td>4.13</td>
</tr>
<tr>
<td>DPM-15</td>
<td>19.11</td>
<td>9,12,15-Octadecatrienoic acid ethyl ester (z,z,z)</td>
<td>C&lt;sub&gt;20&lt;/sub&gt;H&lt;sub&gt;34&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>306</td>
<td>3.82</td>
</tr>
<tr>
<td>DPM-16</td>
<td>19.48</td>
<td>Octadecanoic acid ethyl ester</td>
<td>C&lt;sub&gt;20&lt;/sub&gt;H&lt;sub&gt;40&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>312</td>
<td>1.22</td>
</tr>
<tr>
<td>DPM-17</td>
<td>24.75</td>
<td>1,2-Benzene dicarboxylic acid diisoctyl ester</td>
<td>C&lt;sub&gt;24&lt;/sub&gt;H&lt;sub&gt;38&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;</td>
<td>390</td>
<td>9.89</td>
</tr>
</tbody>
</table>
The peak areas (%) were high for n-Hexadecanoic acid (13.41%), 1,2-Benzenedicarboxylic acid diisooctyl ester (9.89%), 9,12,15-Octadecatrienoic acid methyl ester (z,z,z) (9.66%), 1,2-Benzenedicarboxylic acid Bis(2-methylpropyl) ester (8.02%), 4-hydroxy-2methylpyrrolidine-2-carboxylic acid (7.94%) and Dibutyl phthalate (7.75%).

Figure 4.9. to Fig. 4.14. show the comparison of mass spectra of the compounds **DPM-3** with RT 8.88, **DPM-4** with RT 9.99, **DPM-5** with RT 11.30, **DPM-11** with RT 16.47, **DPM-13** with RT 18.85 and **DPM-14** with RT 19.01, with those of the standard compounds and were identified as Tetradecane, 4-hydroxy-2-methylpyrrolidine-2-carboxylic acid, Hexadecane, Hexadecanoic acid ethyl ester, 9,12,15-Octadecatrienoic acid methyl ester (z,z,z) and Linoleic acid ethyl ester respectively.

The mass spectra of the other compounds identified from the methanolic extract of the leaves of *Dipteracanthus patulus* (DPM) are presented in Fig. 4.15. to Fig. 4.23. viz., Octane 3,3-dimethyl- (Fig. 4.15.), 2,6-Dimethyl-6-trifluoroacetoxoctane (Fig. 4.16.), 1,2-Benzenedicarboxylic acid bis (2-methylpropyl) ester (Fig. 4.17.), Hexadecanoic acid methyl ester (Fig. 4.18.), Dibutyl phthalate (Fig. 4.19.), n-Hexadecanoic acid (Fig. 4.20.), 9,12,15-Octadecatrienoic acid ethyl ester (z,z,z) (Fig. 4.21.), Octadecanoic acid ethyl ester (Fig. 4.22.) and 1,2-Benzenedicarboxylic acid diisooctyl ester (Fig. 4.23.).
Fig. 4.9. Mass spectrum of the compound DPM-3 and its comparison with that of NIST Library spectrum
Mass spectrum of the compound DPM-4 (RT 9.99)

NIST spectrum

Comparison of mass numbers

Fig. 4.10. Mass spectrum of the compound DPM-4 and its comparison with that of NIST Library spectrum
Fig. 4.11. Mass spectrum of the compound DPM-5 and its comparison with that of NIST Library spectrum.
Mass spectrum of the compound DPM-11 (RT 16.47)

![Mass spectrum of DPM-11 and NIST spectrum](image)

Comparison of mass numbers

Fig. 4.12. Mass spectrum of the compound DPM-11 and its comparison with that of NIST Library spectrum
Fig. 4.13. Mass spectrum of the compound DPM-13 and its comparison with that of NIST Library spectrum.
Mass spectrum of the compound DPM-14 (RT 19.01)

NIST spectrum

Comparison of mass numbers

Fig. 4.14. Mass spectrum of the compound DPM-14 and its comparison with that of NIST Library spectrum
Fig. 4.15. Mass spectrum of Octane 3,3-dimethyl-
Name: 2,6-Dimethyl-6-trifluoroacetoxyoctane

Formula: C_{12}H_{21}F_{3}O_{2}

MW: 254 CAS#: 61986-67-2 NIST#: 215969 ID#: 27204 DB: mainlib

10 largest peaks:

<table>
<thead>
<tr>
<th>Peak</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>69</td>
<td>999</td>
</tr>
<tr>
<td>43</td>
<td>845</td>
</tr>
<tr>
<td>55</td>
<td>698</td>
</tr>
<tr>
<td>41</td>
<td>631</td>
</tr>
<tr>
<td>70</td>
<td>615</td>
</tr>
<tr>
<td>57</td>
<td>584</td>
</tr>
<tr>
<td>56</td>
<td>435</td>
</tr>
<tr>
<td>42</td>
<td>292</td>
</tr>
<tr>
<td>71</td>
<td>279</td>
</tr>
<tr>
<td>39</td>
<td>238</td>
</tr>
</tbody>
</table>

Fig. 4.16. Mass spectrum of 2,6-Dimethyl-6-trifluoroacetoxyoctane
Name: 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
Formula: $C_{16}H_{22}O_4$
MW: 278 CAS#: 84-69-5 NIST#: 229506 ID#: 19826 DB: replib

10 largest peaks:
149 999 57 261 150 94 41 91 29 72 56 65 223 64 104 51 76 35 167 34

Fig. 4.17. Mass spectrum of 1,2-Benzenedicarboxylic acid bis(2-methylpropyl) ester
Name: Hexadecanoic acid, methyl ester
Formula: C_{17}H_{34}O_2
MW: 270 CAS#: 112-39-0 NIST#: 107527 ID#: 9001 DB: replib
Other DBs: Fine, TSCA, EPA, HODOC, NIH, EINECS
Contributor: Chuck Anderson, Aldrich Chemical Co.
10 largest peaks:
74 999 | 87 585 | 43 546 | 41 419 | 143 247 | 75 169 | 55 174 | 29 158 | 69 133 | 129 106 |

Fig. 4.18. Mass spectrum of Hexadecanoic acid methyl ester
Name: Dibutyl phthalate  
Formula: C₁₆H₂₂O₄  
MW: 278 CAS#: 84-74-2 NIST#: 114974 ID#: 19769 DB: replib  
Other DBs: Fine, TSCA, RTECS, EPA, HODOC, NIH, EINECS, IRDB  
10 largest peaks:  
149 999 | 150 88 | 41 67 | 76 64 | 104 58 | 223 47 | 205 40 | 29 39 | 65 38 | 93 36 |

Fig. 4.19. Mass spectrum of Dibutyl phthalate
Name: n-Hexadecanoic acid
Formula: C₁₆H₃₂O₂
MW: 256 CAS#: 57-10-3 NIST#: 8185 DB: mainlib
Other DBs: Fine, TSCA, RTECS, EPA, HODOC, NIH, EINECS, IRDB
Contributor: Chemical Concepts
10 largest peaks:
43 999 | 73 905 | 60 838 | 41 749 | 57 634 | 55 616 | 29 414 | 69 310 | 71 285 | 61 218

Fig. 4.20. Mass spectrum of n-Hexadecanoic acid
Name: 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-
Formula: C₂₀H₃₄O₂
MW: 306 CAS#: 1191-41-9 NIST#: 14550 ID#: 9700 DB: replib
Other DBs: None
10 largest peaks:
79 999 | 67 829 | 41 735 | 95 707 | 81 605 | 55 604 | 93 556 | 108 510 | 80 472 | 29 374

Fig. 4.21. Mass spectrum of 9,12,15-Octadecatrienoic acid ethyl ester, (Z,Z,Z)-
Name: Octadecanoic acid, ethyl ester
Formula: C_{20}H_{40}O_{2}
MW: 312 CAS#: 111-61-5 NIST#: 151365 ID#: 11018 DB: replib
Other DBs: Fine, TSCA, RTECS, HODOC, NIH, EINECS, IRDB
Contributor: Chemical Concepts
10 largest peaks:
88 999 | 101 606 | 43 531 | 55 340 | 41 332 | 29 321 | 57 305 | 73 166 | 69 154 | 28 151 |

Fig. 4.22. Mass spectrum of Octadecanoic acid ethyl ester
Name: 1,2-Benzene dicarboxylic acid, diisooctyl ester  
Formula: C$_{24}$H$_{38}$O$_4$  
MW: 390  CAS#: 27554-26-3 NIST#: 113206 ID#: 19804 DB: replib  
10 largest peaks:  
_149_ 999_ | _167_ 350_ | _57_ 341_ | _70_ 264_ | _41_ 225_ | _71_ 224_ | _55_  
218_ | _43_ 200_ | _150_ 107_ | _83_ 100_ | 

Fig. 4.23. Mass spectrum of 1,2-Benzene dicarboxylic acid diisooctyl ester
4.4.3.2.1. GC-MS analysis of the column chromatographic fraction of the methanolic extract of the leaves of *Dipteracanthus patulus* (DPMF)

GC-MS chromatogram of DPMF revealed 6 peaks indicating the presence of 6 compounds (DPMF-1 to DPMF-6) (Fig. 4.24.). When the mass spectra of these compounds were compared with those of the compiled data for known compounds, 5 compounds were identified (Fig. 4.25. to Fig. 4.29.). Compound DPMF-1 (RT 12.917) was identified as L-alanine 1,1-dimethylethyl ester hydrochloride (103), Compound DPMF-2 (RT 16.308) was identified as Clivorine-(12-acetyloxy)-14,15,20,21-tetradehydro-15,20-dihydro-8-n (104), Compound DPMF-3 (RT 24.917) was identified as 5H-Cyclopropa[3,4]Ben[1,2-e]azulen-5-one (105). Compound DPMF-4 (RT 27.192) was identified as 2-Methyl-4-benzyl-(n-benzyl trifluoroacetamide) oxazole. Compound DPMF-5 (RT 28.333) was identified as Methyl-12-n-hexyl-octadecanoate (106).
Fig. 4.25. Comparison of mass spectrum of compound DPMF-1
Mass spectrum of compound DPMF-2 (RT 16.308)

Scan # 1718
Base Peak: 43.55 (69976)

Fig. 4.26. Comparison of mass spectrum of compound DPMF-2 with compiled data
Mass spectrum of compound DPMF-3 (RT 24.917)

Fig. 4.27. Comparison of mass spectrum of compound DPMF-3
Mass spectrum of compound DPMF-4 (RT 27.192)

Mass Peak #: 31 Ret. Time: 27.192
Scan #: 3024
Base Peak : 43.55 (97275)

No | SI Mol.Wtg. | Mol.Form | Compound Name | CAS No. | Entry | LIB#
--- | ---------- | -------- | -------------- | ------- | ------ | ----
1 | 56 | 374 | 2-Methyl-4-benzyl-5(N-benzyltrifluoroacetamide)oxazole | 114656 | 1 | 1 |
2 | 64 | 276 | N,N-Bis(1,3-dimethylbutyl)-1,4-benzenediamine | 84143 | 1 | 1 |
3 | 52 | 359 | 5-[(Z)-2-2'-dimethylpropyldene]-2,1,4-bis(1-methyl-1-nitroethyl)tetrhydropyridine | 80055-06-5 | 111107 | 1 |
4 | 51 | 209 | Propanoic acid, 2,2-dimethyl, silver(I) salt (CAS) | 7224-58-5 | 52968 | 1 |
5 | 49 | 364 | 2,5-DINITRO-1,4-BIS(1,1,2,2-TETRAMETHYLPROPYL)BENZENE | 68667-15-0 | 112353 | 1 |

**Fig. 4.28.** Comparison of mass spectrum of compound DPMF-4
Mass spectrum of compound DPMF-5 (RT 28.333)

Fig. 4.29. Comparison of mass spectrum of compound DPMF-5
4.4.4. Estimation of minerals in *Acalypha fruticosa* and *Dipteracanthus patulus*

Table 4.8. and Table 4.9. show the results of quantitative estimation of minerals in the dried powder of *Acalypha fruticosa* and *Dipteracanthus patulus*. Quantitative determination of mineral elements in *Acalypha fruticosa* indicated that the concentration of macro elements (K, Na, Ca, Mg and S) ranged from 0.01% to 4.23% and that of the microelements (Zn, Cu, Fe, Mn, Bo and Mo) ranged from 0.02 ppm to 87.62 ppm and in *Dipteracanthus patulus* the concentration of macro elements ranged from 0.16% to 4.16% and the microelements ranged from 0.02 ppm to 84.59 ppm. Of the macro elements analyzed, calcium was present in high amount followed by magnesium and potassium in *Acalypha fruticosa*. In *Dipteracanthus patulus* the concentration of magnesium was high followed by calcium and potassium. Among the minor elements, iron and manganese were present in higher quantity in both the plants. The concentration of copper and zinc was more in *Dipteracanthus patulus* when compared to *Acalypha fruticosa*. 
Table 4.8.  Estimation of minerals in the aerial parts of *Acalypha fruticosa*

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of the minerals</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sodium</td>
<td>0.01%</td>
</tr>
<tr>
<td>2</td>
<td>Potassium</td>
<td>2.87%</td>
</tr>
<tr>
<td>3</td>
<td>Calcium</td>
<td>4.23%</td>
</tr>
<tr>
<td>4</td>
<td>Magnesium</td>
<td>3.16%</td>
</tr>
<tr>
<td>5</td>
<td>Sulphur</td>
<td>0.59%</td>
</tr>
<tr>
<td>6</td>
<td>Zinc</td>
<td>7.65 ppm</td>
</tr>
<tr>
<td>7</td>
<td>Copper</td>
<td>0.46 ppm</td>
</tr>
<tr>
<td>8</td>
<td>Iron</td>
<td>87.62 ppm</td>
</tr>
<tr>
<td>9</td>
<td>Manganese</td>
<td>59.16 ppm</td>
</tr>
<tr>
<td>10</td>
<td>Boron</td>
<td>0.92 ppm</td>
</tr>
<tr>
<td>11</td>
<td>Molybdenum</td>
<td>0.02 ppm</td>
</tr>
</tbody>
</table>
Table 4.9. Estimation of minerals in the leaves of *Dipteracanthus patulus*

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Name of the minerals</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sodium</td>
<td>0.16%</td>
</tr>
<tr>
<td>2</td>
<td>Potassium</td>
<td>3.29%</td>
</tr>
<tr>
<td>3</td>
<td>Calcium</td>
<td>4.13%</td>
</tr>
<tr>
<td>4</td>
<td>Magnesium</td>
<td>4.16%</td>
</tr>
<tr>
<td>5</td>
<td>Sulphur</td>
<td>0.64%</td>
</tr>
<tr>
<td>6</td>
<td>Zinc</td>
<td>7.92 ppm</td>
</tr>
<tr>
<td>7</td>
<td>Copper</td>
<td>0.81 ppm</td>
</tr>
<tr>
<td>8</td>
<td>Iron</td>
<td>84.59 ppm</td>
</tr>
<tr>
<td>9</td>
<td>Manganese</td>
<td>51.20 ppm</td>
</tr>
<tr>
<td>10</td>
<td>Boron</td>
<td>0.52 ppm</td>
</tr>
<tr>
<td>11</td>
<td>Molybdenum</td>
<td>0.02 ppm</td>
</tr>
</tbody>
</table>
4.5. DISCUSSION

Medicinal plants are of great importance to the health of individuals and communities. The quality and therapeutic efficacy of herbal drugs is dependant on the active constituents which are present in the plant. The most important of these bioactive constituents of plants are terpenoids, alkaloids, tannins, flavonoids and phenolic compounds. The medicinal value of the plants lies in some chemical substances that produce a definite physiological action on the human body.

Acalypha is the fourth largest genus in the family Euphorbiaceae with about 450 species. Some species are widely used as traditional medicines for treating skin diseases, venereal infections, head ache, inflammation and urinary disorders (Morton, 1981). Despite the diversity on this genus, to date only few species have been chemically studied (Gutierrez-Lugo et al., 2002). The chemical investigation of Acalypha afforded the isolation of different classes of natural products like flavonoids, tannins, sterols, diterpenes and amides as well as cyanogenic glycosides (Nahrstedt et al., 1982; Amakura et al., 1999; Gutierrez-Lugo et al., 2002; Reiersen et al., 2003; Astudillo et al., 2004; Nahrstedt et al., 2006 and Hungeling et al., 2009). In the present study the aerial parts of Acalypha fruticosa was selected for phytochemical evaluation on the basis of its wound healing activity.

Species of Ruellia (Acanthaceae) have been utilized in folkloric medicine to cure eye sore, wounds, scalds, gonorrhea, syphilis, renal infections, tooth ache and stomach pain (Murugesamudaliar, 1985 and Kirtikar and Basu, 1980). In view of the medicinal properties attributed to the leaves of
Dipteracanthus patulus (syn. Ruellia patula), chemical investigation of Dipteracanthus patulus has been carried out. The chemistry of Acanthaceae shows a great diversity of different compounds such as alkaloids, iridoids, lignans, flavonoids, terpenoids and phenylpropanoid glycosides.

Screening of phytochemicals and quantitative estimation of secondary metabolites in Acalypha fruticosa and Dipteracanthus patulus revealed that they are rich in flavonoids, tannins and phenols. These phytochemicals are known to show medicinal activity (Sofowora, 1993). The presence of terpenoids and flavonoids in Acalypha fruticosa confirms with the reports of Mothana et al. (2007). Moreover the species of Acalypha were reported to contain alkaloids, tannins, flavonoids and terpenoids (Musa et al., 2000). The studies on A. wilkesiana, A. hispida and A. communis have demonstrated the isolation of gallic acid, corilagin, geraniin and triterpenoids of cycloartane-type (Adesina et al., 2000 and Gutierrez-Lugo et al., 2002) and cyanogenic glycosides (Hungeling et al., 2009). Soladoye et al. (2008) reported the presence of alkaloids, tannins, saponins and cardenolides in A. fimbriata, A. hispida, A. ornata, A. racemosa and A. wilkesiana.

Screening and estimation of phytochemicals in the leaves of Dipteracanthus patulus (syn. Ruellia patula) revealed the presence of flavonoids, steroids, saponins, and phenols. Steroids were isolated and estimated from Ruellia prostrata and Ruellia patula (Banerjee, 1984 and Muthumani et al., 2009). Ahmad et al. (1993) have isolated lignans from the extract of the whole plant of Ruellia patula. Studies on Ruellia tuberosa (Nair and Subramanian, 1974 and Lin et al., 2006) and Ruellia praetermissa (Salah et al., 2001) demonstrated the presence of flavonoids.
GC-MS analysis of the ethanolic extract of *Acalypha fruticosa* revealed high concentration of 1,2-Benzene dicarboxylic acid diisoctyl ester, and the presence of n-Hexadecanoic acid and 9,12-Octadecadienoic acid. Presence of anti-oxidant like Hexadecanoic acid and anti-inflammatory compound, 9,12-Octadecadienoic acid ester may possibly play a role in curing skin diseases. Hexadecanoic acid was reported from fruit pulp of *Tamarindus indica* (Pino et al., 2004) and root of *Salvia hypoleuca* (Bigdeli et al., 2005). In the present study seventeen known chemical compounds were identified in the methanolic extract of the leaves of *Dipteracanthus patulus*. Twenty two compounds in chloroform extract and 12 compounds in ethanolic extract of aerial parts of *Gmelina asiatica* were identified by Gas chromatography- Mass spectrometry analysis, out of which 1,2-Benzenedicarboxylic acid diisoctyl ester and Monolineoleoyl glycerol trimethyllysilyl ether were the major constituents (Merlin et al., 2009). GC-MS analysis of the reddish brown fraction of the methanolic extract of the leaves of *Dipteracanthus patulus* (DPMF) contained L-alanine 11-dimethylethyl ester hydrochloride, Clivorine-(12-acetyloxy)-14,15,20,21-tetradehydro-15,20-dihydro-8-n, 5H-Cyclopropa[3,4]Benz[1,2-e]azulen-5-one, 2-Methyl-4-benzyl-(n-benzyl trifluoroacetamido) oxazole and Methyl-12-n-hexyl-octadecanoate.

Quantitative determination of elements in *Acalypha fruticosa* indicated that the concentration of macroelements ranged from 0.01% to 4.23% and the microelements ranged from 0.02 ppm to 87.62 ppm and in *Dipteracanthus patulus* the concentration of macroelements ranged from
0.16% to 4.16% and the microelements ranged from 0.02 ppm to 84.59 ppm. Heavy metals like cadmium, arsenic, lead and mercury were not detected in the powdered samples of *A. fruticosa* and *D. patulus*. Zinc is a constituent of important multiple metalloenzymes including collagenase, DNA and RNA polymerases that are needed to repair wounds. Analysis of minerals in *Acalypha fruticosa* and *Dipteracanthus patulus* indicated the presence of zinc and magnesium. Topical zinc-containing treatments, have improved healing of skin wounds (Agren and Wilkinson, 1999). Trace minerals such as manganese, copper and silicon were known to be important in the biochemistry of tissue healing (Pereira and Felcman, 1998).

Obiajunwa *et al.* (2002) have reported the concentration of major and trace elements of twenty medicinal plants commonly used in Nigeria. Therapeutic role of certain medicinal plant materials has been correlated with the presence of specific elements in their composition (Ahmad *et al*., 1989).

The presence of substantial amount of calcium in *A. fruticosa* is probably due to the presence of calcium oxalate crystals (Druses) in the plant parts as shown in the cross sections of the various parts of the plants. Topical zinc-containing treatments have improved healing of wounds (Agren and Wilkinson, 1999). Zinc is essential for the normal maturation of the epidermis, a process referred to as terminal differentiation (Molokhia and Portnoy, 1969). Magnesium is a cofactor for many enzymatic reactions including collagen synthesis. Copper is a cofactor in protein synthesis and is essential for wound healing. Copper is a required cofactor for the enzyme lysyl oxidase, which plays
a role in the cross-linking and strengthening of connective tissue (Rucker et al., 1998). Iron is required for hydroxylations of proline and lysine, both the amino acids are essential for collagen synthesis (Patel, 2005). In the present study the result on the analysis of minerals support the traditional use of *Acalypha fruticosa* and *Dipteracanthus patulus* in wound healing. The presence of magnesium, copper, iron, manganese and zinc may be responsible for the wound healing activity.
4.6. REFERENCES


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