CHAPTER 5

DISCUSSION

Natural products have been the most successful source of drugs for ever. Researches on drug discovery especially in phytodrug investigation have become one of the frontier areas in phytochemistry. Plants are probably the best cell factories that can be exploited to produce a wide range of chemical compounds, especially the low molecular weight secondary metabolites (Hadacek, 2002).

5.1. Pharmacognosy:

Pharmacognosy is defined as the scientific and systematic study of structural, physical, chemical and sensory characters of crude drugs along with their history, method of cultivation, collection and preparation for the market (Evans, 1996). Identification of drugs can be done by morphological, histological and chemical testing. There are five methods of evaluation of crude drugs namely Morphological or Organoleptic, Microscopical or Histological, Physical, Chemical and Biological.

Micromorphology of vegetative and reproductive plant organs is the object of research to resolve the taxonomic problems of critical species and genera. Of the several traits on leaf surface, the stomata are perhaps the most significant from the point of view of systematic and phylogeny. Stomata that are highly characteristic of the epidermis occur in widely divergent parts of the plants including common foliage leaves. Stomatal size is an ecologically important attribute. The type, size, distribution and frequency of stomata have been recognized to be specific to the taxa below the family and these characters were
used as significant parameters in the angiosperm taxonomy as well as phylogeny. The importance of epidermal cell characters is now well established in taxonomic considerations of angiosperms (Parveen et al., 2000). Microscopical evaluation of the plant drugs helps to identify the organized drugs by their known histological characters and used to confirm the structural details of the drugs from plant origin.

The anatomical features of *T. sphaerocarpa* such as the astomatic nature of the adaxial epidermis of leaf, the epidermal cell number, stomatiferous abaxial epidermis, rubiaceous type of stomata in various sizes, orientation and frequency, palisade spongy ratious, vein islet number, veinlet termination number and unicellular conical trichomes of the leaf traits are the characteristic to the plant. The macerated elements showed characteristic vessel elements with tails on one side or on both sides. Anatomy of the leaf, stem and root reveled unique features such as hemispherical epidermal cells on the stem and midrib region of leaf with arches of thick cuticle, discrete vessels in the secondary xylem, distribution of tannineferous idioblasts. All these characters are typical to this plant which would be very useful in correct botanical identification of crude samples.

Histochemical localization methods provides the authentic data on the availability of chemical compounds by simple and quick methods. Histochemical analysis is highly essential that will aid the pharmacognosist to locate chemical substances and its properties in terms of cells, tissues and parts (Johansen, 1940).

Histochemical localization is performed for starch, alkaloid, protein, tannin and lignin. The present study reports the presence of starch, alkaloid and
protein in leaf, stem and root; tannin in leaf and stem. Lignin only in root. Mucilage is totally absent in all the plant part studied.

Fluorescence analysis showed that, the leaf powder is mostly green to dark green in daylight whereas it is orange in UV light. In stem, it is mostly yellow in both day light and UV light. In root, it is mostly yellow in day light and pale yellow to dark yellow in UV light. In fruit, it is mostly light brown in day light and creamy white to brown in UV light. When physical and chemical methods are insufficient, as often happens with the powdered drugs, there are methods such as fluorescence studies and microchemical tests to identify the powdered drugs and their adulterants. In addition to this preliminary phytochemical, histochemical tests using free hand sections of fresh parts, microtome sectioning are used in identifying the adulterated ones. Maceration methods also included, in which the type of vessels, tracheids, fibres etc. are considered in determining the genuiness of the drug (Kulkarni and Surekha, 1981). In *Tricalysia sphaerocarpa*, the libriform type of fibres, with thick lignified walls and fairly very narrow lumen are seen.

A glimpse at the literature reveals that there is very little information on the anatomical features of Rubiaceae members. Virtually *Tricalysia sphaerocarpa* the present test plant has remained unexplored. Therefore, the present study marks the first comprehensive report on the anatomical features of leaves, stem and root of the test plant. Along with the physico-chemical studies, it would pave the way for its botanical identification and to distinguish from adulterants and substitutions during herbal drug quality control.
5.2. Phytochemistry:

Physicochemical properties are important parameters in detecting adulteration on improper handling of the drug. In the evaluation of crude drug, ash value and extractive values are important parameters. The estimation of ash value is useful for detecting low-grade products, exhausted drugs and the drug with excess of sandy matter. The determination of extractive values with array of solvent gives information about extractable polar and non polar as well as total extractable plant constituents (Rajan et al., 2013). Physicochemical evaluation of crude drug involves the determination of the identity, purity and quality. Purity depends upon the absence of foreign matter, whether organic or inorganic. While quality refers essentially to the concentration of the active constituents in the drug that makes it valuable to medicine. The present study reveals that the moisture content, total ash, acid insoluble ash and water soluble ash are high in stem when compared to leaf, root and fruit.

5.2.1. Phytochemical Screening

The preliminary phytochemical colour reactions reveals the marked presence of alkaloids, cardiac glycosides, flavonoids, glycosides, reducing sugar and non-reducing polysaccharide(starch) in methanolic extract, water extract and powder as such in leaf, stem, root and fruit. Proteins, phenolic group, steroids, and saponins are very low or sparingly observed in leaf, stem, root and fruit. Terpenoids are present in leaf, stem and root, but it is absent in fruit. Flavonoids encompass a huge array of biologically active compounds that are omnipresent in plants, many of which have been used in established eastern medicine for
thousands of years (Gurudev Singh Raina, 2013). Flavonoids are also shown to inhibit microbes which are resistant to antibiotics (Linuma et al., 1994).

Phenols the aromatic compounds with hydroxyl group are widespread in plant kingdom. They occur in all parts of the plants. Phenols are said to offer resistance to diseases and pests in plants. Phenolic compounds could be a major determinant of antioxidant potentials of food plants and could therefore be a natural source of antioxidants and therefore phenolic compounds have been associated with the health benefits derived from consuming high levels of fruits and vegetables (Kähkönen et al., 1999). Hence presence of phenolic compounds in Tricalysia sphaerocarpa plays an important role in antioxidation. Saponins are a special class of glycosides which have soapy characteristics (Fluck, 1973), and also an active antifungal agents (Sadipo et al., 1991). Tannins are water – soluble polyphenols that are present in many plant foods and precipitate proteins. Tannins have been reported to prevent the development of microorganisms (antimicrobial agents) by precipitating microbial protein and making nutritional proteins unavailable for them (Sadipo et al., 1991). The growth of many fungi, yeasts, bacteria and viruses is inhibited by tannins (Chung et al., 1998). Tannins are reported to have various physiological effects like anti–irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects. Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention (Ruch et al., 1989; Motar et al., 1985). The phytochemical analysis conducted on Helichrysum longifolium extract revealed the presence of tannins, flavonoids, steroids and saponins (Aiyegoro and

### 5.2.2. GC-MS Analysis

Methanolic extract of leaves, stem, root and fruit were subjected to GC-MS analysis. This analysis were carried out to detect the possible compounds present in the active fraction. Phytochemicals were extracted best in methanol (Bhaigyabati *et al.*, 2011).

**Leaf**

In the present study, from the methanol extract of leaf, totally 30 chemical compounds were identified of which 9 belong to fatty acids, four to aliphatic and aromatic bicyclics, two each to aromatic hydrocarbons groups, aromatic nitrile groups, aromatic dicarboxylic esters groups. Of which one compound belonged to each of the class terpenoids, barbiturates, aromatic alcohols group, aliphatic aldehydes group, aromatic ketones group, aromatic ethers, phenolic group and to pyrimidinedione group. Among this, eicosanoic acid is found to be present as major constituent, followed by octadecanoic acid. Oleic acid is an unsaturated fatty acid present in several plants and being unsaturated is considered as a healthy source of fat in the diet. Many fatty acids are known to have antibacterial and antifungal properties (Russel, 1991). Dodecanoic acid, tetradecanoic acid,
hexadecanoic acid, octadecanoic acid and oleic acids are among the fatty acids known to have potential antibacterial and antifungal activity (McGraw et al., 2002; Seidel and Taylor, 2004). Oleic acid has been found to be fungistatic against a wide spectrum of moulds and yeasts. For example, it was observed to cause a delay of 6-8 hour in the germination of fungal spores, and was also found to be effective at concentrations as low as 0.7% v/v (Sheba et al., 1999). It has also been disclosed that these fatty acids have potential antibacterial and antifungal principle for clinical application (Altieri et al., 2008). Docosanoic acid also called as Behenic acid is a normal carboxylic acid, which is a saturated fatty acid. Commercially, behenic acid is often used to give hair conditioners and moisturizers due to their smoothing properties. It is also used in lubricating oils, as solvent evaporation retarder in paint removers. As Amide an anti-foam is used in the manufacturing of detergents, floor polishes and dripless candles. Reduction of behenic acid yields behenyl alcohol. Pracaxi oil from the seeds of Pentaclethre macroloba is a natural product with one of the highest concentrations of behenic acid, and is used in hair conditioners. Nonadecanoic acid found in ox fats and vegetables oils is used by certain insects as a pheromone. Nonadecanoic acid has also been reported from the genus Streptomyces, along with its biological functions as anti-tumor agent and inhibition of IL-12 production (Yoo et al., 2002). Nonadecanoic acid has already been isolated from several sources, including a fungus (Juzlova et al., 1996), marine sponge (Mishra et al., 1996), and plant (Hogg and Gillan, 1984; Fukunaga et al., 1989), and exhibits inhibitory effects on fibrinolysis and plasmin activity (Kawashiri et al., 1986).
Squalene, an isoprenoid compound structurally similar to beta-carotene, is an intermediate metabolite in the synthesis of cholesterol. In humans, about 60 percent of dietary squalene is absorbed. It is transported in serum generally in association with very low density lipoproteins and is distributed ubiquitously in human tissues, with the greatest concentration in the skin, where it is one of the major components of skin surface lipids. Squalene is not very susceptible to peroxidation and appears to function in the skin as a quencher of singlet oxygen, protecting human skin surface from lipid peroxidation due to exposure to UV and other sources of ionizing radiation. Supplementation of squalene to mice has resulted in marked increases in cellular and non-specific immune functions in a dose-dependent manner. Squalene may also act as a "sink" for highly lipophilic xenobiotics. Since it is a nonpolar substance, it has a higher affinity for un-ionized drugs. In animals, supplementation of the diet with squalene can reduce cholesterol and triglyceride levels. In humans, squalene might be a useful addition to potentiate the effects of some cholesterol-lowering drugs. The primary therapeutic use of squalene currently is as an adjunctive therapy in a variety of cancers. Although epidemiological, experimental and animal evidence suggests anti-cancer properties, to date no human trials have been conducted to verify the role this nutrient might have in cancer therapy regimens. Phthalic acid, di(2-propyl pentyl)ester and squalene was found in the wood extractives of *Melaleuca leucadendrda* (Xu *et al.*, 2013). Squalene has already been reported from the acetone extract of the glandular hair of fruit of *Mallotus philippensis* (Velanganni, 2012), and also from the root of *Bulbophyllum kaitense* (Kalairasan *et al.*, 2012).
n-hexadecanoic acid has anti inflammatory property. The rigorous use of medicated oils rich in n-hexadecanoic acid for the treatment of rheumatic symptoms in the traditional medical system of India, Ayurveda (Aparna et al., 2012). n-hexadecanoic acid, oleic acid are the two major compounds present in the oil of Chasmanthera dependens (Modupe Ogunlesi et al., 2010). 9,12,15-octadecatrenoic acid, methyl ester, (Z,Z,Z-), oleic acid, 9,12-octadecadienoic acid(Z,Z)- has been reported from the ethanolic extract of stem and root of the plant Mallotus philippensis (Velanganni et al., 2011). 2,3,5,6,tetrafluoroanisole was already reported from Dinochloa puberula by Py-GC/MS and it used as raw material for bioenergy and rare biomedicines (Qiang et al., 2008). 2,4,(1H,3H)-Pyrimidinedione is used as an antiviral agent, and it reduces cellular cytotoxicity and inhibits HIV type 1 and HIV type 2 (Buckheit et al., 2007). Phthalic acid, di(2-propyl pentyl)ester and oleic acid was identified from the chloroform extract of marine Kocuria sp. SRS88 by GC/MS and the chloroform extract showed antibacterial activity (Ranganathan Sahadevan et al., 2014). Ethyl benzonitrile was found to be the major constituents of the methanolic extract of leaf of Gaultheria fragrantissima (Padmavathy et al., 2014). The oil yielded from the ethanolic extract of the seeds of Brachystegia eurycoma showed n-hexadecanoic acid, octadecanoic acid, docosanoic acid, bether-sitosterol, eicosanoic acid and the oil showed antibacterial activity (Okenwa Uchenna Igwe et al., 2013). Cyclobarbital is used as an anesthetic, anticonvulsant, sedative, hypnotic, veterinary euthanasia agent.
Stem

From the methanol extract of stem, totally 17 chemical compounds were identified of which one belongs to aliphatic hydrocarbons groups, four to steroid groups, five to fatty acid esters. Of which one compound belongs to the class sugars, one to the class tocopherols, one to aromatic nitrile. Among this, octadecanoic acid was found to be present as major constituent, followed by n-hexadecanoic acid. n-hexadecanoic acid has also reported from the stem of *Bulbophyllum kaitense* (Kalairasan and Ahmed John, 2011). n-hexadecanoic acid, octadecanoic acid, 9,12,15-octadecatrenoic acid, methyl ester, \((Z,Z,Z-)\), oleic acid, 9,12-octadecadienoic acid\((Z,Z-\) has been reported the ethanolic extract of stem and root of the plant *Mallotus philippensis* (Velanganni and Kadamban, 2011). Steroid are abundant in nature, many derivatives of steroid have physiological activity (Vollhardt et al., 1994). Steroids are used in medicine in the treatment of cancer, arthritis or allergies and in birth control (Okwu et al., 2010). Stigmasterol isolated from plants were reported to be involved in the synthesis of many hormones like progesterone, androgens, estrogens and corticoids with several pharmacological prospects such as antiosteoarthritic, antihypercholesterolemic, antitumor, hypoglycaemic, antimutagenic, antioxidant, anti-inflammatory and CNS effects. Stigmasterol does seem to be play a role in reducing inflammation, which may because it is a precursor to chemical compounds which can limit inflammatory processes. Sterols like stigmasterol have also been recommended for their cholesterol lowering abilities, although more study is needed to determine which compounds perform this function, and
how they work in the body. It has been already reported form the pseudobulb of *Bulbophyllum kaitense* (Kalairasan and Ahmed John, 2011). Sitosterol is already recorded from the ethanolic extract of leaf of *Mallotus philippensis* (Velanganni *et al.*, 2011). τ-Tocopherol is the analog of tocopherol (vitamin E). In cosmetics and personal care products, tocopherol and other ingredients made from tocopherol, including tocopherol esters are used in the formulation of lipstick, eye shadow, blushers, face powders and foundations, moisturizers, skin care products, bath soaps and detergents, hair conditioners, and many other products. Similar results were reported from the pseudobulb of *Bulbophyllum kaitense* (Kalairasan and Ahmed John, 2011).

**Root**

Totally 8 compounds were identified from the methanol extract. Of which five belongs to heterocyclics groups, one to aromatic ester groups, one to fatty esters and one to triterpenoid group. Among this, benzo(b)thiophene, 4-methyl was found to be present as major constituent, followed by 2-methyl-5-p-dimethylaminophenyl oxadiazol. Oxadiazol is a compound showing strong antifungal activity (Zhang *et al.*, 2013). 9,19-Cyclolanostane derivates was also isolated from the roots of *Actaea pachypoda*. Cyclolanostane Triterpene diglycosides isolated from the aerial parts of *Cimicifuga foetida* shows immunosuppressive effect (Pan *et al.*, 2009). Cyclolanostane Triterpene was also isolated from the ethanolic extract of the stems of *Kadsura heteroclite* (Wang *et al.*, 2006). Tryptophan is a compound which is useful for insomnia, depression and anxiety. Its also lower blood pressure in Hypertension patients, stimulate the
production of antibodies, reduce inflammation. Tryptophan supplementation may inhibit the development of full-blown AIDS in persons infected with HIV virus (www.biogenesis-antiaging.com).

**Fruit**

Totally 10 compounds were identified of which three belong to heterocyclic group, one to aliphatic aldehyde groups, one to thiosulphate group, one to thiophosphates group, one to antibiotic and three to the other unclassified groups. Among this, S,S-3,8-Diazaundecamethylene bis[hydrogen thiosulfate] was found to be present as major constituent, followed by 5,8,15,18,23-pentaoxa-1,12-diazabicyclo(10,8,5)-pentacosane. Deoxyspergualin (DOS), a substance composed of a guanidinic and a spermidine moiety, was originally described as an antitumor agent (Takeuchiii et al., 1981). Deoxyspergualin (DSG) has been found to have an antitumour and immunosuppressive activity. It also acts as an antimalarial agent (Ramya et al., 2007). Methyldeoxyspergualin (MeDSG) in *in vitro* culture studies of DSG shows good stability in aqueous solution and retains strong immunosuppressive activity (Odaka et al., 1998). Methylpiperidin isolated from plants shows antipsychotic therapeutic potential (Fuchigami et al., 2012).

Octadecanoic acid, n-hexadecanoic acid and 9,12,15- octadecatrienoic acid (Z,Z,Z-) are the common compounds seen both in stem and leaf. Most of the compounds obtained through GC-MS analysis from the methanolic extract of leaf, stem, root and fruit of *Tricalysia sphaerocarpa* show antibacterial, antifungal and antiviral properties. Some of them have antitumour, anti-inflammatory, antiasthma, antiarthritic, diuretic, antipsychotic, anesthetic, anticonvulsant,
sedative, antiarthritic, antioxidant and anticancer properties and hence the plant
*Tricalysia sphaerocarpa* have high medicinal value.

5.3. Pharmacology

5.3.1. Antioxidant Activity

The antioxidant activity of various extracts (petroleum ether, chloroform, methanol, water) of *Tricalysia sphaerocarpa* was determined by DPPH, FRAP, Hydrogen peroxide and SOD Scavenging assay. The present study reveals, the maximum activity in chloroform extract, followed by the methanolic extract using DPPH, FRAP and Hydrogen peroxide assay, but in SOD assay, the maximum activity was observed in methanolic extract. Using DPPH radical scavenging method, methanolic extract of some medicinal plants like *Camellia sinensis*, *Eugenia caryophyllus*, *Piper cubeba*, *Zingiber officinale*, *Trigonella foenum-graecum* and *Elettaria cardamonum* was found to have significant antioxidant activity (Khalae *et al.*, 2008). Al-fartosy (2011) reported strong antioxidant activity as well as strong reducing power (increase in the extract concentration increases the activity) and ferrous ion chelating abilities from the methanolic extract of *Inula graveolensa*. Higher antioxidant potential of the *Samanea saman* extracts (petroleum ether, ethyl acetate, chloroform, aqueous and HCl extracts) was observed in both DPPH scavenging assay and reducing power assay (Arulpriya *et al.*, 2010). *Crataegus. monogyna* flowers, leaves and fruits had H$_2$O$_2$ radical scavenging, total antioxidant activity (Keser *et al.*, 2012). Antioxidant potential of various extracts of *Cassia fistula* was determined by the DPPH, FRAP, Fe$^{3+}$ reducing power, and hydrogen peroxide scavenging assay.
Methanolic extracts of *Cassia fistula* showed the highest amount of reducing capacity (Irshad et al., 2012). Riaz et al., (2012) reported highest total antioxidant activity from the chloroform fraction of *Dodonaea viscosa*. The methanolic extract of *Leucas plukenetti* Whole plant plays an important role in the modulation of oxidative stress (Subhangkar Nandy et al., 2012). The methanolic leaf extract of *Moringa peregrine* exhibited the scavenging activity on DPPH assay (Dehshahri et al., 2012). The ethyl acetate fraction of *Tagetes erecta* ethanol extract was found to be the most effective in DPPH assay (Miglena Valyova et al., 2012).

**5.3.2. Anti-Depressant Activity**

The present findings obtained from FST, TST and HBT clearly reveal the methanolic extract of *Tricalysia sphaerocarpa* elevate the suppressed mood in animal models. The decrease in the immobility time was quite close to that of the standard i.e. Imipramine. It clearly reveals that the animals treated with methanol extract 200 mg/kg showed better response than those treated with standard drug. Saroj Kothari et al., (2010) reported the methanolic extract of *Aegle marmelos* leaf showed significant antidepressant and anxiolytic activities. All doses of the aqueous extract of *Melissa officinalis*, produced a significant reduction in immobility along with an increase in climbing behavior which is similar to those which have been observed with imipramine (Emamghoreishi and Talebianpour, 2009). The methanol extract at the dose of 100mg/kg of the leaves of *Citrus paradise* var. *foster* markedly increased the average time spent in the open arms in EPM and methanol extract at the dose of 400mg/kg showed a significant decrease
in the time spent immobile by mice in FST (Vikas Gupta et al., 2009). The methanolic extract of *Foeniculum vulgare* possesses significant antidepressant activity due to its reduction in the immobility period (Jamwal Neetu Singh et al., 2013). The ethanolic extract of *Caryophyllus aromaticus* proposed antidepressant-like effect of higher dose concentration (200mg/kg) and significantly increased swimming time and decreased immobility time (Sangavai et al., 2013). Caffeine, as a psychomotor stimulant, suggest a possible positive effect on dopaminergic activity of caffeine augmentation (10 mg/kg or lower dose) with antidepressant agents for depression treatment (Pravin Popatrao Kale et al., 2010). *Celastrus paniculatus* seed oil showed significant antidepressant-like activity (Valeca et al., 2014).

### 5.3.3. Anti-Diabetic Activity

The present study revealed that all dosess of *Tricalysia sphaerocarpa* methanolic stem extract in normal fasted rats, significantly (P<0.05) reduced the blood glucose levels up to 6 hr. except the lowest dose. The maximum hypoglycemic activity was induced by 500 mg/kg dose at 4 hr. by 18%. In alloxan-induced diabetic rats, it significantly (P<0.01) reduced the blood glucose levels up to 3 hour except the lowest dose. The maximum hypoglycemic activity was induced by 500 mg/kg dose at 3 hour. The present study indicates that alloxan induced tissue injury is reversed by continuous administration of *T. sphaerocarpa* extract with subsequent decrease in blood sugar. Oral administration of *T. sphaerocarpa* methanolic extract of 500 mg/kg showed significant (P<0.01) plasma glucose lowering effect in 12 and 16 days of
treatment. Oral administration of the extract showed a significant (P<0.01) decrease in body weight in all the tested concentrations, compared to control groups. The group treated with 500 mg/kg showed the decrease the lysosome enzyme levels, SGOT, SGPT, Alkaline phosphatase, and also in % of lipid peroxidation. It also reduced the enzymatic antioxidants like CAT, GPx, compared to diabetic controls. Microscopically examined pancreas section of normal rat group, diabetic control group, Test extract (125, 250 and 500 mg/kg) and the standard showed that normal architecture of pancreas with acini of serous epithelial cells along with nest of endocrine cells separated by fibrocollaenoous stroma into lobules. No fibrosis or inflammation was found. Similar results reported by previous workers are in conformity into the present findings. The antidiabetic potential of the methanolic extract of *Operculina turpethum* stem and root was evaluated in the Streptozotocin- induced type 2 diabetic models (Pulipaka *et al.*, 2012). The antidiabetic effects of the methanol and acetone extract of *Acalypha indica* Linn. was evaluated in normal and Alloxan induced diabetic rats. Decreased blood glucose level of the test animals shows that the extract exhibit significant antidiabetic activity when compared to diabetic control group (Masih *et al.*, 2011). The aqueous and methanolic extract of *Gongronema latifolium* leaves showed antidiabetic activity in alloxan induced diabetic rats (Akah *et al.*, 2011). The methanolic and ethanolic extracts 200 mg/kg b.wt. of seeds of *Annona squamosa* as significant hypoglycemic activity in both normal and Alloxan induced diabetic rats (Ravinder Sangala *et al.*, 2011). Aqueous and cold extracts of *Terminalia catappa* exhibited significant anti-hyperglycemic
activities in alloxan-induced hyperglycemic rats without significant change in body weight. They also improved conditions of DM as indicated by parameters like bodyweight, and lipid profiles along with serum (Ahmed et al., 2005). Manikandan et al., (2013) reported the methanolic extract of Psidium guajava leaves, showed its in vitro anti-diabetic activity. The aqueous and methanolic extracts of aerial parts, viz. leaves, stem and seeds of the plant, Cassia occidentalis possessed anti-hyperglycemic/anti-diabetic activity against alloxan induced animal model (Arya et al., 2013). The methanol extract of Costus pictus(120mg/kg.p.o.) showed significant (p<0.001) reductions of blood glucose and serum enzymes (SGOT, SGPT, ALP) in alloxan induced diabetic rats (Nandhakumar Jothivel et al., 2007).

Thus the present study will be useful in the following ways.

- In proper botanical identification of the crude drug of the plant studied through pharmacognostical investigation
- To identify the chemicals responsible for the medicinal properties of the plant through various phytochemical studies
- Pharmacological investigations on antioxidant activity, antidepressant activity and antidiabetic activity of Tricalysia sphaerocarpa will provide the first scientific report in medicinal science
- It will also provide clues for new drug discovery.
Figure 1: Extractive values of various parts of *Tricalysia sphaerocarpa* by Batch process.

Figure 2: Extractive values of various parts of *Tricalysia sphaerocarpa* by Successive process
Figure 3: GC-MS chromatogram of Methanolic Leaf Extract of *Tricalysia sphaerocarpa*

Figure 4: GC-MS chromatogram of Methanolic Stem Extract of *Tricalysia sphaerocarpa*
Figure 5: GC-MS chromatogram of Methanolic Root Extract of *Tricalysia sphaerocarpa*

![Figure 5: GC-MS chromatogram of Methanolic Root Extract of *Tricalysia sphaerocarpa*](image)

Figure 6: GC-MS chromatogram of Methanolic Fruit Extract of *Tricalysia sphaerocarpa*

![Figure 6: GC-MS chromatogram of Methanolic Fruit Extract of *Tricalysia sphaerocarpa*](image)
Figure 7(a): Compounds identified from GC-MS analysis of *Tricalysia sphaerocarpa*

- 2,5-Dimethylbenzonitrile
- 2-Ethylbenzonitrile
- 2,3,5,6-Tetrafluoro-4-Methylanisole
- n-Hexadecanoic acid
- Tetradecanoic acid
- Eicosanoic acid
- Oleic acid
- 9,12-Octadecadienoic acid (Z,Z-)
- Docosanoic acid
- Bis(2-Ethylhexyl)Phthalate
- 3-O-Methyl-D-Glucose
Figure 7(b): Compounds identified from GC-MS analysis of *Tricalysia sphaerocarpa*

- Eicosanoic acid, methyl ester
- Octadecanoic acid
- Ergost-5-en-3-ol
- Stigmasterol
- Cyclobarbital
- 1,2-benzenedicarboxylic acid bis(2-methylpropyl) ester
- 9,12,15-octadecatrienoic acid, (zzz)-
- 9,12-Octadecadienoic acid, methyl ester, (E,E)-
Figure 7(c): Compounds identified from GC-MS analysis of *Tricalysia sphaerocarpa*

Hexadecanoic acid, methyl ester

Octadecanoic acid, methyl ester

9Z-octadeca-9,17-dienal

1H-Benzimidazole, 5,6-dimethyl-

1H-Indene-2-ethanol, 2,3-dihydro-

Oxitriptan

Benzoic acid, 4-(3-hydroxy-3-methyl-1-butynyl)-methyl ester
Figure 7(d): Compounds identified from GC-MS analysis of *Tricalysia sphaerocarpa*

2-[[3-cyclohexylaminopropylamino]ethyl thiophosphate  
EPPS

S, S’-3,8-diazaundecamethylene bis[hydrogenthiosulfate]

2-methyl-5-p-dimethylaminophenyl oxadiazol  
Benzo(b)thiophene, 4-methyl

Hexadecanoic acid, 1a, 2, 5, 5a, 6, 9, 10, 10a, octahydro-4-(hydroxymethyl)-1, 1, 7, 9-tetramethyl-6, 11-dioxo-1H-2, 8a-methanocyclopenta(a)cyclopropa(e)cyclodecen-5-yl ester
Figure 7(e): Compounds identified from GC-MS analysis of *Tricalysia sphaerocarpa*

- 4,13,20-tri-O-methylphorbol 12-acetate
- 2-myristoyl pantetheine
- 5,8,15,18,23-pentaoxa-1,12-diaza bicyclo (10,8,5)-pentacosane
- dl-5-hydroxytryptophan
- 3-chloro-2,4-dimethyl-12-thia-1,5,6a,11,tetraaza- indeno[2,1-a]fluorine
- 4-octadecenal
Figure 7(f): Compounds identified from GC-MS analysis of *Tricalysia sphaerocarpa*

- **N-[4-(4-chlorophenyl)isothiazol-5-yl]-1-methylpiperidin-2-imine**
- **Deoxyspergualin**
- **Squalene**
- **Phthalic acid, di(2-propylpentyl) ester**
- **9,17-Octadecadienal, (Z)-**
- **Nonadecanoic acid**
Figure 8: Anti oxidant activity - DPPH Scavenging assay:

![DPPH Scavenging assay graph](image)

Figure 9: Anti oxidant activity - Iron chelating activity (FRAP)

![Iron chelating activity graph](image)
Figure 10: Anti oxidant activity - Hydrogen peroxide assay

Figure 11: Anti oxidant activity - Superoxide dismutase (L-methionine and NBT assay)
CHAPTER 6
SUMMARY AND CONCLUSIONS

Chemical investigations of wild medicinal plants used by the indigenous people of world show unknown compounds with promising biological activities. Phytochemical analysis of plants, used in folklore has yielded a number of compounds with various pharmacological activities. Hence medicinal plants are important substances for the study of their traditional uses through the verification of pharmacological effects and can be natural composite sources that can act as disease curing agents. The integrated research in drug discovery have attracted and provided multidisciplinary research platforms. The present work has been concentrated to bring out the medicinal potential of *Tricalysia sphaerocarpa*.

The important conclusion derived from this study is summarized in three aspects viz, 1. Pharmacognostical studies for easy identification of plant species, 2. Phytochemical analysis to identify the chemicals responsible for the medicinal properties of the plant, 3. To understand the Pharmacological properties of the chemicals through *in vivo* and *in vitro* studies i.e., the antioxidant, antidepressant activity and antidiabetic activity.

*Tricalysia sphaerocarpa* belongs to the coffee family (Rubiaceae), which is commonly called as wild coffee, locally called as irrukulimaram in tamil, vella by Srilankans and kadukafibija in kanada. The roasted seeds of *Tricalysia sphaerocarpa* are used as a coffee substitute. Along with *Tinospora giloy*, *Argemone satyanashi*, *Tricalysia sphaerocarpa* is traditionally used for sleep.
Pharmacognostical parameters like leaf constants, microscopy, physico-chemical analysis, fluorescence analysis are a few of the basic protocol for standardization of crude drugs. Hence, in the present work the pharmacognostical standardization has been performed.

Microscopical analysis of cleared leaf showed the characteristic polyhedral vein islets and vein terminations. The examination of the macerated materials showed the characteristic fibres, vessel elements and broken parenchyma cells. The epidermal studies revealed that the number of epidermal cells/mm², type of trichome, occurrence of stomata on the lower surface only, the number of stomata/mm², type of stomata, the stomatal index are characteristic to this plant.

The anatomical studies revealed the presence of hemispherical shaped epidermal cells with very thick arches of cuticle in leaf and stem that extends to the radial walls also. Presence of tannineferous idioblast, discrete vessel elements with simple perforation and tails at one or both ends are the salient features present in *Tricalysia sphaerocarpa*. All the characters are typical to this plant which would be very useful in correct botanical identity of crude samples.

The histochemical localization tests revealed the presence of starch, alkaloid and protein in all the plant parts studied, tannin in leaf and stem, lignin only in root and the absence of mucilage in all the parts.

In fluorescence analysis, leaf powder is mostly green to dark green in daylight whereas it is orange in UV light. In stem, it is mostly yellow in both day light and UV light. In root, it is mostly yellow in day light and pale yellow to dark
yellow in UV light. In fruit, it is mostly light brown in daylight and creamy white to brown in UV light.

Of the physico-chemical parameters studied, the moisture content (20%), total ash (5.16%), acid insoluble ash (1.06%) and water soluble ash (3.90%) were high in stem when compared to all other parts of the plant. In both the batch process and successive process the highest extractive values were recorded in methanol extract of leaf, stem, root and fruit, when compared to other solvents.

The preliminary phytochemical screening of leaves, stem, root and fruit revealed the presence of phytoconstituents like alkaloids, carbohydrates, flavonoids, glycosides, proteins, phenolic group and saponins in methanolic extract, water extract and powder as such.

By GC-MS analysis, totally 65 chemical compounds (17 chemical compounds from stem, 30 from leaf, 8 from root and 10 compounds from fruit) were identified from the methanolic extract. Octadecanoic acid, n-hexadecanoic acid and 9,12,15-octadecatrienoic acid (Z,Z,Z-) are the three common compounds seen both in stem and leaf.

Antioxidant activity was significantly higher in the chloroform extract, followed by the methanolic extract of stem. By DPPH scavenging method, the percentage of scavenging activity was found to be more in chloroform extract, followed by methanol, petroleum ether and water extracts. All the extracts showed dose concentration dependent activity in all the tested concentration. By FRAP assay, the maximum value was observed in chloroform extract, followed by methanol, water and petroleum ether extracts. By Hydrogen peroxide assay,
Chloroform extract showed the maximum value, followed by methanol, petroleum ether and water extracts. By superoxide dismutase assay, the maximum value was observed in methanol extract, followed by chloroform, water and the minimum value was observed in petroleum ether extract. This activity may be due to the presence of secondary metabolites such as flavonoids, tannins, and steroids, all of which are known antioxidants.

In acute toxicity, no mortality was observed in the animals treated with the dose of 2000 mg/kg methanol extract of stem. There were no signs of any toxicity. The studies on anti-depressant activity clearly revealed that the animals treated with methanol extract at 200 mg/kg had better response than those treated with standard drug (Imipramine). In anti-diabetic activity, the methanolic extract of stem, significantly reduced the blood glucose level in both normal fasted rats and alloxan induced diabetic rats. It also reduces the body weight, and decrease the enzymatic levels like SGOT, SGPT, Alkaline phosphatise, CAT and GPX. It also reduced the lipid peroxidation level in all the tested concentrations. Microscopically examined pancreas section of normal rat group, diabetic control group, test extract (125, 250 and 500 mg/kg) and the standard showed the normal architecture of pancreas with acini of serous epithelial cells along with nest of endocrine cells separated by fibrocollaenoous, stroma into lobules. No fibrosis or inflammation was found. The various extracts (petroleum ether, chloroform and methanol) of stem possess significant antioxidant activity and the methanolic stem extract possesses significant anti-depressant and anti-diabetic activities.
Thus the study concludes that the plant may be used as a potential drug for antioxidation, depression and diabetes. The results of pharmaconostical, phytochemical and pharmacological studies which are reported for the first time from *Tricalysia sphaerocarpa*, may pave way for new drugs discovery.
Plate 1
Morphology of *Tricalysia sphaerocarpa*

Habit
Basal portion

Trunk
A twig with fruits

T.S and L.S of fruit showing flat seeds
Plate 2
Leaves of *Tricalysia sphaerocarpa*

Dorsal surface

Ventral surface

T.S. of Lamina

Midrib

Leaf blade

Cu: Cuticle; Ep: Epidermis; X: Xylem; Ph: Phloem; GT: Ground tissue; Pc: Palisade cells; Sc: Spongy cells

Leaf epidermal peel of *Tricalysia sphaerocarpa*

Adaxial Epidermis without Stomata

Abaxial Epidermis with Stomata

ICR: Inter Costal Region; CR: Costal Region
Plate 3
Size and Orientation of Stomata in *Tricalysia sphaerocarpa*

Various sizes of Stomata

Stomatal opening
Stomata enlarged

Degenerated Stomata
Unicellular Trichome

GS: Giant Stomata; MS: Medium sized Stomata; SS: Small Stomata; BS: Blind Stomata; DS: Degenerated Stomata; HS: Half Stomata.
Plate 4

Vein islet and Veinlet termination of *Tricalysia sphaerocarpa*

Stem epidermal peel of *Tricalysia sphaerocarpa*

VI: Vein islet; VLT: Veinlet termination; S:Stomata.
Plate 5

T.S. of Stem of *Tricalysia sphaerocarpa*

Portion enlarged

Outer portion

Epidermis enlarged

Xylem showing vessels

Pith portion enlarged

Sc: Sclerenchyma cells; Ve: Vessel element; TI: Tannineferous Idioblast.
Plate 6

T.S. of Root of *Tricalysia sphaerocarpa*

Entire view

portion enlarged

Xylem showing vessels

SG: Starch grains; Ve:Vessel.
Plate 7

Macerated elements of stem of *Tricalysia sphaerocarpa*

Vessel elements  Single tailed vessel member  Single fibre

Vessel member with pits  Double tailed vessel member

Parenchyma cells  Trachea

P: Pits; PP: Perforation plate; Ta: Tail.
Plate 8

Effect of Methanolic Stem extract of *Tricalysia sphaerocarpa*

Anti - Depressant Activity

Forced swimming test (FST)

Tail suspension test (TST)

Hole Board Test (HBT)
Plate 9

Effect of Methanolic Stem extract of *Tricalysia sphaerocarpa*

Anti –Diabetic Activity

Histopathological Studies

Normal Control                  Diabetic Control                  Test extract (125)

Test extract (250)                        Test extract (500)                     Glibenclamide (5mg)