6. SUMMARY

Ever since his appearance on this planet, man has tried to live in harmony with nature. The relationship between man and plants is in practice for time immemorial. Natural products have played an important role in the development of drugs and drug leads for various diseases. The secondary metabolites from natural sources are good candidates for drug development because being elaborated within the living systems, they are perceived to exhibit more similarities to drugs and show more biological friendliness than totally synthetic drugs. Plant derived compounds continue to provide valuable therapeutic agents, in both modern medicine and in traditional system. Thus, plants remain a vital source of drugs and now-a-days much emphasis has been given to phytomedicine and many medicinal plant species are being screened for their pharmacological activities. The present study aims to report the pharmacognostical and pharmacological activities of leaf, fruit and seed of *Elaeocarpus serratus* L. (Elaeocarpaceae). The morphological, anatomical, physicochemical and phytochemical analyses were used as criteria for pharmacognostical investigation.

*Elaeocarpus serratus* L. is a tree that grows up to 18m tall in evergreen to semi-evergreen forests up to 1600m altitude. The bark is brownish and smooth. The leaves are simple, alternate, spiral, clustered at twig ends and turn red when senescent. Inflorescence is a raceme. Flower petals are white, laciniate; peduncle up to 8cm long; pedicels 5-8mm long. Calyx is composed of 5 lobes, free, reddish and hairy. Petals are 5 in number and free. Stamens are numerous, free, filamentous; anthers 2-celled, with tufts of hairs at tips. Fruit is a drupe; containing a much tubercled, 1-seeded stone.

The anatomical features of *E. serratus* were investigated. The leaf showed a thick plano convex midrib, parenchymatous ground tissue with tannin distributed in most of the cells. The vascular strands are triangular in outline and are surrounded by a thick sclerenchyma sheath. There is a circular, small single bundle placed within the wing. Palisade cells possess tannin contents. Calcium oxalate crystals are abundant in the leaf mesophyll and veins. The stem consists of thick and fissured periderm followed by parenchymatous cortex with dense accumulation of tannin. The cortex is followed by the phloem zone. Secondary xylem vessels occur mostly in radial
multiples of 2-6 cells. The fruit is a drupe with epicarp, mesocarp and endocarp. The mesocarp is thick, fleshy and parenchymatous with dense deposition of tannin. The conspicuous feature of the endosperm cells is the presence of spherical bodies of smaller and larger sizes. Rosette of calcium oxalate crystals and starch grains are seen in the endosperm.

Since the standardization of crude drug is an integral part for establishing its correct identity, the organoleptic investigation of fresh and powdered samples of leaf, fruit and seed of *Elaeocarpus serratus* were also carried out. Microscopic observations of the leaf powder of *E. serratus* showed cyclocytic type of stomata in abaxial peeling of the epidermis and the adaxial epidermal peeling was apostomatic. Minute prismatic crystals and unicellular trichomes having granular inclusions are seen on the epidermal cells of *E. serratus*. The stem powder includes wide fibres, narrow fibres, fibriform sclereids and vessel elements. Fruit powder consists of parenchymatous cells without inclusions. The seed coat powder includes only brachy sclereids.

Ash content, its extractive value in different solvents, behaviour of the plant powder with different chemical reagents, mineral and vitamin content were analysed and the values were recorded. The total ash content of *E. serratus* was highest in the seed (2.75 ± 0.23%) and lowest in the fruit (2.33 ± 0.2%). The acid soluble ash values of the plant parts were lower than the acid insoluble ash values. The percentage solubility of the plant parts in alcohol was higher than the value of solubility percentage in water. The maximum extractive value was obtained using the solvent ethanol (70.21 ± 4.02%), followed by water (56.42 ± 3.03%) and acetone (45.23 ± 2.04%) in leaf of *E. serratus*. Minimum extractive value was observed in benzene and petroleum ether extract of leaf (8.9 ± 0.16; 8.02 ± 9.11%, respectively).

A total of 10 (inclusive of macro and micro elements) were determined in the powdered samples of various parts of *E. serratus*. The concentration of mineral elements analyzed in the study decrease in the order Ca >Na >Mg >Fe >Zn >Mn >Se. Among the various macro elements studied calcium was present in high amount. Iron was present in higher concentration among the micro elements studied. The heavy metals like nickel and cadmium were completely absent in the study material. The
water soluble vitamins B₁, B₂, B₆, and B₁₂ were detected in the leaf, fruit and seed of *E. serratus*. Among the plant parts studied, the leaves contained the maximum amount of vitamins B₁ and vitamin B₁₂. Similarly, the vitamins B₂ and vitamin B₆ was found to be higher in the fruit sample. Generally the seeds possessed moderate amounts of B-complex vitamins. The fatty acids namely, palmitic acid, oleic acid, linolenic acid and stearic acid were estimated in the leaf, fruit and seed of *E. serratus*. All the four fatty acids were present in maximum level in fruit sample. The leaves also showed appreciable amounts of fatty acids.

The phytochemical screening of powder of leaf, fruit and seed of *E. serratus* in various extracts i.e. hexane, petroleum ether, benzene, chloroform, acetone, ethanol and water was performed. The result showed the presence or absence of carbohydrate, protein, alkaloids, terpenoids, triterpenes, phenols, flavonoids, tannins, saponins, steroids, glycosides, coumarin, quinine, anthraquinone, starch, gum and fixed oils. Generally the solvents ethanol, water and acetone were more efficient in extracting the phytochemicals than the other solvents.

The phytoconstituents in the various plant parts of *E. serratus* were estimated quantitatively. The leaf sample recorded high amounts of protein and carbohydrates. The maximum content of phytochemicals was as follows: tannin in leaf; flavonoids, phenols, saponin, starch in fruit; alkaloid, steroids and anthraquinone in seed. Presence of the phytoconstituents contributed significantly to the antioxidant capacity of this plant.

Chemical profiling of the ethanolic extracts of *Elaeocarpus serratus* by Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed the presence of 30 compounds in the leaf, fruit and seed. The active principles with their retention time, molecular formula, molecular weight and percentage of peak area were identified. Among the identified compounds fatty acid esters and alcohols were more in number followed by hydrocarbons, aldehydes, alkenes, fatty acids and amides. Methanol (20.57%) was the most prevailing major compound in the leaf, n-octanol (25.91%) in the fruit and n-propanol (19.12%) in the seed. The minor compounds like farnesol (0.51%), N-(2-Deuteroallyl)-2-fluoro-N-methylaniline (0.52%), and
1-Propylthio-3,3,3-trifluoropropyl acetate (0.61%) were detected in the leaf, fruit and seed, respectively.

The leaf, fruit and seed extracts of *E. serratus* obtained by different solvents (ethanol, acetone and water) were subjected to screening for their possible antioxidant activity using five complementary test systems namely Hydroxyl radical (‘OH) scavenging assay, Superoxide radical (O$_2$•⁻) scavenging assay, Nitric oxide radical (NO⁺) scavenging assay, ABTS⁺⁺ scavenging assay and β–carotene/linoleic acid peroxidation inhibition assay.

*E. serratus* extracts displayed a dose-dependent scavenging activity against the hydroxyl (‘OH) species. All the plant parts of *E. serratus* generally registered high hydroxyl radical quenching ability. The maximum inhibition exhibited by the ethanolic extract of fruit (IC$_{50}$ = 24.08 ± 0.27µg/ml) and leaf (IC$_{50}$ = 24.30 ± 0.19µg/ml) which were comparable with standards BHA (7.97 ± 1.07µg/ml) and gallic acid (2.7±1.35µg/ml). The ethanol extracts were more efficient in scavenging the hydroxyl radicals than the acetone and water extracts.

The various extracts of *E. serratus* were capable of scavenging the superoxide radicals in a dose-dependent manner. Among the samples tested, the ethanol extract of seed showed the maximum scavenging of superoxide radicals with an IC$_{50}$ value of 41.56 ± 0.18µg/ml, followed by acetone extract of leaf (IC$_{50}$ = 59.52 ± 0.79µg/ml). The ethanol extract of seed (IC$_{50}$ = 41.56 ± 0.18µg/ml) was more or less equal to the standard antioxidant gallic acid (IC$_{50}$=38.27±0.28µg/ml) in quenching the superoxide radicals.

In the present study, *E. serratus* extracts compete with oxygen to react with nitric oxide and thus inhibits generation of the anion. The highest NO⁺ scavenging activity was noticed in the acetone extract of seed (IC$_{50}$ = 36.23 ± 0.26µg/ml). The activity was better than BHA (IC$_{50}$ = 43.37 ± 1.26µg/ml) and closer to that of gallic acid standard (IC$_{50}$=29.76±0.81µg/ml). The ethanol extract of leaf (IC$_{50}$=45.94±0.16µg/ml) and acetone extract of fruit (IC$_{50}$ = 64.12 ± 0.44µg/ml) also exhibited good scavenging activity which was comparable to that of the standards.
*E. serratus* exhibited potent ABTS$^+$ scavenging activity with maximum activity seen in the ethanol extract of leaf (18428.1 ± 23.08µmol/g) and water extract of fruit (18270.8 ± 32.2µmol/g). The lowest ABTS$^+$ scavenging activity was noted in the acetone extract of seed (7578.8 ± 99.5µmol/g). *E. serratus* extracts possessed greater antioxidative activity by prohibiting the bleaching of β-carotene and inhibiting linoleic acid oxidation. The ethanol and water extracts of leaf showed the maximum inhibition of lipid peroxidation (79.43 ± 5.27% and 78.33 ± 9.67%, respectively). In general, all the extracts showed above 60% inhibition of β-carotene bleaching activity.

The *in vivo* toxicological evaluation of ethanolic extracts of *E. serratus* leaf, fruit and seed revealed that the plant extracts were quite safe even at a high dose of 5000mg/kg b.w. p.o. and had no acute toxicity on animal model. As a part of the present investigation, the anti-arthritic activity, cardio protective activity and anti-diabetic activity of the ethanolic extracts of the plant parts of *E. serratus* were estimated using *in vitro* and *in vivo* assays.

The *in vitro* anti-arthritic activity of *Elaeocarpus serratus* was estimated using inhibition of protein denaturation and proteinase activity. The ethanol extracts of the leaf and seed of the study species *E. serratus* is capable of controlling the denaturation of protein and also promoted proteinase inhibitory activity against inflammation diseases. The *in vivo* anti-arthritic activity of the test plant was studied using Freund’s Complete Adjuvant (FCA) method. The *E. serratus* leaf and seed ethanolic extracts inhibited chronic inflammation at the dose of 400mg/ kg b.w. p.o.

The cardio protective activity of the ethanolic extract of leaf and seed of *E. serratus* was assessed. The lipid profile showed that the levels of total cholesterol (TC), triglycerides (TGL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) significantly decreased (p<0.05) whereas, the level of high density lipoprotein (HDL) significantly increased (p<0.05) with the administration of the plant sample. The biochemical parameters were analyzed to confirm the cardio protective activity of the plant extract. Oral pretreatment of *E. serratus* extracts resulted in significant decrease (p<0.05) in the levels of serum marker enzymes aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphate.
(ALP) as well as cardio marker enzymes lactate dehydrogenase (LDH) and creatine phosphokinase (CK-MB).

Anti-diabetic activity of ethanolic extract of fruit of *E. serratus* was evaluated by both *in vitro* and *in vivo* studies. The *in vitro* evaluation of anti-diabetic potential was done using α-amylase and α-glucosidase inhibitory activity. The ethanolic extract of plant inhibited both α-amylase and α-glucosidase enzymes in a dose-dependent manner. Insulin dependent Diabetes mellitus was induced using streptozotozin (STZ). Body weight of the animals and their blood glucose levels were used as criteria to measure the *in vivo* anti-diabetic activity of the test plant. The administration of the plant sample significantly restored the blood glucose levels to near normal value which was comparable to standard drug treatment. The body weight of the animals was determined. Administration of STZ significantly decreased (p<0.05) the body weight of experimental animals. However, pretreatment with the plant sample significantly increased (p<0.05) the weight of the animals to near normal value.

The effects of ethanolic extract of fruit of *E. serratus* on certain serum liver markers were analyzed to confirm the anti-diabetic activity of the plant. The diabetic rats group showed increased oxidative stress and reduced liver functions. At the same time, the diabetic groups administrated with ethanol extracts of fruit of *Elaeocarpus serratus* showed remarkable reduction in the levels of AST, ALT and ALP comparable to the standard drug treated group. However the level of serum protein and albumin increased significantly in the animals pretreated with the plant extract. In the present study, the levels of carbohydrate metabolizing enzymes such as hexokinase (HK), phosphoglucoisomerase (PGI), glucose-6-phosphatase (G-6-P) and fructose 1,6-diphosphatase (F-1,6-DP) in plasma and liver tissue were also estimated. Pretreatment with the ethanolic extract of fruit of *E. serratus* decreased the levels of HK, PGI and decreased the levels of G-6-P and F-1, 6-DP significantly (p<0.05).

The present study registered remarkable antioxidant, anti-arthritis, cardio protectiv e and anti-diabetic activities for the ethanolic extract of *Elaeocarpus serratus*. 