5. DISCUSSION

5.1 Pharmacognostical investigation of *Elaeocarpus serratus*

Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained (Gokhale, 1979; Mukherjee, 2002; Trease and Evans, 2002). Standardization is an essential measure of quality, purity and authenticity. The standardization of crude drug is an integral part for establishing its correct identity. Before any crude can be included in an herbal pharmacopoeia, pharmacognostic parameters and standards must be established. In order to standardize a drug, various botanical, pharmacognostical and phytochemical parameters like macroscopical, microscopical, powder characteristics, organoleptic characters, ash values, extractive values, vitamin studies, fatty acids, heavy metal analysis and preliminary qualitative and quantitative phytochemical analysis of various solvent extracts must be analysed. As there is no pharmacognostic work recorded so far on *Elaeocarpus serratus*, the present work was undertaken to lay down the standards which could be useful for establishing its authenticity.

5.1.1. Macroscopic Analysis

The macroscopical characters of the plant can serve as diagnostic parameters to provide the standards, to identify the crude drug and to avoid adulteration of drugs (Dinesh Kumar *et al.*, 2012). *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, smooth or blaze orange red. Branchlets are terete, glabrous, with scars of fallen leaves. Leaves are simple, alternate, spiral, clustered at twig ends. The petiole is 1.2 - 4cm long, swollen at both ends, glabrous; lamina is elliptic, apex is acuminate or obtuse, base is acute, margin is serrate. The leaves are glabrous. The leaves turn red when senescent. The midrib is slightly raised above. Inflorescence is a raceme. Flower petals are white, laciniate. Peduncle is 8cm long and pedicels are 5-8mm long. Calyx is composed of 5 lobes, free, 5-7mm long, ovate-lanceolate, reddish and hairy. Petals are 5 and free. Stamens are many, free, filamentous, anthers 2-celled, with tufts of hairs at tips. Fruit is a drupe, oblong, ellipsoid or ovoid to 2.5cm long; containing a much tubercled, 1-seeded stone.
5.1.2. Microscopic Analysis

5.1.2.1. Microscopic Evaluation of Fresh Plant Parts

Microscopic evaluation is an indispensable tool for identification of medicinal herbs and is one of the essential parameters in modern monograph (Sai Saraswathi et al., 2011). Anatomical features of plants have been considered as highly dependable guide lines for diagnosis of fragmentary plant (Metcalf and Chalk, 1950; Kokoski et al., 1958; Singh et al., 2010). Many structural features have established as specific at the species or generic level. Especially, many qualitative characters, such as petiolar vasculature, venation pattern, trichome morphology and pattern of secondary growth in stem / root and quantitative characters such as stomatal number, stomatal index value and palisade ratio, vein islet and vein termination value determination are much reliable features in systematic anatomy as well as pharmaceutical studies (Mohan et al., 2011; Kumar et al., 2011). In the present study on *Elaeocarpus serratus*, detailed analysis was made on the anatomical features of this taxon.

*Elaeocarpus serratus* leaf showed a thick plano convex midrib (700 µm and 900 µm wide). The ground tissue was parenchymatous with tannin distributed in most of the cells. The vascular strands are thick and wide, triangular in outline and conjoint, collateral and closed. The entire vascular strands are surrounded by a thick sclerenchyma sheath. There is a circular, small single bundle placed within the wing. The wing bundle is also collateral, comprising a cluster of xylem and a cap of phloem. The lamina is even and smooth. Palisade cells possess tannin contents. Calcium oxalate crystals are abundant in the leaf mesophyll and veins. In the mesophyll the crystals (up to 30µm in diameter) are drugs. Along the veins, the crystals (up to 20µm long) are prismatic type. The major lateral veins and veinlets are thick and straight. The vein-islets are wide, polygonal in outline and have distinct vein boundaries. The vein-terminations are mostly repeatedly branched forming dendroid outline with in the islets. The basal part of the petiole is boat shaped in sectional view. Some of the cells have dense amorphous inclusions which are surrounded by a rosette of parenchyma cells. Priya Shaival et al. (2012) and Bharti (2010) observed the pharmacognostical characteristics of the leaves of *Elaeocarpus ganitrus* and reported
the presence of prisms of calcium oxalate in the leaf mesophyll and midrib. The vascular bundles were reported to be conjoint, collateral and closed. This finding was similar to the present study. Calcium oxalate crystals constitute one of the major ergastic storage products in plant tissues. Generally, frequency, distribution and size of crystals are used as diagnostic characters in microscopical and powder studies of herbal drugs. Extensive survey of crystals in plants is mostly related to the taxonomic studies of plants (Solereder, 1908; Chattaway, 1956 and Arnott and Pautard, 1965).

The stem consists of thick and fissured periderm. There is a broad parenchymatous cortex with dense accumulation of tannin. The cortex is followed by a zone of dense sclereids which is gradually transformed into phloem zone. The phloem zone consists of mixed masses of sclerechyma and radial fiber of sieve elements Secondary xylem vessels occur mostly in radial multiples of 2-6 cells. Xylem rays are fairly prominent. All these parameters have been reported for the first time in *E. serratus*.

The fruit is a drupe with epicarp, mesocarp and endocarp. The mesocarp is thick, fleshy and parenchymatous. The cells possess dense deposition of tannins. The endosperm is cellular. The conspicuous feature of the endosperm cells is the presence of spherical bodies of smaller, larger sizes. The chemical nature of the spherical bodies is not known. Rosette of calcium oxalate crystals frequent in the outer zone of the endosperm. Starch grains are also sparsely seen in the endosperm. Similarly, Bharti (2010) reported the presence of starch grains in the seed powder of *Elaeocarpus ganitrus*. The presence of cellular endosperm, tannins, brachysclereids, calcium oxalate were also reported by Singh *et al.* (2010) in *Elaeocarpus ganitrus*.

The results of the study provide a protocol of diagnostic features of *E. serratus* which can be readily employed for diagnosis of the plant from any other simulating species of plants. In conclusion, these parameters which are being reported for the first time could be useful in setting some diagnostic indices for the identification and preparation of a monograph of *E. serratus* plant.
5.1.2.2. Microscopic Evaluation of Dry Powder Plant Parts

The powder characters of a drug are mainly used in the identification of the drug in the powder form (Baral et al., 2011). Organoleptic examination refers to evaluation by means of organ of sense and includes the macroscopic appearance of the leaf and seed, its odour and tastes, occasionally the sound or ‘snap’ of its fracture and the feel of the leaf and seed to the touch (Nitin Kumar, 2010). The morphological and organoleptic characters of the leaf were studied (Mukherjee, 2002; Evans, 2009). Microscopic observations of the leaf powder of *E. serratus* showed cyclocytic type of stomata (a stoma is surrounded by 5 or 6 subsidiary cells) in abaxial peeling of the epidermis and the adaxial epidermal peeling was apostomatic (without stomata). On the contrary, Vijayan et al. (2010) investigated the leaf of *Elaeocarpus blascoi* and reported the presence of cyclocytic stomata on the abaxial epidermis and the adaxial epidermis was apostomatic. Further the microscopic study of leaf powder of *E. ganitrus* revealed the presence of anomocytic stomata (Bharti, 2010). In medicinal plants, trichome and crystal characters have been reported to act as biomarkers to identify the plant even in the raw material or powder form (Ragusa et al., 2001; Gohil et al., 2007; Jayeola, 2009). Minute prismatic crystals and unicellular trichomes having granular inclusions are seen on the epidermal cells of *E. serratus*. Analogous to the present study, Vijayan et al. (2010) reported the presence of trichomes and calcium oxalate crystals in the leaf of *Elaeocarpus blascoi*. Similarly, calcium oxalate crystals were reported in the leaf powder of *E. ganitrus* (Bharti, 2010). 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.

5.1.3. Physiochemical Studies of Plant Parts

Physicochemical standards such as total ash value help us in determining both physiological ash (plant tissue) and non-physiological ash (extraneous matter like sand and soil). The physico-chemical evaluation of a crude drug involves the determination of identity, purity and quality (Neelufar Sharma and Mohana Lakshmi, 2011). It is an important parameter in detecting adulteration or improper handling of drugs (African Pharmacopoeia, 1986). Ash values used to determine quality and
purity of crude drug. Acid insoluble ash indicates the presence of various impurities like carbonate, oxalate and silicate. Ash values can be used as reliable and for detecting adulteration. These studies help in the identification of the plant materials (Nayak and Patel, 2010).

The sensory and related characteristic of a plant show that they are non-hazardous, and can be handled within normal laboratory conditions (Ameh et al., 2010). The results of organoleptic study offer a scientific basis for the traditional use of *E. serratus* which possess characters like green colour (fresh leaves and fruit), brown colour (seeds), characteristic odour and taste. The leaf, fruit and seed powders when treated with various chemicals exhibited various colours. Bharti, (2010) and Priya Shaival et al. (2012) studied the organoleptic characters in the leaf powder of *Elaeocarpus ganitrus*. As there was no previous record on organoleptic study in *E. serratus*, the present work gives a lead in this aspect taken up and studied.

In the present study the leaf, fruit and seed powders treated with chemicals like hydrochloric acid, nitric acid, sulphuric acid, ferric chloride, acetic acid, ammonia, Potassium hydroxide solution, iodine solution, ethyl acetate, silver nitrate, α-naphthol solution, sodium hydroxide, potassium iodide and water showed various shades of green, yellow, orange, brown and black when seen on naked eye. Similar studies were done by Sutar et al. (2010) in the leaf powder of *Coccinia indica*.

Total ash is the measure of the total amount of material left after burning and includes ash derived from the part of the plant itself and acid insoluble ash. The latter is the residue obtained after boiling the total ash with dilute hydrochloric acid, and burning the remaining insoluble matter (AOAC, 2005). 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 water soluble ash value was more or less the same in the leaf, fruit and seed (45.23 ± 0.27, 45.66 ± 0.34 and 44.63 ± 0.29%, respectively). The acid soluble ash value of the plant parts was 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. Low ash value indicates the purity of the plant parts. Inorganic elements present in the raw drug indirectly indicate the water soluble ash.
(Kokate et al., 2004). Present report showed minimal ash value, which indicated that the raw drug is free from foreign matter. Vijayan et al. (2010) noticed total ash value of 7.23%, acid insoluble ash value of 4.20%, water soluble ash value of 5.44% in the leaf powder of Elaeocarpus blascoi. Singh et al. (2010) reported the total ash value of 1.5%, acid insoluble ash value of 1.1%, water-soluble ash value of 0.96% in the seed powder of Elaeocarpus ganitrus.

Extractive values are also useful to evaluate the chemical constituents present in the crude drug and help in estimation of specific constituents soluble in particular solvent (Kokate, 2010; Thomas et al., 2008; Kumar et al., 2011). It is primarily useful for the determination of exhausted and adulterated drugs. Extractive values are indicative of the presence of the polar or nonpolar extractable compounds in a plant drug (Pradeep Kumar et al., 2011). In the present study 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). Water soluble extractive of 6.45% was stated by Vijayan et al. (2010) in leaves of Elaeocarpus blascoi. Different extractive value of the samples shows a path for isolation of different active constituents present in the extracts. Water soluble extractive value indicated the presence of highly polar chemicals such as flavonoids, protein, carbohydrates, etc. Hexane extractable value indicated the presence of fatty acid straight chain compounds such as linoleic acid, stearic acid, pentacosanol, noncosane etc. Hexane extractive values denote the presence of fixed oil and fat. Ethanol extractive value of Mangifera indica seed kernel powder showed the presence of phenolic compounds (Rajan et al., 2011). Earlier studies have revealed extractive value profile in several medicinal plants (Neelufar Sharma and Mohana Lakshmi, 2011; Prakash and Rao, 2012).

Studies on physiochemical constants of E. serratus can serve as a vital source of information for the quality control of the crude drug. Also the physiochemical analysis in the present research states that the results were found to be within the standard limits.
5.1.4. Evaluation of Minerals in the Plant Parts

Minerals are a large family of nutrients essential to the human body, although some of them are present in the body in very small percentages, probably several parts per million (Tianshi, 1997). They are designated as essential mineral elements because of their metabolic role in the body, and their absence cause deficiency symptoms in animals. Essential mineral elements of nutritional importance are classified into the macro (or major) elements (calcium, phosphorus, potassium, sodium, chlorine, sulphur, magnesium) and the micro (or trace) elements (iron, zinc, copper, molybdenum, selenium, iodine, manganese, cobalt). Both the macro and micro essential mineral elements are believed to have one or more catalytic functions in the cell. Some are firmly bound to the proteins of enzymes, while other are present in prosthetic groups in chelated forms such as cytochromes, haemoglobin, vitamin B_{12} (McDonald et al., 1995). Apart from taking part in various metabolic processes in the body, they also play an important role in the growth, development, immunity regulation, mitotic cell division, propagation and genetic expression of the body. They differ from other nutrients such as proteins, amino acids, carbohydrates, and fats and oils because they cannot be synthesised by human but rather transferred from one form to another, and can only be ingested from food and water (Tianshi, 1997). The most important pathway of minerals to transport into human is from soil to plant and from plant to human (Kirmani et al., 2011).

In the present study, among the various macro elements studied calcium was present in high amount in all the plant parts (125.6mg/g). Calcium forms the structural component of cell walls, activates enzymes and influences water movement in cell. Calcium is necessary for cell growth and cell division, bone formation and blood coagulation. Its importance almost becomes double during pregnancy (Lalitha and Ramani, 2012). Sodium is a mineral element and an important part of the human body. It controls the volume of fluid in the body and helps maintain the acid-base level. Sodium level in the blood that is too low is dangerous and can cause seizures and coma. Very high sodium levels can lead to seizures and death. The major source of sodium uptake is the common salt which is used in cooking as well in widely used
in industries and for dyeing purposes (Kirmani et al., 2011). The concentration of sodium was fairly high (45-46mg/g) in *E. serratus*.

Maximum concentration of magnesium was registered in the fruit (43.45mg/g) closely followed by the leaf and seed (42.63mg/g) of *E. serratus*. Magnesium is an important mineral element in connection with circulatory diseases such as ischemic heart disease and calcium metabolism in bone. It is critical structural component of chlorophyll molecule and is necessary for the functioning of plant enzymes in the production of carbohydrates, sugars and fat (Hassan and Umar, 2006).

Iron is an essential element for synthesis of haemoglobin. Iron together with hemoglobin and ferodoxin play a vital role in metabolism. Deficiency of iron in plants produces chlorosis (Hussain et al., 2011). It facilitates the oxidation of carbohydrates, protein and fat to control body weight, which is very important factor in diabetes (Khan et al., 2008). The quantity of iron was moderate in the plant parts of *E. serratus* (leaf 4.82 mg/g; fruit 4.56mg/g and seed 4.23mg/g). Zinc is an essential micronutrient which is involved in many biochemical reactions in the plants. It is required for the optimum crop growth and it is taken in divalent form by the plants (www.spraygro.com.au/documents/zincnutrition.pdf). In the present study, zinc was found in trace amounts [leaf (0.143mg/g), fruit (0.1343mg/g and seed (0.1186 mg/g ].

Manganese is also an essential metallic compound that plays a vital role in the photosynthesis, nitrogen metabolism and in the formation of other compounds that are required for the plant metabolism (www.ecochem.com/t_micronutrients.html.). It is necessary for the functioning of the pituitary gland and the brain. Zinc and manganese are considered as antioxidant micro nutrients and their presence could therefore boost the immune system (De, 1990).Trace amount of manganese was found in the fruit and leaf (0.1134mg/g and 0.1132 mg/g respectively). In the present research, selenium was below the instrumental detection limit for all samples of *E. serratus*. Although selenium is essential for the growth of certain plants (Shrift, 1969); the accumulation of selenium in the plant may cause toxicity (Rios and Waterman, 1997).

This study indicates that *E. serratus* accumulates a diverse array of elements, some of which are important for biological functioning. The elucidation of element
specification in *E. serratus* plant parts helps interpret the potential therapeutic properties of the plant. There could be phyto-chemicals with therapeutic properties that act alone or in conjunction with the elements that are important. The present data on elemental concentration in this medicinal plant might be useful to set standards for prescribing the dosage and duration of administration of these herbal medicines.

5.1.5. Evaluation of Heavy Metals in the Plant Parts

Many medical herbs used in formulating these medicines can present a health risk due to the presence of toxic ingredients like heavy metals. The toxicity of heavy metals depends upon the chemical form of elements. Heavy metals are dangerous in the form of their cations and are highly toxic when bonded to the short chains of carbon atoms (Hussain, 2006). Concentration of essential and non-essential heavy metals in medicinal plants beyond permissible limit is a great concern to public safety all over the world (Kirmani *et al.*, 2011). The heavy metal content in the plant induces stress to the plant and causes various disease, other stress related concerns, etc. So, the need to maintain the quality control of the products obtained from the medicinal plants has attained a great interest particularly in the field of pharmaceutical industries (Sathiavelu *et al.*, 2012). In the present investigation, the concentration of arsenic and mercury was less than 1ppm in all the plant parts studied. The heavy metals like nickel and cadmium were completely absent. Comparing the obtained values to the standard WHO guideline values, we can conclude that the heavy metal concentration was found to be less than the detection limit and hence do not possess any harm in the products obtained out of them.

5.1.6. Evaluation of Vitamin in the Plant Parts

The vitamins are important in the body as their deficiencies adversely affect the metabolism of the body. Lack of the vitamins impairs the normal formation of intercellular substances throughout the body (Okwu, 2006). The reducing property is beneficial in certain physiological process. The vitamin, though in trace amount are very essential for the body metabolism. Deficiency of thiamin in the diet is the cause of the disease beriberi (Taylor, 1972). A deficiency of riboflavin does not result to any
specific and identifiable disease and one is apart therefore to under estimate its importance.

The vitamin B-complex refers to all of the known essential water-soluble vitamins except for vitamin C. These include thiamine (vitamin B₁), riboflavin (vitamin B₂), niacin (vitamin B₃), pantothenic acid (vitamin B₅), pyridoxine (vitamin B₆), biotin, folic acid and the cobalamins (vitamin B₁₂). Each member of the B-complex has a unique structure and performs unique functions in the human body. Vitamins B₁, B₂, B₃, and biotin participate in different aspects of energy production, vitamin B₆ is essential for amino acid metabolism, and vitamin B₁₂ and folic acid facilitate steps required for cell division. Each of these vitamins has many additional functions. Mainly B-complex vitamins play different specific and vital functions in metabolism and their lack or excess produces specific diseases.

In *E. serratus* among the plant parts studied, the leaves contained the maximum amount of vitamin 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 than the leaf and fruit. Similarly, Pachkore *et al.* (2012) elucidated the Vitamin B₁ and B₂ composition of the seed of *Ocimum basilicum* (0.64mg/100g; 0.38mg/100g) *Ocimum gratissimum* (0.54mg/100g; 0.48mg/100g) and *Ocimum sanctum* (0.48mg/100g; 0.24mg/100g). Lalitha and Ramani (2012) detected 0.648ppm of vitamin B in the leaf powder of *Naravelia zeylanica*.

**5.1.7. Evaluation of Fatty acid in the Plant Parts**

Fatty acids are the basic components of most naturally occurring lipids in both animals and plants. Recently the biological importance of fatty acids has gained considerable importance in food nutrition evaluation and in the diagnosis of certain diseases and pharmacology. Monounsaturated and polyunsaturated fatty acids (MUFA and PUFA) have been used in lowering the risks of heart diseases, against inflammation and in enhancing the immunity or immune system (Hussain *et al.*, 2012). In the present study, the fatty acids such as palmitic acid, oleic acid, linolenic acid and stearic acids were present in maximum level in fruit sample of *E. serratus*.
(3.11 ± 1.01; 2.20 ± 1.01; 3.11 ± 1.01 and 1.34 ± 0.1%, respectively). The leaves also showed appreciable amounts of fatty acids. Palmitic acid, oleic, linolenic and stearic acid are long chain fatty acids (British Pharmacopoeia (2001). Similar to the present research, Hawash et al. (2009) revealed that the Jatropha curcas oil consists of many fatty acids such as palmitic 18.22%, stearic 5.14%, Oleic 28.46% and Linoleic 48.18% acids. Likewise, Zhang et al. (2010) identified and quantified palmitic acid (38.82%, 4.74 ± 0.09mg/g), linoleic acid (27.92%, 2.31 ± 0.07mg/g), stearic acid (14.14%, 1.54 ± 0.04mg/g) and oleic acid (11.04%, 1.02 ± 0.03 mg/g) in the alcohol extract of Panax japonicas.

5.1.8. Phytochemical Studies in the leaf, fruit and seed of E. serratus

5.1.8.1. Qualitative phytochemical Analysis

Everything of this world directly or indirectly depends on plants. The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. The medicinal value of plants lies in the bioactive phytochemical constituents that produce definite physiological effects on human body (Edeoga et al., 2005). Phytochemicals are non-nutritive plant chemicals that have disease preventive properties (Kumar et al., 2011). These phytoconstituents work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions. Scientists have identified thousands of phytochemicals, although only a small fraction has been studied closely (Djeridane et al., 2006).

Phytochemicals are basically divided into two groups, i.e. primary and secondary constituents; according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acid, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids, saponins, phenolic compounds, flavonoids, tannins and so on (Koche et al., 2010). Phytochemical screening plays a vital role in the pharmacological and chemical study of the medicinal plants. It may suggest feasible pharmacological effects of its extracts or fractions in comparison of identified phytochemicals groups, highlighting a close relationship with its main therapeutic uses (Arcanjo et al., 2011). Presence or absence
of certain important compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigation. Various tests have been conducted to find out qualitatively the presence or absence of bioactive compounds.

Information concerning the presence of phytochemical constituents in *E. serratus* is limited. However, literature survey revealed that, inadequate work has been done on phytochemicals analysis of this potentially medicinal plant. Therefore the present study was designed to carry out the qualitative and quantitative phytochemical screening of leaf, fruit and seed of *E. serratus*. Different chemical compounds such as carbohydrate, protein, alkaloids, terpenoids, triterpenes, phenols, flavonoids, tannins, saponins, steroids, glycosides, coumarin, quinine, anthraquinone, starch, gum and fixed oils etc. were detected in *E. serratus* which could make the plant useful for treating different ailments as having a potential of providing useful drugs of human use. This is because the pharmacological activity of any plant is usually traced to a particular compound. The majority of phytoconstituents were found in ethanol, water and acetone extracts than the other solvents.

Comparable to the present research, Sriti *et al.*, (2011) screened the leaves of *E. serratus* using the solvents petroleum ether, benzene, chloroform and acetone. While from the chloroform extract alkaloids and anthraquinone glycosides were found, flavonoids and anthraquinone glycosides were present in the acetone extract. In addition, from the petroleum ether extract flavonoids were detected. On the other hand, anthraquinone glycosides were found in the benzene extract. Kothale and Rothe (2012) performed the phytochemical screening of leaf and stem *Elaeocarpus tuberculatus* in various extracts i.e. petroleum ether, benzene, chloroform, acetone, ethanol, methanol, rectified spirit and water shows that there is presence of alkaloids, carbohydrates, proteins, tannins, saponin, anthraquinone glycosides, flavonoids, cardiac glycosides and phenolic compounds, quinine, steroids. The majority of phytoconstituents are found in acetone, ethanol, methanol, rectified spirit and water extracts. Steroids present in the leaves tests are good but not traced in acetone, chloroform and water extracts. Only stem extract in acetone, test was positive.
Coumarin test found weak presence in leaf than in stem. Saponin found positively in leaf in ethanol, methanol and water only. Stem extracts had shown positive results in above solvents and in petroleum ether also. Quinone present in stem found positive results in acetone, ethanol, methanol, sprit and water. In leaf it shown positive result in acetone only while, fixed oil and fats are totally absent in all extracts of stem and leaf.

In phytochemical investigation of seed of *Elaeocarpus ganitrus*, petroleum ether showed the presence of phytosterols along with fats and fixed oils. Chloroform extract had phytosterols. Ethanol extract gave the tests for alkaloids, flavonoids, carbohydrates, proteins and tannins. Water extract showed the presence of proteins, tannins and carbohydrates (Singh *et al.*, 2010). The above studies were on par with the present study.

5.1.8.2. Quantitative phytochemical Analysis

The green plants are the storehouses of many chemical components. Metabolites are organic compounds synthesized by organisms using enzyme-mediated chemical reactions called metabolic pathways. Primary metabolites comprise many different types of organic compounds, including, carbohydrates, lipids, proteins, and nucleic acids and have functions that are essential to growth and development and are therefore present in all plants. In contrast, the secondary metabolites are the substances, which are produced by plants as defense chemicals. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, steroids, terpenoids, carbohydrates and phenolic compounds (Pascaline *et al.*, 2011). There is no doubt that plants have continued to offer a large range of natural compounds belonging to different molecular families which have various properties on humankind as earlier reported by Zabri *et al.* (2008).

In the present finding, appreciably high amounts of carbohydrate and protein were registered in the leaf sample (9.52 ± 0.39%; 13.56 ± 0.33%, respectively). The other samples also showed substantial quantities of these primary metabolites. Similar to the present study, Rasool *et al.* (2010) estimated the total carbohydrates (0.375 ± 0.0012%) and protein (0.44 ± 0.0025%) in whole plant of *Prunella vulgaris*. The
results of Rajan et al. (2011) quantified carbohydrates (49mg/kg) and protein (33.6mg/kg) in *Mangifera indica* seed kernel powder. Our results also confirm high values of carbohydrates and proteins.

*E. serratus* appears to be rich in bioactive compounds are widely used for various activities. Findings from this work suggest that the maximum amount of phenol was present in the ethanolic extract of fruit (0.113 ± 0.002%), followed by the leaf (0.112 ± 0.007%) and seed (0.104 ± 0.001%). Likewise, among the three solvents (acetone, methanol and water) investigated, the methanolic extract of leaf, stem bark and fruit of *Elaeocarpus tuberculatus* registered the maximum total phenol content (70.05 ± 9.67; 62.51 ± 4.35 and 34.21 ± 0.83% tannic acid equivalent, respectively). At the same time, the water extracts of the studied plant parts contained comparatively lesser amount of total phenolic (Jayashree, 2011).

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh et al., 2007). They possess biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han et al., 2007). Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. (Ali et al., 2008). Phenolic compounds are well known as antioxidant and scavenging agents against free radicals associated with oxidative damage (Ferguion et al., 2006). Similarly, several workers reported on the total phenolic content in plants. According to Satish Kumar et al. (2008) the total phenolic content in the ethanolic extract of leaves of *Elaeocarpus ganitrus* was found to be 56.79 ± 1.6mg gallic acid equivalents/g of dry material. They suggested that 85% of the antioxidant capacity of *E. ganitrus* was due to the contribution of phenolic components. In addition to their roles as antioxidants, these compounds exhibit a wide spectrum of medicinal properties, such as anti-allergic, anti-inflammatory, antimicrobial, anti-thrombotic, cardio-protective and vasodilatory effects (Middleton et al., 2000; Balasundram et al., 2006).

In the present work, *Elaeocarpus serratus* registered the maximum amount of flavonoids in the ethanolic extract of fruit (0.204 ± 0.001%), followed by the leaf
(0.201 ± 0.004%) and seed (0.198 ± 0.007%). In contrast to the present research, *Elaeocarpus tuberculatus*, registered the maximum amount of total flavonoids in the acetone extract of leaf (96.67 ± 3.84mg/g dry weight), and minimum amount of total flavonoids was recorded in the water extract of fruit (6.40 ± 1.46mg rutin/g dry weight) (Jayashree, 2011). In the same way, Chand *et al.* (1977) reported the presence of flavonoids, including quercetin as one of the phytoconstituents of *Elaeocarpus sphaericus*. According to Satish Kumar *et al.* (2008) the total flavonoids in the ethanolic extract of leaves of *Elaeocarpus ganitrus* were found to be 18.58 ± 0.3mg rutin equivalents/g of dry material. Singh *et al.* (2000) reported the presence of flavonoids in the methanol extract of *Elaeocarpus sphaericus* fruits.

Flavonoids are important secondary metabolite of plants capable of both preventing and eliminating the effects of reactive oxygen species (Gow-Chin *et al.*, 2002; Kaur and Kapoor, 2002; Prior, 2003; Cai *et al.*, 2004). They possess an ideal structural chemistry for free radical scavenging activity both *in vitro* and *in vivo* condition (Kong *et al.*, 2003). Flavonoids are Flavonoids contain hydroxyls, which are responsible for the radical scavenging effect (Younes, 1981; Das and Pereira, 1990; Mensor *et al.*, 2001; Hou *et al.*, 2003). Flavonoid compounds strengthen the walls of capillary vessels and improve blood circulation in the heart muscle. They have a spasmodilistic, diuretic, anti-aggregation effect on blood platelets, and also have anti-inflammatory, anti-ulcerative, anti-allergic and anti-hepatotoxic action (Ferrali *et al.*, 1997; Brown *et al.*, 1998; Sugihara *et al.*, 1999).

The interests in phenolic compounds, particularly tannins, have considerably increased in recent years because of their broad spectrum of chemical and diverse biological properties, which include the antioxidant effects (Larson, 1988) and radical scavenging properties (Agrawal, 1989). Many tannin containing drugs are used in medicine as astringent. They are used in the treatment of burns as they precipitate the proteins of exposed tissues to form a protective covering (Handa and Kapoor, 1992). They are also medically used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns, piles and antidote (Ali, 1994).

Quantitative phytochemical studies in the present research revealed the presence of appreciable amounts of tannin in the ethanolic extract of leaf
(0.123 ± 0.014%) fruit (0.114 ± 0.002%) and seed (0.102 ± 0.003%). Likewise, *Elaeocarpus tuberculatus*, the maximum amount of tannin was recorded in the acetone extract of stem bark (66.78 ± 1.64mg/g dry weight), followed by the methanol extract of leaf and fruit (58.14 ± 0.50 and 29.86 ± 0.50mg/g dry weight, respectively). Generally, the water extracts of leaf, stem bark and fruit showed minimum amount of tannin in the present study. Similarly, Rahman et al. (1998) isolated tannin from the leaves of *Elaeocarpus grandiflorus* and Singh et al. (2000) reported the presence of tannins in the chloroform and methanol extracts of *Elaeocarpus sphaericus* fruits. Tannins were detected by Zakaria (2007) in the leaves of *Muntingia calabura*, *Dicranopteris linearis* and *Melastoma malabathricum*.

Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity (Nobori et al., 1994). Several workers have reported the analgesic (Antherden, 1969; Harborne, 1973), antispasmodic and antibacterial (Stray, 1998; Okwu and Okwu, 2004), chest pain, arthritis (Njoku and Obi, 2009; Aiyegoro and Okoh, 2010), anti-inflammatory, antioxidant (Nasreen et al., 2010) properties of alkaloids.

In the current work, *E. serratus* showed the maximum amount of alkaloid in the ethanolic extract of seed (0.946 ± 0.02%). Minimum amounts were noticed in the leaf (0.017 ± 0.001%) and fruit (0.003 ± 0.004%). Similarly, Mbaebie et al. (2012) quantified the amount of alkaloids in stem bark of *Schotia latifolia* (9.80 ±0.01%). The results of Rajan et al. (2011) revealed the presence of alkaloids (0.64 mg/kg) in *Mangifera indica* seed kernel powder. Nasreen et al. (2010) investigated the methanol and water (6:4) extract of stem of *Tinospora cordifolia* and found that it possessed 0.2998mg/100gm of alkaloids.

Steroids have been reported to have antibacterial properties (Raquel, 2007) and they are very important compounds especially due to their relationship with compounds such as sex hormones (Okwu, 2001). In the present work, substantial amounts of steroids were noticed in the ethanolic extract of seed (0.855 ± 0.005%), leaf (0.342 ± 0.012%) and fruit (0.331 ± 0.004%). Similarly, Mbaebie et al. (2012) quantified the amount of steroids in stem bark of *Schotia latifolia* (18.20±0.01 %).
Saponin is one of the anti-nutrients commonly found in plant foods to have both adverse effects and health benefits. However, saponins have also been shown to reduce blood glucose and insulin responses to starchy foods and the plasma cholesterol and triglycerides. In addition, saponins have been related to reduce cancer risk (Thompson, 1993). It has the potential to lower cholesterol levels in humankind due to its hypocholesterolemic effect (Osagie, 1998). Saponins, a group of natural products occur in moderate amounts in the ethanolic extracts of fruit (0.304 ± 0.002%), leaf (0.301 ± 0.004%) and seed (0.289 ± 0.006%) of *E. serratus*.

Zakaria (2007) demonstrated the presence of flavonoids, saponins, triterpenes and steroids in *Muntingia calabura, Dicranopteris linearis, Melastoma malabathricum, Bauhinia purpurea* and *Corchorus capsularis*. Similarly, Mbaebie et al. (2012) quantified the amount of saponins in stem bark of *Schotia latifolia* (6.80 ± 0.00%). Patil et al. (2011) detected 31.19% of saponins in methanolic extract of tuberous root of *Chlorophytum glaucum*. Koche et al. (2010) estimated the amount of saponins in aqueous leaf extracts of *Ocimum sanctum* (0.58 ± 0.11%), *Hyptis suaveolens* (0.30 ± 0.02%), *Physalis minima* (2.82 ± 0.22%), *Tephrosia villosa* (1.80 ± 0.31%), *Cleome viscosa* (2.0 ± 0.10%), and *Galphimia glauca* (0.80 ± 0.24%). In plants, the presence of steroidal saponins like, cardiac glycosides appear to be confined to many families and these saponins have great pharmaceutical importance because of their relationship to compounds such as the sex hormones, cortisones, diuretic steroids, vitamin D etc., (Evans and Saunders, 2001). From plant sapogenins, a synthetic steroid is prepared and to treat a wide variety of diseases such as rheumatoid arthritis, collagen disorders, allergic and asthmatic conditions (Claus, 1956).

In the current work anthraquinone occurred only in trace amounts in the various plant parts of *E. serratus*. Likewise, anthraquinone and its derivatives were reported in *Boerhaavia diffusa* which had antifungal activities (Wuthi-Udomlert et al., 2010). Further, the phytochemical investigations of *E. serratus* revealed the maximum amount of starch in the ethanolic extract of all the samples viz., (5.52 ± 1.02%) in leaf, (5.66 ± 2.01%) in fruit and (4.23 ± 3.01%) in seed.
5.1.9. GC-MS Analysis of *E. serratus*

Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbals and its formulations. Also the WHO has emphasized the need to ensure the quality of medicinal plants products using modern controlled technique and applying suitable standards (Sharma *et al*., 2010). Gas Chromatography-Mass Spectrometry (GC-MS) is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino acid and nitro compounds (Sampath Kumar and Ramakrishnan, 2011; Johnson *et al*., 2011; Muthulakshmi *et al*., 2012). Many plants emit substantial amounts of phytogenic volatile organic compounds which include alkanes, alkenes, alcohols, aldehydes, ethers, esters and carboxylic acids (Ciganek *et al*., 2007; Battino *et al*., 2007). All volatile organic compounds emitted from plants can originate from biogenic and or anthropogenic sources. The biological activity of volatile compounds is dependent on the synergistic or additive effects of the constituent types present at different concentrations. Volatile components from plants can cause a number of positive or negative interactions (Vokou *et al*., 2003). Defense, communication or protection against extreme environmental conditions may be the reasons for these emissions of volatile compounds (Niinemets *et al*., 2004).

*Elaeocarpus serratus* is used as diuretic and as a cardiovascular stimulant. The leaves are used in the treatment of rheumatism and as antidote to poison, while the fruits are locally prescribed for the treatment of diarrhea and dysentery. The fruit juice of *E. serratus* is given for stimulating secretions from taste buds thus increasing appetite in patients (Ghani, 1998). Taking into consideration of the medicinal importance of this plant, the ethanol extract of leaf, fruit and seed of *E. serratus* were analyzed for the first time using GC-MS. Perusal of literature reveals that information on the GC-MS analysis of the plant is totally lacking. Hence, the objective of the present study is to identify the phytochemical constituents with the aid of GC-MS technique. This work will help to identify the compounds of therapeutic value.
Chemical profiling of the ethanolic extracts of *Elaeocarpus serratus* by GC-MS analysis revealed the presence of 30 compounds in the leaf, fruit and seed. Among the identified compounds fatty acid esters and alcohols were more in number followed by hydrocarbons, aldehydes, alkenes, fatty acids and amides.

Hexadecanoic acid methyl ester which is a palmitic acid ester is one of the identified phytochemicals in the leaf of *E. serratus*. It is used as an antioxidant, pesticide, nematicide, hypocholesterolemic, flavouring agent, lubricant and anti-androgenic (Dr. Duke's Online phytochemical and ethnobotanical database). Analogous to the present study, Vinay Kumar *et al.* (2011) identified hexadecanoic acid methyl ester in the methanol extract of *Spirulina platensis*. Gopalakrishnan and Vadivel (2011) subjected the ethanolic extract of *Mussaenda frondosa* to GC-MS analysis and identified twenty chemical constituents namely (-)-Quinic acid, 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, Hexadecanoic acid ethyl ester, Linoleic acid ethyl ester, Oleic acid, decahydro-2-methoxy,1,2,3-Benzenetriol, Naphthalene, etc. In contrast to the above study, the present investigation reported the presence of Hexadecanoic acid methyl ester instead of Hexadecanoic acid ethyl ester.

Mohan *et al.* (2010) detected 15 compounds in the ethanolic extracts of spines from the bark of *Zanthoxylum rhetsa* using GC-MS analysis. The compounds identified by them were Hexadecanoic acid ethyl ester, Hexanoic acid ethyl ester, Octadecanoic acid 2-hydroxy-1,3-propanediyl ester, 1,2-Benzenedicarboxylic acid, diisoocetyl ester, Dodecanoic acid, Tetradecanoic acid, n-Hexadecanoic acid, 9,12-Octadecadienoic acid,(Z,Z). The compounds belonging to fatty acid ester group reported by the authors were similar in present work.

Ze-kun and Haixia (2012) analyzed the components present in the volatile oil from *Bupleurum chinense* using GC-MS method. 27 compounds were identified including τ-muurolo, β-caryophyllene, aromadendrene, citronellyl acetate, elemene, guaiene, β-farnesol, elemol and citronellyl acetate. The compounds farnesol and citronellyl isobutyrate are present in the ethanolic leaf and seed extract of *E. serratus*. The compound farnesol is an acyclic sesquiterpene alcohol and it has been suggested to function as a chemopreservative and anti-tumor agent (Joo and Jetten, 2009). It is also used as a deodorant in cosmetic products because of its anti-bacterial activity.
(Kromidas et al., 2006). The anti-fungal activity of farnesol has also been reported (Ramage et al., 2002). Citronellyl isobutyrate is an ester of propanoic acid which is widely used as flavoring agent and is known to possess insect repellent and antimicrobial properties (Schmidt et al., 2005; Jirovetz et al., 2006 and Azeez, 2008).

Yassa et al. (2009) analyzed the essential oil of fresh petals of *Rosa damascena* and identified 17 phytocompounds like linalool, nerol, geraniol, n-nonadecene, n-tricosane, hexatriacontane and n-pentacosane. The bioassay-guided fractionation of extract led to the isolation of three flavonol glycosides: quercetin-3-O-glucoside, kaempferol-3-O-rhamnoside and kaempferol-3-O-arabinoside. The compound n-tricosane present in the fruit and seed of *E. serratus* is common from the present study. The saturated aliphatic hydrocarbon n-tricosane has known anti-microbial property (Kordali et al., 2009; Agnihotri et al., 2012).

Agnihotri et al. (2012) analysed the phytocompounds from the essential oil of fruit of *Amomum subulatum*. They detected the major aliphatic hydrocarbons like 2-methylheptane, 2-methylnonane, n-hexane, n-non-2-ene and n-heptane, n-tricosane and n-docosane. n-octanol, n-nonanol, n-decanol, n-heptadecanol, n-dodecanol, n-tridecanol and n-tetradecanol occurred as the main aliphatic alcohols. n-tetracosanoic acid was the predominant fatty acid followed by hexadecanoic acid (2.5%) and arachidic acid. The higher alkane (n-tricosane and n-docosane) and alcohol groups (n-octanol, n-dodecanol) are common from the present study.

Loghmani-Khouzani et al. (2007) identified 18 components from the essential oil of petals and whole flower of *Rosa damascena* by GC-MS. According to GC-MS results β-citronellol, nonadecane, geraniol, hencicosane, farnesol, docosane and octadecane were some of the components. Similarly, Bakkour et al. (2011) identified 8 major chemicals from the essential oil of seeds of *Punica granatum*, *Vitis vinifera* and *Cucurbita maxima*, using GC-MS. Only three of the identified chemicals (farnesene, docosane and tetracosane) were found in all three samples, but in varying proportions. The results of the GC-MS analyses on the essential oils of the aerial parts, n-hexane extract of the bulbs and the hydrolyzed methanolic extract of the bulbs of *Ornithogalum procerum* revealed the presence of docosane (5.52%) (Delazar et al.,
The compound docosane (2.19%) present in the seed of *E. serratus* was common in the present study.

Ricinoleic acid one of the components identified in the ethanolic extract of leaf of *E. serratus*. It is an unsaturated omega-9 fatty acid. Ricinoleic acid exerts analgesic, anti-inflammatory effects (Vievia *et al.*, 2000) and anti-microbial activity (Vinay Kumar *et al.*, 2011). It was hypothesized by Lampe *et al.* (1998) that lipids kill microorganisms by leading to disruption of the cellular membrane as well as bacteria, fungi and yeasts because they can penetrate the extensive meshwork of peptidoglycan in the cell wall without visible changes and reach the bacterial membrane leading to its disintegration.

Fatty alcohols are emulsifiers and emollients (Smolinske and Susan, 1992) to make skin smoother and prevent moisture loss. They are used to control viscosity and dispersion characteristics in cosmetics, personal care products and pharmaceutical ingredients such as medications for the treatment of eczema (Kato *et al.*, 1987). The fatty alcohols like methanol, n-dotriocontanol, n-dodecanol, n-pentadecanol, n-hexadecanol, n-propanol and n-undecanol are present in the ethanolic extract of leaf, fruit and seed of *E. serratus*. Similarly, Ashok Kumar *et al.* (2010) detected the presence of methanol and n-dodecanol in the methanolic extract of roots of *Hibiscus micranthus* by using GC-MS. Likewise; Prakash Babu *et al.* (2009) observed n-dodecanol (2.94%) in the petroleum ether extract of bark of *Albizia lebbeck* using GC-MS analysis. Alcohols are known to possess antioxidant (Abdelwahab *et al.*, 2010) and bactericidal rather than bacteriostatic activity (Muthuchelian *et al.*, 2011). In the current research, the compound bis-(3,5,5-trimethylhexyl) ether (6.30%) was detected in the ethanolic extract of *E. serratus* leaf. Similarly, Prakash Babu *et al.* (2009) noticed bis-(3,5,5-trimethylhexyl) ether (2.49%) in the petroleum ether extract of bark of *Albizia lebbeck* using GC-MS study.

In the present investigation the following aldehydes 2-fluoro hexadecanal, pentadecanal, 4-methyl-4-nitro-5 oxoheptanal were present in the ethanolic extract of fruit and seed of *E. serratus*. Aldehydes are known to possess powerful anti-microbial activity (Muthuchelian *et al.*, 2011). It has been proposed that an aldehyde group conjugated to a carbon to carbon double bond is a highly electronegative arrangement,
which may explain their activity (Moleyar and Narasimham, 1986), suggesting an increase in electronegativity increases the anti-bacterial activity (Kurita et al., 1979; Kurita et al., 1981). Such electronegative compounds may interfere in biological processes involving electron transfer and react with vital nitrogen components, e.g. proteins and nucleic acids and therefore inhibit the growth of the microorganisms.

Our systematic investigation reveals the potential of *E. serratus* as a good source of bioactive compounds such as fatty acid esters, alcohols, hydrocarbons, aldehydes, alkenes, fatty acids and amides that justify the use of this plant for its various ailments by traditional practitioners. The study also suggests that the various parts of *E. serratus* produced different ratios of bioactive compounds and offers further research interest in the study of these active bio-compounds as antioxidant, anti-microbial, anti-inflammatory agents, and analgesic agents.

Establishing standards is an integral part of establishing the correct identity and quality of a crude drug. Before any drug can be included in the pharmacopoeia, these standards must be established. The majority of the information on the identity, purity and quality of the plant material can be obtained from its macroscopy, microscopy and physio-chemical and phytochemical parameters. As there is no record on pharmacognostical work on *Elaeocarpus serratus*, the present work was undertaken to produce some pharmacognostical standards. The above studies provide information in respect of their identification, chemical constituents and physicochemical characters which may be useful for pharmacognostical study and standardization of herbal drugs.

**5.2. Evaluation of *In vitro* Antioxidant Activity of *E. serratus***

Reactive oxygen species play an important role in oxidative stress related to the pathogenesis of various important diseases. Such as anaemia, asthma, arthritis, inflammation, Cardio vascular diseases, Parkinson’s disease, Mongolism, ageing. In healthy individuals, the production of free radicals is balanced by the antioxidative defense system ((Hertog et al., 1997; Kumpulainen and Salones, 1999; Pourmorad et al., 2006).
Diminished antioxidant system occasioned by increased free radical generation during normal metabolic function such as respiration, digestion, immune response and growth or introduced from environment (Nagavani et al., 2010) have been reported to play an important role in the induction of oxidative stress (Prasanna and Purnima, 2011). It is widely accepted that antioxidants are radical scavengers which protect the human body against free radicals. Hence, the use of antioxidant as supplement is recommended as a possible remedy in the control of diseases (Huong et al., 1998; Jayaprakasha et al., 2004; Badami et al., 2008). Restriction of the use of synthetic antioxidants due to their possible undesirable effects on human health has led to a growing interest in natural antioxidants of plant origin in recent years (Wang et al., 2011).

Moreover, knowledge and application of such potential activities of reducing oxidative stresses in vitro has prompted many investigators to search for potent and cost effective antioxidants from various plant sources (Cesquini et al., 2003). However, the potential of higher plants as sources for new drugs is still largely unexplored (Oke and Hamburger, 2002 and Sim et al., 2010). There is increasing interest in phytochemicals such as polyphenols, saponins, tannins, alkaloids and terpenes due to their potentially positive effect against certain diseases. They can act as free radical scavengers neutralizing dangerous ROS which are responsible for antioxidant properties (Cheeseman and Slater, 1993; Wargovich et al., 2001 and Seal et al., 2012).

Also the antioxidant potential of different plant extracts and pure compounds can be measured using in vitro assays. Various mechanisms such as free radical scavenging (by acting as a hydrogen electron donor or direct reaction with them), reducing capacity, metal ion chelation inhibition of radical producing enzymes, increase in the expression of antioxidant enzymes, inhibition of both lipid peroxidation and LDL oxidation have been studied to explain how plant extracts could be used as effective antioxidants (Rice-Evans, 1997). However, single method is not recommended for the evaluation of the antioxidant activities of different plant extracts, due to their complex composition (Nuutila et al., 2003; Shahidi, 2008 and Kuate et al., 2011).
In the current research, the antioxidant activity of acetone, ethanol and water extracts of leaf, fruit and seed of *Elaeocarpus serratus* was assessed using the *in vitro* assays such as 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. Since free radicals are of different chemical entities, it is essential to test the plant extract against many free radicals to prove its antioxidant activity. Hence, a large number of *in vitro* methods were used for screening.

Similarly, the antioxidant activity of methanol extract of *Indigofera cassioides* was investigated (Senthil Kumar *et al.*, 2012) using various *in vitro* methods. The assays revealed potent antioxidant activity and free radical scavenging activity of the leaves of the plant. According to them, the potent antioxidant activity might be attributed to high phenolic and flavonoid content. Likewise, Badami *et al.* (2008) observed the powerful antioxidant activity of petroleum ether, chloroform, ethyl acetate and methanol extracts of *Enicostemma axillare* using many methods. Among the extracts, the petroleum ether, chloroform and ethyl acetate extracts exhibited potent activities. However, the efficacy of each extract differed against various free radicals depending on the specific assay methodology, reflecting the complexity of the mechanism and diversity of the chemical nature of phytoconstituents present.

5.2.1. Hydroxyl Radical (·OH) Scavenging Assay

Hydroxyl radical is the most deleterious and reactive among oxygen species and it bears the shortest half-life compared to other free radicals (Sundrarajan, 2006; Adesegun, 2009). It induces severe damage to the adjacent biomolecules (Gulcin, 2006; Awah *et al.*, 2010). Hydroxyl radical can also react with lipid, polypeptides, saccharides, nucleotides and organic acids especially thiamine and guanosine, thereby causing cell damage (Jiao *et al.*, 2005). The presence of antioxidants could block the initiation of hydroxyl radical formation and break free radical chain reaction (Nabushi and Uchikura, 2010).

Jayashree, (2011) investigated the hydroxyl radical scavenging activity of acetone, methanol and water extracts of leaf, stem bark and fruit of *Elaeocarpus*
*E. tuberculatus* had efficient in quenching the hydroxyl radicals. The water extract of leaf and methanol, acetone and water extracts of stem bark of *E. tuberculatus* exhibited moderate \( \cdot \text{OH} \) scavenging activity with IC\(_{50}\) values of 18.20 ± 1.37, 18.75 ± 1.88, 19.65 ± 2.23 and 20.55 ± 1.42\( \mu \)g/ml, respectively. Similarly, Satish Kumar *et al.* (2008) confirmed that the ethanolic extract of leaf of *Elaeocarpus ganitrus* showed only moderate hydroxyl radical scavenging activity. Only 13.43% inhibition was noted with 500\( \mu \)g/ml of *E. ganitrus*. In contrast, in the present study, the administration of ethanol extracts of leaf, fruit and seed to the reaction mixture significantly inhibited the hydroxyl radical activity. The maximum inhibition exhibited by the ethanolic extract of fruit (IC\(_{50}\) = 24.08 ± 0.27\( \mu \)g/ml) and leaf (IC\(_{50}\) = 24.30 ± 0.19\( \mu \)g/ml) which were comparable with standards BHA (7.97 ± 1.07\( \mu \)g/ml) and gallic acid (2.7 ± 1.35\( \mu \)g/ml). Hence, the ethanol extracts of leaf, fruit and seed of *E. serratus* were potent scavengers of \( \cdot \text{OH} \) species than the other solvents used.

Similarly the ethanolic extract of seed of *Xylopia parviflora* and catechin was significantly more effective than hydroethanolic and water extracts, respectively. Generally, all extracts possess some antioxidant activity, with the ethanolic extracts being more effective than catechin and hydroethanolic extract in scavenging free radicals and ROS (Kuate *et al.*, 2011). The results of Samuel *et al.* (2012) explained that the hydroxyl scavenging activity of ethyl acetate extract of *Ageratum houstonianum* leaves was found to be high (71.99 ± 1.10%) at 500\( \mu \)g/ml, where as in hexane and methanol extracts of *A. houstonianum* the highest activity was found to be 64.61 ± 0.52 and 68.29 ± 0.91% inhibition respectively, at 500 \( \mu \)g/ml. The IC\(_{50}\) values of the extracts of *Millettia pulchra* and *Pitosporum moluccanum* displayed hydroxyl scavenging activities as 0.16-0.67\( \mu \)g/ml (Lee *et al.*, 2005). Certain biologically active agents containing phenolic rings have the capacity to scavenging hydroxyl radicals by virtue of aromatic hydroxylation at the orthoposition, and it is shown to have a protective effect on ischemia-induced cerebral neuron damage (Lipinski, 2011).

The extract of *Eclipta alba* exhibited dose-dependent inhibition of oxidation of dimethyl sulfoxide indicating hydroxyl radical scavenging activity up to 22.11% at the concentration of 100\( \mu \)g/ml (Majumdar *et al.*, 2008). These results are in
agreement with the statement of Opoku et al. (2002); Mahakunakorn et al. (2004) and Katalinic et al. (2006). The scavenging ability of *Solanum surattense* leaf extract exhibited more pronounced hydroxyl radical scavenging activity compared to α-tocopherol in a dose-dependent manner. In hydroxyl radical scavenging assay the IC$_{50}$ value of the extract was 154.03mg/ml (Sridevi et al., 2013). Phytochemical studies of the plant leaf extract revealed various bioactive compounds which may be acting synergistically. The phenolic compounds have direct antioxidative activity due to their hydroxyl groups (Patel et al., 2011; Thirumali et al., 2011).

### 5.2.2. Superoxide Radical (O$_2^{−}$) Scavenging Assay

Superoxide oxygen centered anion radical is known to be a very harmful species to cellular components as a precursor of more reactive species (Halliwell and Guttridge, 2007). It is formed from mitochondrion electron transport system. Further endogenously, superoxides could be produced in large amounts by various metabolic and physiological processes (Blaszczyk et al., 1994; Bedard et al., 2001 and Govindarajan et al., 2003). This species is produced by a number of enzyme systems in auto-oxidation reaction and by non-enzymatic electron transfers (Gulcin et al., 2005). Formation of superoxide radical leads to a cascade formation of other reactive oxygen species in cell such as hydrogen peroxide, hydroxyl radical or singlet oxygen in living system (Lee et al., 2004). Superoxide scavenging ability of plant extracts might primarily be due to the presence of flavonoids, alkaloids and phenolics (Zheng and Wang, 2001; Kosanic and Rankovie, 2011).

In the present study, superoxide radical scavenging activity of ethanolic extract of seed (IC$_{50}$ value of 41.56±0.18µg/ml) and acetone extracts of leaf (IC$_{50}$=59.52±0.79µg/ml) compared with standard gallic acid (IC$_{50}$=38.27± 0.28µg/ml) suggest that the plant extracts might serve as potent scavenging of free radicals. Therefore, studying the scavenging effects of *E. serratus* on superoxide radicals is one of the most important ways clarifying the mechanism of antioxidant activity. Likewise, *Elaeocarpus tuberculatus* had strong superoxide radical scavenging activity (Jayashree, 2011). Inhibition of superoxide radicals was proportional to the amount of the extracts added. The superoxide radical scavenging potential was higher in *E.tuberculatus*. The acetone and methanol extracts of leaf and methanol extract of
fruit of *E. tuberculatus* exhibited higher superoxide radical scavenging activity ($IC_{50} = 21.81\pm0.67, 25.92\pm1.40$ and $29.17\pm 6.01\mu g/ml$, respectively) than the standard gallic acid ($IC_{50} = 38.27 \pm 0.28\mu g/ml$).

Similarly, Kuate *et al.* (2011) studied the effects of water, hydroethanolic and ethanol extracts of seed of *Xylopia parviflora* and *catechin* on superoxide radical scavenging activity. However, the highest scavenging ability was exhibited at the dose 1mg/ml by the hydroethanolic extract followed by the ethanolic extract and *catechin*. The water extract had the lowest scavenging activity. Panda *et al.* (2011) concluded that the ethanol extract from the aerial parts of *Cocculus hirsutus* significantly inhibited the production of superoxide anion radicals which can be comparable to the standards. These findings are in agreement with the present conclusions. The leaf extract of *Mojarana hortensis* were tested for their scavenging effect on the *in vitro* generation of $O_2^-·$ radical (Radha and Padma, 2012). The results showed that the methanolic extract of *M. hortensis* exhibited highest superoxide radical scavenging activity than other extracts studied. In contrast, the present study exposed the highest superoxide radical scavenging activity of the ethanolic extract of *E. serratus* in comparison with the other extracts studied. Sreelatha and Padma (2009) demonstrated that the extracts of both mature and tender leaves of *Moringa oleifera* had very good superoxide scavenging activity.

On a similar note (Sridevi *et al.*, 2013) studied the scavenging effect of *Solanum surattence*, on the inhibition of superoxide and found that it was in a concentration-dependent manner. The percentage of inhibition was greater than ascorbic acid standard and at all concentrations studied. The $IC_{50}$ value of the extract was 145.22$\mu g/ml$. The inhibition of superoxide generation by *S. surattence* may be due to the presence of phytochemicals such as flavonoids, alkaloids and phenolics (Kosanic and Rankovic, 2011).

### 5.2.3. Nitric Oxide (NO$^-$) Scavenging Assay

Nitric oxide (NO$^-$) is a reactive free radical generated from sodium nitroprusside in aqueous solution at physiological pH and reacts with oxygen to form nitrite. It is well known that nitric oxide play an important role in various
inflammatory processes such as carcinomas, juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis (Hazra et al., 2008). Nitric oxide is a free radical product in animal cells involved in the regulation of various physiological process. However excess production of nitric oxide radical potentially toxic associated with several diseases (Ialenti et al., 1993; Yabuki et al., 1999).

In the current study, the highest nitric oxide scavenging activity was noticed in the acetone extract of seed (IC$_{50}$=36.23 ± 0.26µg/ml) followed by the ethanol extract of leaf (IC$_{50}$=45.94±0.16µg/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). This might be due to antioxidant principle present in the extracts which competes oxygen to react with nitric oxide there by inhibiting the generation of nitrite. Therefore, E. serratus may have the property to counteract the effect of NO’ formation and in turn, may be of considerable interest in preventing the ill effects of excessive NO’ generation in vivo.

In connection with the present result Kim et al., (1998) found that Labiatae family exhibited strong suppressing activity upon NO’ production and thus provided further convincing evidence to illustrate, at least partially, as anti-inflammation, anti-cancer or antioxidant. It seems that the extracts do not only exert NO’-suppressing effect through direct scavenging of NO’ radicals but also through inhibition of NOS catalytic activity and suppression of iNOS expression (Sheu et al., 2001). The inhibition of nitric oxide radicals by the E. serratus leaf and fruit extracts may offer scientific evidence to treat inflammatory diseases.

The observation corroborated with the report of Jayashree, (2011) all the plant parts of E. tuberculatus in the various solvent extracts showed strong activities on NO’ scavenging with the IC$_{50}$ value of extracts lesser than that of the standards BHA (43.37 ± 1.26µg/ml) and gallic acid (29.76 ± 0.81µg/ml). The acetone, methanol and water extracts of the leaf and stem bark showed higher activities. Further, Kuate et al. (2011) evaluated the NO’ scavenging activity of ethanol, hydroethanolic and water extracts of seed of Xylopia parviflora and catechin. Overall, the ethanol and hydroethanolic extracts showed the highest NO’ scavenging ability compared to the water extract and catechin. Nitric oxide assays was adopted by Deepa et al. (2009) to
complete the antioxidant effect of the ethanolic extract of the leaves of *Commiphora caudate* and *C. varpubescens*.

The water extracts of leaf, fruit and seed of *E. serratus* presented moderate activity in scavenging the nitric oxide radicals. On a similar note, Oyedemi *et al.* (2010) revealed that the aqueous bark extract of *Strychnos henningsii* displayed moderate activity in scavenging nitric oxide radical by directly competing with oxygen, nitric oxide and its derivative (Marcocci *et al.*, 1994).

### 5.2.4. ABTS•⁺ Radical Scavenging Assay

ABTS•⁺ is stable free radical bluish in colour. The antioxidant assay is based on the reduction of ABTS solution by plant extracts. ABTS radical scavenging activity is relatively recent one, which involves a more drastic radical, chemically produced and is often used for screening complex antioxidant mixtures such as plant extracts and biological fluids. The ability in both the organic and aqueous media and the stability in a wide pH range raised the interest in the use of ABTS for the estimation of antioxidant activity (Wang *et al.*, 1998; Yu *et al.*, 2002).

In the current research, *E. serratus* extracts 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). Similar trend was noticed in *E. tuberculatus* extracts with maximum activity seen in the methanol extract of stem bark (19960.6 ± 28.9µmol/g) (Jayashree, 2011). According to Satish Kumar *et al.* (2008) the ethanolic extract of leaf of *Elaeocarpus ganitrus* displayed 55.77% inhibition of ABTS•⁺ at 500µg/ml. IC₅₀ value was found to be 297.12µg/ml *E. ganitrus*. This is in agreement with the present study.

Further, Kuate *et al.* (2011) analysed the antioxidant activity of ethanol, hydroethanolic and water extracts of seeds of *Xylopia parviflora* using ABTS•⁺ scavenging assay. However, their results showed that the hydroethanolic extract was the best scavenger of ABTS•⁺ followed by the ethanol extract. However, the ABTS•⁺ method, and all the four extracts namely, petroleum ether, chloroform, ethyl acetate and methanol extracts of whole plant of *Enicostemma axillare* showed potent antioxidant activity with IC₅₀ values ranging from 13.26±24.36mg/ml
The extracts from *Felicia muricata* leaf was fast and effective scavengers of ABTS$^{••}$ radical. The percentage inhibition was 94.55%, 99.21%, 98.66%, 97.27% and 99.27% in water, methanol, acetone, ethanol and BHT respectively at 0.05mg/ml, the highest concentrations tested (Ashafa *et al*., 2010).

Additionally, *Leonotis leonorus* extract was an effective scavenger of ABTS radicals. The ABTS radical scavenging activity of the extract was comparable to that of BHT and rutin in a concentration-dependent manner. At 0.8mg/ml plant extract, BHT and rutin scavenged ABTS radicals by 78.02, 89.86 and 91.1%, respectively (Oyedemi and Afolayan, 2011). The presence of polyphenolics compounds could be responsible for this observation which has been reported of oxidizing the proton radical generated within the system (Mathew and Abraham, 2006). This result showed similar trend with that of Adedapo *et al*. (2008) on methanol stem extracts of *Acokanthera oppositifolia* and *Adenia gummifera*. The *Indigofera cassioides* extract showed potent antioxidant activity in ABTS method which is comparable to the standard used. Here the extract’s radical scavenging activity showed a direct role of its phenolic compound in free radical scavenging Senthil Kumar *et al*. (2012). All these findings are in corroboration with the present study.

### 5.2.5. β-carotene / Linoleic Acid Peroxidation Inhibition Assay

The mechanism of bleaching of β-carotene is a free radical mediated phenomenon resulting from the hydro peroxides formed from linoleic acid. β-carotene, in this model system, undergoes rapid discoloration in the absence of an antioxidant. The linoleic acid free radical, formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups, attacks the highly unsaturated β-carotene molecules. As β-carotene molecules lose their double bonds by oxidation, the compound loses its chromophore and characteristic orange colour, which can be monitored spectrophotometrically. The presence of different extracts can hinder the extent of β-carotene bleaching by neutralizing the linoleate-free radicals and other free radical formed in the system (Jayaprakasha *et al*., 2001).

In the present research, all the solvent extracts of leaf, fruit and seed of *Elaeocarpus serratus* showed above 60% inhibition of β-carotene bleaching activity.
Maximum inhibition of lipid peroxidation was shown by the ethanol and water extracts of leaf (79.43±5.27 and 78.33±9.67%, respectively). Similarly, in β-carotene bleaching assay, the various solvent extracts of leaf, stem bark and fruit of *Elaeocarpus tuberculatus* showed moderate to high antioxidant capacity. The acetone extract of fruit and leaf of *E. tuberculatus* showed considerably strong antioxidant response with an inhibition of 89.50±5.41 and 87.91±3.92%, respectively which was comparable with that of the standard BHA and gallic acid (91.37±2.2 and 94.11±1.5%, respectively) (Jayashree *et al*., 2011).

Further, Preethi *et al.* (2010) studied the inhibitory effect of petroleum ether, chloroform, ethyl acetate, butanol and methanol extracts of fruits of *Muntingia calabura* (Elaeocarpaceae) on lipid peroxidation. The methanol (IC$_{50} =110.4 \pm 0.64\mu g/ml$) extract was able to inhibit lipid peroxidation efficiently. The ethyl acetate and petroleum ether extracts exhibited moderate lipid peroxidation inhibitory activity with IC$_{50}$ values of 190.2±0.62μg/ml and 240.2± 0.04μg/ml, respectively. Chloroform and butanol extracts showed minimum activity (IC$_{50} = 490.23 \pm 0.24\mu g/ml$ and 540.1 ± 0.02μg/ml, respectively).

In a similar study, Badami *et al.*(2008) showed that in *Enicostemma axillare*, the petroleum ether (IC$_{50}$ value 100.00±1.52mg/ml) and chloroform extracts (IC$_{50} = 94.66 \pm 2.40 \text{ mg/mL}$) exhibited more potent or comparable antioxidant activity to (BHA IC$_{50} =112.66\pm 1.32 \text{ mg/mL}$) standards used. Dubey and Batra, (2009) showed that the ethanol extract of the *Thuja occidentalis* inhibited lipid peroxidation in a dose-dependent manner. The extract at 300μg/ml exhibited maximum inhibition (61.516±0.131%) of lipid peroxidation nearly equal to the inhibition produced by vitamin C. The IC$_{50}$ value was found to be 195.60μg/ml. In the lipid peroxidation assay the methanolic extract of *Crataeva magna* showed good effectiveness and inhibition values of 25.27±1.1% at 100mg/ml (Sridhar *et al*., 2012).

Correspondingly, Gezer *et al.* (2006) found that inhibition values of both *Ramaria flava* ethanol extract and the standards (BHA and α-tocopherol) increased with concentration in β-carotene/linoleic acid system. For example, in 80μg/ml concentration, *R. flava* extract, BHA and α-tocopherol showed 73.3, 96.4 and 98.6% of inhibition, respectively, whereas in 160μg/ml concentration the values were
94.7, 98.9 and 99.2% of inhibition, respectively. Therefore the high inhibition value of *R. flava* extract was due to the high concentration of phenolic compounds. This was in accordance with the present investigation. Aktumsek *et al.* (2012) evaluated the *in vitro* antioxidant activity of methanol extracts of the root and aerial parts of *Glycyrrhiza echinata* using β-carotene/linoleic acid bleaching assay. In term of β-carotene bleaching effect, those samples exhibited the following order:BHT>BHA>aerial >root. Aerial and root extracts exhibited 79.84 and 74.28 % inhibition activity.

Additionally, the aqueous extract of ethanol, acetone extracting two different parts of *A. nilotica* showed peroxidation inhibiting activity. At the concentration of 0.5mg in reaction mixture, peroxidation inhibiting percentage of both ethanol and acetone extracts of leaf sample comparable to each other (25.72% and 25.86%) whereas acetone extract of bark 30.39% is found to be higher than ethanol extract (21.11%). However the highest antioxidant activity was observed for α-tocopherol in linoleic acid emulsion system (Shyamala Gowri *et al.*, 2011). Similar results have also been observed in different solvent extracts of leaf and stem of *Cassia fistula* (Siddhuraju *et al.*, 2002). The results of the present investigation are in agreement with the observations of the above previous works.

From the result obtained in the present study, the antioxidant activity of *in vitro* assays tested, indicate that the *E. serratus* extracts are sufficient source of natural antioxidant which might the helpful in preventing the process of various oxidative stresses. Radical scavenging may protect tissues from free radicals, thereby preventing diseases. Even though it is unclear whether active constituents in plant extracts, such as those from *E. serratus* parts are active against free radicals after being absorbed and metabolized cells in the body, the radical scavenging assay were gained acceptance for their capacity to rapidly screen materials of interest. Therefore it can be recommended for the *in vivo* pharmacological activities based on the antioxidative activities like anti-arthritis, cardio protective and anti-diabetic.

**5.3. Evaluation of Pharmacological Activity of *E. serratus***

Pharmacology, a biomedical science, deals with the research, discovery, and characterization of chemicals which show biological effects and the elucidation of
cellular and organismal function in relation to these chemicals (Vallance and Smart, 2006). This field encompasses drug composition and properties, synthesis and drug design, molecular and cellular mechanisms, organ or organ system mechanisms, signal transduction or cellular communication, molecular diagnostics, interactions, toxicology, chemical biology, therapy, and medical applications and anti-pathogenic capabilities.

Plants have been used in treating human diseases for thousands of years, as reflected by the aphorism famous German-Swiss physician Paracelsus, “The art of healing comes from nature and not from the physician. Therefore, the physician must start from nature with an open mind”. The medicinal qualities of plants are of course due to chemicals. Plants synthesize many compounds called primary metabolites that are critical to their existence and a dazzling array of additional components, called secondary metabolites, which play a vital role in combating diseases (Cox and Balick, 1994). These natural substances can promote health and alleviate illness and are proved to be non-toxic, relatively less expensive and globally competitive. Also many plant species have been investigated in the search for novel phytomedicines but generally there is still a demand to find more information concerning the medicinal potential of plant species as they are safe and also bioactive.

5.3.1. Toxicological Evaluation of Plant Extracts

In the present investigation, toxicological evaluation of ethanolic extracts of leaf, fruit and seed of *Elaeocarpus serratus* showed that the plant extracts were quite safe even at a high dose of 5000mg/kg b.w. per day p.o. and had no acute toxicity on albino rat. Correspondingly, Vijayakumar *et al.* (2012) reported the ethanolic extract of *Elaeocarpus sphericus* seed was not produced any mortality even at the highest dose (2500mg/kg, p.o.) after 3 days and found to be safe. Additionally, Juvekar *et al.* (2009) have revealed that aqueous extract of *Elaeocarpus ganitrus* did not show any behavior changes, toxic reaction or mortality. The extract was found to be safe at the dose of 2000mg/kg, p.o. Similarly, Sridhar *et al.* (2011) described the methanolic extract of leaf of *Muntingia calabura* showed that the maximum dose of 2000mg/kg, p.o. and had no toxicity and mortality study.
5.3.2. Evaluation of *In vitro* Anti-arthritic Activity of Plant Extracts

Denaturation of protein is one of the cause of rheumatoid arthritis was documented. Denaturation of protein is one of the causes of arthritis production of auto-antigen in certain arthritic diseases may be due to *in vivo* denaturation of proteins (Gutteridge, 1995; Kris-Etherton *et al*., 2004). The mechanism of denaturation probably involves alteration in electrostatic hydrogen, hydrophobic and disulphide bonding (Grant *et al*., 1970). From the result of present study it can be stated that all the extracts of *E. serratus* of various parts are capable of controlling the production of auto-antigen and thereby it inhibiting denaturation of protein and membrane lysis leading to arthritic activity.

Similarly, Sharavan *et al*. (2011) the methanolic extract of *Bacopa monniera* has showed significant activity at various concentrations (50, 100, 250, 500, 1000, 2000μg/ml) and its effect was compared with the standard drug diclofenac sodium. The maximum percentage inhibition of protein denaturation of *B. monniera* was observed as 90.34 ± 0.85% at 2000μg/ml. The presence of active principle such as steroids, flavonoids, alkaloids, bacosides and triterpenoids and related polyphenols may be responsible for the above activity (Deshpande and Jadhav, 2009; Ramalingam, 2010).

The methanolic extract of leaves of *Coldenia procumbens* showed more inhibition of protein denaturation (52.84%) at 250μg/ml and its effect was compared with the standard drug diclofenac sodium. Hence it can be stated that the methanolic extract was capable of controlling the production of auto-antigen and inhibits denaturation of protein in rheumatic diseases (Lavanya *et al*., 2010). Also, Satish Kumar and Vivek Kumar (2011) evaluated the crude methanolic leaves extract of *Asystasia dalzelliana* for its possible anti-arthritic activity by inhibition of protein denaturation method. Methanolic extract upon the column chromatography yielded five fractions named (AD-01, AD-02, AD-03, AD-04, and AD-05) and were screened for their anti-arthritic activity. Among the five fractions tested, AD-3 and AD-4 shown good anti-arthritic activity when compared with standard diclofenac sodium. The protein denaturation inhibition of AD-3 and AD-4 fraction was found to be 52.84 and 64.56%, respectively. Likewise, Santosh Kumar *et al*. (2013) evaluated the
in vitro anti-arthritic activity of the ethanolic flower extract of *Callicarpa macrophylla* using inhibition of protein denaturation model and human red blood cell membrane stabilization model. The ethanolic plant extract at different concentrations (50, 100, 200, 400, 800mg/ml) possessed significant anti-arthritic activity as compared to standard drug diclofenac sodium.

Proteinases have been implicated in arthritis reactions. Neutrophils are known to be rich source of proteinase which carries in their lysosomal granule many neutral serine proteinases. Leukocytes proteinases play an important role is the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors (Das and Chatterjee, 1995).

Fascinatingly, in the current study, the leaf and seed extracts of *Elaeocarpus serratus* had remarkable ability on the inhibition of protein denaturation and proteinase inhibition. *E. serratus* leaf extract showed the maximum inhibition of protein denaturation (68.32%) and maximum anti-proteinase activity (64.71%) at the concentration of 400μg/ml and followed by seed extract which showed the maximum inhibition of protein denaturation (62.13%) and anti-proteinase activity (59.34%) at the concentration of 400μg/ml.

Likewise, Lavanya *et al.* (2010) stated that the methanolic extract of *Anisomeles malabarica* was capable of controlling the production of auto-antigen and inhibited denaturation of protein in rheumatic disease. Similarly, Sakat *et al.* (2010) showed that the methanol extract of *Oxalis corniculata* exhibited significant anti-proteinase activity and inhibition of protein denaturation at different concentrations. Similar results were obtained by Mishra *et al.* (2011) According to them, *Glycyrrhiza glabra* (150mg/kg), *Boswellia serrata* (50mg/kg) and combined formulation containing both *G. glabra* and *B. serrata* (100mg/kg), exhibited significant anti-proteinase activity. After investigation of all parameters a better synergistic activity has been observed in the combined formulation containing both *G. glabra* and *B. serrata* (100mg/kg) at equal proportion than the individual one. Interestingly, Meena and Seema, (2011) evaluated the in vitro anti-arthritic activity of methanolic extract of leaves of *Centella asiatica*. The production of auto antigen in certain arthritic disease may be due to denaturation of protein, and proteinase action.
The maximum percentage inhibition of protein denaturation membrane stabilisation and proteinase inhibitory action were observed as 89.76%, 91.63% at 2000μg/ml respectively. This study reveals that methanol extracts are capable of controlling the production of auto antigen and inhibits denaturation of protein and proteinase action in rheumatic disease.

5.3.3. Evaluation of In vivo Anti-arthritic Activity of Plant Extracts

Rheumatoid arthritis (RA), a chronic, inflammatory, systemic auto-immune disease characterized by pain, swelling and stiffness is a major cause of morbidity of the working force throughout world. This has been called the ‘King of Human Miseries’ (Chatterjee and Pal, 1984). It involves the breakdown of cartilage. Cartilage normally protects a joint, allowing it to move smoothly. Cartilage also absorbs shock when pressure is placed on the joint, such as when walk. Without the normal amount of cartilage, the bones rub together, causing pain, swelling (inflammation), and stiffness (Pearson, 1956). It is characterized by both local and systemic inflammation with elevated plasma concentration of pro-inflammatory cytokines and acute phase proteins (Eric and Lawrence, 1996; Lam et al. 2004). RA progresses in three stages. The first stage is the swelling of the synovial lining, causing pain, warmth, stiffness, redness and swelling around the joint. Second is the rapid division and growth of cells, or pannus, which causes the synovium to thicken. In the third stage, the inflamed cells release enzymes that may digest bone and cartilage, often causing the involved joint to lose its shape and alignment, more pain and loss of movement (Atushi et al., 2005).

There are many common types of arthritis viz. rheumatoid arthritis, osteoarthritis, juvenile arthritis, psoriatic arthritis, reactive arthritis, infectious arthritis, etc. (Harish singh et al., 2010). The key risk factors of arthritis includes age, gender, excess weight, injury, dietary pattern, consumption of excess alcohol, life style, heredity, hormonal factors, environmental factors and lack of physical activity (Baranwal et al., 2012). Arthritis is caused by number of pro inflammatory molecules released by macrophages including reactive oxygen species and ecosanoids such as prostaglandins, leukotriines and cytokines (Tripathy et al., 2010). The mechanism of inflammation injury is attributed, in part, to release of reactive oxygen species from
activated neutrophil and macrophages. This over production leads to tissue injury by damaging the macromolecule and lipid peroxidation of membranes. Thus free radicals are important mediators that provoke or sustain inflammatory processes and consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation (Lavanya et al., 2010). Further, the endogenous formation of free radicals can contribute to the inflammatory process (Hernandez et al., 2010).

Conventional treatments for RA, including non-steroidal anti-inflammatory drugs (NSAID’s), disease modifying anti-rheumatoid drugs (DMARD’s) and corticosteroids, aim to reduce the patient’s pain and joint inflammation, minimize loss of function and decrease the progression of joint damage (Srikanth et al., 2012). Even though these conventional drugs are being used till now, the potential side effects give a limitation for their use (Shivanand, 2010). Therefore, a systematic approach should be made to find out the efficacy of plants against inflammation.

Plants are one of the most important sources of medicines. India is known as the “Emporium of Medicinal plants” due to availability of several thousands of medicinal plants in the different bioclimatic zones. The use of natural remedies for the treatment of inflammatory and painful conditions has a long history, starting with ayurveda treatment, and extending to the European and other systems of traditional medicines. Natural products serve as a ‘gold mine’ in the management of inflammatory diseases as they are effective, nontoxic and are considered being excellent candidates for arthritis therapy (Hemamalini et al. 2010).

A large number of medicinal plants have been used and tested and found to contain active principles and curative properties against arthritis. Numerous agents derived from plants can suppress these cell signaling intermediates, including phenols, coumarins, monoterpenes, essential oil, catechin, quinine, carotenoids, flavonoids, alkaloids, anthocyanin and xanthenes (Shah Biren et al., 2006; and Clavin et al., 2007), curcumin (turmeric), resveratrol (red grapes and peanuts), quercetin (onions), polyphenols (tea), guggulsterone (guggul), withanolides (ashwagandha) and genistein (soy) (Aggarwal et al., 2007).
A systematic approach has been made by several researchers to find out the efficacy of plants against arthritis. The anti-arthritic properties of *Rubia cordifolia* was attributed to the presence of anthraquinones such as rubiadin, munjistin and purpurin (Suzuki *et al*., 1984). Similarly, *Polisota hirsuta* exhibited potent anti-arthritic properties in the rat adjuvant-induced arthritis when administrated in combination with standard anti-rheumatic drugs. The plant extract able to suppress the joint inflammation and synovitis (Woode *et al*., 2009). It has been suggested that new therapeutic strategies for chronic forms of arthritis have to aim both suppression of inflammation and suppression of ultimate goals of a better arthritis treatment (Mottonen *et al*., 2006; Atzeni and Sarz-Putini, 2007; Capell *et al*., 2007). Begum and Sadique (1988) showed long term effect of *Withania sominfera* on adjuvant-induced arthritis in rats. More recently Rasool and Varalakshmi (2008) investigated the effect of *W. sominfera* root powder on paw volume in rats.

The *in vivo* studies suggest that curcumin of *Curcuma longa* oral administration has been shown to decrease the paw inflammation (Joe *et al*., 1997; Onodera *et al*., 2000; Liacini *et al*., 2003 and Funk *et al*., 2006). Recent studies indicate that resveratrol suppress the inflammatory gene products (Aggarwal *et al*., 2004; Elmali *et al*., 2005; and Tang *et al*., 2006). Fan *et al*., (2005) examined the effects of an acetone extract of *Bosewellia carterii* gum resin on adjuvant-induced arthritis in rats. The results showed that *B. carterii* extract had significant anti-arthritic and anti-inflammatory properties and suggest that there effects may be medicated via, the suppression of pro-inflammatory cytokines. The standard drug diclofenac and hydroalcoholic extract of *Lawsonia innermis* significantly suppressed the swelling of the paws and also decrease paw volume in both acute and chronic phase which may be due to the suppression of inflammatory medicated released due to induction of FCA. The mechanism of suppressing inflammation is due to antioxidant activity of presence of alkaloids and flavonoids (Kore *et al*., 2011). Similar trend of observation was made in plants like *Trigonella foenum-graecum* (Sharififara *et al*., 2009), *Rosa domascena* (Hajhashemia *et al*., 2010) and *Leucas aspera* (Baburao *et al*., 2010).

In the present study, rats were selected to induce arthritis because they develop a chronic swelling in multiple joints due to accumulation of inflammatory cells,
erosion of joint cartilage and bone destruction. The Freund’s complete adjuvant (FCA)-induced arthritis model in rat is the common model (Samy et al., 2006; Nandhinekannan and Limny et al., 2012). Freund’s Complete adjuvant (FCA) contains heat killed mycobacteria is a water-in-oil emulsion. After subcutaneous injection, FCA induces arthritis that can serve as a model to test the anti-arthritic and anti-inflammatory effects of investigational substances. The effects observed in this model seem to be parallel to that observed human diseases (Pearson and Wood, 1959; Newbould et al., 1963; Jia et al., 2003; Alluri et al., 2009). Due to inoculation of FCA, there was an increase in the ankle diameter where sings as an inflammation of ankle joint. The determination of swelling is apparently simple, sensitive and quick procedure for evaluating the degree of inflammation (Latha et al., 1998 and Paval et al., 2009). The increase in edema of hind paw after adjuvant infection in rat is paralleled by increased extra cellular activities of lysosomal enzymes. These enzymes are involved in the degradation of structural macromolecules in connective tissue and cartilage proteoglycans. They are also capable of destroying extra cellular activities by increased extra cellular activities of lysosomal enzymes. They are also capable of destroying extra cellular structures and may participate in mediating tissue injury in rheumatic diseases (Kesava Reddy and Dhar, 1988).

In the present findings, the anti-arthritic activity of ethanolic extracts of leaf and seed of Elaeocarpus serratus was estimated using the FCA-induced rat paw edema model. Both the plant extracts gave significant reduction (p< 0.05) of rat paw edema at all valuation times. High doses (400mg/kg p.o.) of ethanolic extracts of leaf and seed of E. serratus displayed profound anti-arthritic effect as compared to the control group. The significant ameliorative activity of the extracts of E. serratus and standard drug indomethacin detected in the present study may be due to inhibition of the mediators of inflammation. This study confirmed the efficacy of E. serratus extracts as an anti-arthritic agent and also scientifically justified the use of this plant as an anti-edematous agent in traditional medicine. In a corresponding study, Vijaya Kumar et al. (2012) elucidated the anti-arthritic effects of ethanolic extract of seed of Elaeocarpus sphaericus at a dose of 250mg/kg p.o. on FAC-induced rat. The ethanolic extract showed significant reduction in rat paw edema volume when compared to the standard drug prednisolone.
Equally, Manocha et al. (2011) reported that the methanolic extract of bark of Ficus bengalensis exhibited anti-arthritic activity in a dose-dependent manner. The extract suppressed the development of acute edema of rat paw by agar-induced and FCA-induced. In contrast, the methanolic extract of aerial part of Costus speciosus exhibited a significant anti-arthritic activity in a dose-dependent manner. The methanolic extracts at the dose of 400 and 800mg/kg showed 75.50% and 68.33% protection against paw edema, respectively (Pradeep et al., 2012). In addition to this, petroleum ether, acetone, water extracts bark of Machilus macrantha showed significantly inhibited the formation of the FCA-induced rat paw edema in acute and chronic arthritis (Cuman et al., 2001 and Linardi et al., 2002). The petroleum ether extract of Portulaca oleracea possessed potentially useful anti-arthritic activity (Jagan Rao et al., 2012). Reduction of aqueous extract of the fruits Piper longum significantly suppressed paw swelling from the third week onwards may be due to immunological protection rendered by the plant extracts (Yende et al., 2010).

Inhibition of paw edema and paw diameters observed in formaldehyde models may be due to the ability of the aqueous extract of leaves of Aegle marmelos to inhibit histamine, serotonin and the prostaglandin which are responsible for inflammation (Desai Nilesh et al., 2011). Earlier studies supported the above investigations (Fakata, 2004; Kumar et al., 2008 and Chitme and Patel, 2009; Dhanakhar and Ruhil, 2010). From Freund’s adjuvant-induced arthritis model, the percentage increase in paw volume from 7-21days after the drug administration (Sutharsingh et al., 2011). It was revealed that the reduction of paw volume of rats treated with chloroform and ethanolic extracts of aerial parts of Naravelia zeylenica and standard drugs prednisolone were moderately reduced as that of the control group of animals.

According to Kore et al. (2011) the hydroalcoholic leaf extract of Lawsonia innermis significantly suppressed the swelling of the paw and also decreased the paw volume in both acute and chronic phase which may be due to the suppression of inflammatory mediator released due to induction of Freund’s adjuvant. Alcoholic fraction of Cardiospermum halicabum (250mg/kg b.w. for 15 days) was treated on animals suffering from acute inflammation (hind paw edema). The plant significantly suppressed paw edema which suggested its anti-inflammatory action (Gopala et al., 2010).
Suspension of dried powdered leaves of *Vitex negundo* showed dose related inhibition of primary and secondary lesions induced by adjuvant (Vtpalendu *et al.*, 1999).

Methanolic extract of *Melastoma malabaricum* leaves are a rich source of flavonoids which inhibits inflammation (Sergeev *et al.*, 2006; Vikas Kumar *et al.*, 2012). The first indication of anti-arthritic effect of *M. malabaricum* was observed when the extract administrated (25, 50 and 100mg/kg) showed significant dose-dependent reduction in swelling of paw edema. As a number of disease-modifying anti-rheumatic drugs in monotherapy often have unexpected side effects, combined treatment at lower doses may be necessary in order to expand the margin between efficacy and toxicity (Hisadome *et al.*, 2004, Makinen *et al.*, 2007). Based on this premise, the effect of combined lower doses of *Palisota hirsuta* and methotrexate or dexamethasone on the progression of hind paw inflammation and joint destruction in rat was studied (Woode *et al.*, 2009). The leaf extract in combination with dexamethasone had strong inhibitory effect on arthritis in rat showing synergistic suppression of both the increase in hind paw volume and also joint destruction thus producing a better remission of adjuvant-induced arthritis than *P. hirsuta* leaf extract or dexamethasone alone.

Standard drug and aqueous extract of *Piper longum* significantly suppressed the swelling of the paws. Reduction of paw swelling may be due to immunological protection rendered by the plant extract. Piperine was reported to display anti-inflammatory activity (Parmar *et al.*, 1997). Harpalani *et al.* (2011) studied the FCA-induced arthritis revealed the significant prevention of injected paw inflammation by aqueous extract of *Euphorbia antiquorum* 400mg/kg on 12th and 16th day and that by ethanolic extract of *E. antiquorum* at the dose of 400mg/kg.

Further, Purushoth Prabhu *et al.* (2012) showed the ethanolic extract of whole plant of *Merremia emarginata* significantly inhibited the development of joint swelling when compared to the standard drug methotrexate (5mg/kg) at the dose of 400 mg/kg p.o. Likewise, Vishal and Chandrashekhar (2012) scientifically validated that the ethanolic extract of *Asystasia dalzelliana* leaves at the dose of 800mg/kg possessed a significant anti-arthritic activity than the lower doses of 200mg/kg and
400mg/kg. The observed anti-arthritis activity of extract may be due to the presence of phytoconstituents such as alkaloid and flavonoids.

Free radical stress has been implicated in the etiopathology of many human diseases such as arthritis, atherosclerosis, diabetes, neurodegenerative disorders and aging (Satlisha et al., 2011). Free radicals are highly reactive molecules derived from the metabolism of oxygen (Temraz and EL-Tantawy, 2008). Some of them play a positive role in biochemical (energy production), immunological (phagocytosis) and physiological (regulation of cell growth and intercellular signaling) processes. However, when they are produced in excess and cannot be destroyed, their accumulation in the body generates a phenomenon called oxidative stress. Inability to destroy or remove excess free radicals has been attributed to many reasons such as decreases in antioxidant endogenous enzymes (superoxide dismutase, glutathione peroxidase and catalase) synthesis or activities and reduction in non-enzymatic protection (α-tocopherol, ascorbic acid, β-carotene, and uric acid) (Lien et al., 2008). Excessive free radicals that are generated are capable of reacting with unsaturated lipids thereby initiating self-perpetuating chain reactions of lipid peroxidation in the membranes (Salvemini and Cuzzocrea, 2003). Free radicals also known as reacting oxygen species can also cause oxidation of sulphhydryl groups in proteins and strand scission in nucleic acids (Kaul et al., 1993). Research evidence has showed that a potent scavenger of these free radical species may serve as a possible preventive intervention for free radical mediated diseases (Alluri et al., 2009).

Reactive oxygen species are highly reactive transient chemical species (nitric oxide, superoxide anion, hydrogen peroxide and hydroxyl radical) with the potential to initiate cellular damage (to proteins, lipids, etc.) to joint tissues especially in rheumatoid arthritis (Rasool and Varalakshmi, 2007; Choi, 2007). The damaging effect of oxygen free radicals and the accompanying lipid peroxidation in vivo plays a very important role in mediating pathological processes (Gutteridge et al., 1979). Oxygen-derived free radicals like superoxide (O$_2^-$) hydrogen peroxide (H$_2$O$_2$) and hydroxyl radicals (‘OH) formed in all aerobic cells have deleterious effect in inflammatory conditions like arthritis.
The antioxidant marker enzymes such as CAT, SOD, GST, GPx and GSH which are the first line of cellular defense against oxidative injury, decomposing O$_2$ and H$_2$O$_2$ before they interact to form more reactive (‘OH) radicals. SOD is a class of metal-containing proteins, catalysing the dismutation of superoxide radical anions into H$_2$O$_2$ and molecular oxygen (Scandalios, 2001).

SOD may play an important role in protecting cells against reactive oxygen species. Increased Super Oxide Dismutase (SOD) activity observed in arthritic rats appears to be reflux mechanism to guard against extracellular oxygen free radicals. Increased production of NADPH from hexose monophosphate shunt during arthritis may cause an increase in SOD activity (Marklund et al., 1987). SOD mainly acts by quenching of superoxide and active oxygen free radical, produced in different aerobic metabolism (Mac Millan-Crow et al., 1998). GPx protects the cell against cell damage resulting from the increased level of peroxides. Liver has been reported to be a major site of lipid peroxide metabolism. Lipid peroxides are metabolized in the liver by GPx (Kasama et al., 1988). This may be the reason for the absence of noticeable change in liver lipid peroxides in arthritis.

The main role of catalase (CAT) is to detoxify H$_2$O$_2$. CAT is an enzymatic antioxidant widely distributed in all animal tissues and its activity is higher in liver and erythrocytes. In rheumatoid arthritis, its concentration is very low, to expect considerable protection against H$_2$O$_2$ (Blake et al., 1981; Geetha et al., 1998). Glutathione is endogenously synthesized in the liver and is the first line of defense against peroxidation. In arthritic rats, liver glutathione was not affected. This might be due to the fact that liver glutathione is utilized and resynthesized with great rapidity. The observed non-enzymic antioxidants depression in adjuvant-induced arthritis is associated with the protracted inflammatory phase of the disease. GSH has an important function in maintaining cellular redox status (Rennenberg, 1980).

Lipid peroxide (LPO) used as biomarker to show the index of oxidative stress, and causes cell membrane fluidity, decreased the membrane potential and increased permeability to ions (Halliwell and Gutteridge, 1989; Kim et al., 2005). This report is in harmony with the study made by Tabassum et al. (2010) for Ocimum sanctum, Camellia sinensis and Striynchos nuxvomica (Chitra et al., 2011). In view of the
above conclusions, the present study supported that the administration of ethanolic extracts of leaf and seed of *E. serratus* showed significant increase in the activities of antioxidant enzymes (SOD, CAT, GPx, GST). However, the level of LPO was significantly decreased. The earlier study also supported by liver lipid peroxide levels were found to be decreased in arthritic conditions of *Withania somnifera* root. (Rasool and Varalakshmi, 2007).

Olorunnisola *et al.* (2012) observed an elevation of antioxidant defense enzymes in the rats treated with the methanolic extract of rhizomes of *Tulbaghia violacea*. Since the synovial fluid has lesser activities of SOD and CAT, the oxygen-derived species are not scavenged and react with the joint components causing significant damage (Wong *et al*., 1981). The scavenging enzymes present in the circulating system are utilized at higher levels there by showing a reduced activity in arthritis. SOD and CAT are major enzymes involved in detoxification of reactive oxygen species in most cells (Sivaraj *et al*., 2011). While the observed dose-dependent increase in antioxidant status in the extract treated groups may be due to enhancement of antioxidant enzymes synthesis by acting in the antioxidant response element in the enhance region at the promotor site of the gene that codes for the enzymes (Ayoola *et al*., 2001).

On treatment with methanolic extracts of *Mukia maderaspatana*, the activities of SOD, CAT, GPx and GST were brought to near normal levels, which may be attributed to the free radical scavenging activity of phytochemicals present in the drugs (Sanz *et al*., 1984). This fact was on par with the results of the present study. Chang *et al.* (2011) also observed that the status of antioxidant level in the serum of carageenan-induced rat was brought to normal when it was pretreated with ethanolic extract of *Phellinus linteis*.

Hydroalcoholic extract of aerial parts of *Leucas aspera* played a significant role to maintaining the oxidative homeostasis as in manifested by increase in GSH along with increased activity of SOD, GPx and CAT, indicating its promising role as an antioxidant (Kirpa *et al*., 2010). This is in concord with earlier studies done on other plant extracts (Ramprasath *et al*., 2005; Ismail *et al*., 2008). Similarly, Methanolic fraction methanolic extract of *Wedelia calendulacea* shows significant
anti-arthritic activity in FCA-induced arthritic animals. Enhanced oxidative stress in terms of measured by the enzymes SOD, CAT and LPO were observed in arthritic control and methotrexate treated group (Panchal et al., 2011). Earlier studies also support the above findings (Agarwal and Rangari et al., 2003; Rajkapoor et al., 2009).

The lupeol isolated from Russelia equisetiformis extract demonstrated its anti-arthritic action by reducing the alterations induced arthritis animals in the levels of lipid peroxide, SOD, GPx and CAT. The hexane extract of R. equisetiformis possessed anti-inflammatory activity against inflammatory disease conditions (Olorunju et al., 2012). Lupeol and its esters have been reported to reduce the level of enzymes (SOD, GPx and CAT) involved arthritic-induced animals (Agarwal and Rangari, 2003).

This finding justifies the preclinical efficacy and safety data, the E. serratus leaf and seed ethanolic extracts could be considered as safe and effective intervention for arthritis. Etiopathogenesis of rheumatoid arthritis still remains obscure despite extensive research. Although the pathophysiology basis of rheumatoid arthritis is not yet fully understood, reactive oxygen species have been implicated in its pathogenesis. A further research is on underway with its active principles to identify the extract mechanism of action.

5.3.4. Evaluation of In vivo Cardio protective Activity of Plant Extracts

Cardio diseases have emerged as one of the foremost causes of death in both developed and developing countries and it is predicted by the year 2020. This disease will persist as the major and the most common threat to human life (Anonymous, 1997). Myocardial infarction (MI) usually results from an abrupt reduction in coronary blood flow to a segment of the myocardium, which initiates continuum of progressively more severe cellular changes that unless interrupted by early reperfusion, inevitably culminate in cell death and tissue necrosis (Hearse, 1998). There is substantial evidence that ischemic tissue generates oxygen-derived free radicals (Muruganandan et al., 2002). In this regard, animal experiments suggests that increase in free radical formation and subsequent oxidative stress associated with the occurrence of a relative deficit in the endogenous antioxidant reserve is one of the
mechanism for the development of congestive heart failure (Singal and Kirshenbaum, 1990; Das and Maulik, 1995). Damage to the myocardial cells arises due to the generation of toxic reactive oxygen species such as superoxide radical, hydrogen peroxide and hydroxyl radical (Vaage and Valen, 1993).

Isoprenaline or Isopronerol (Isoprenaline hydrochloride 1-(3,4-dihydroxyphenyl)-2-iso-propylaminoethanol) a synthetic catecholamine and a β-adrenergic agonist causes severe oxidative stress in the myocardium, resulting in infarct like necrosis of the heart muscles (Wexler, 1978). Catecholamine’s rapidly undergo auto-oxidation and has been suggested that the oxidative products of catecholamines are responsible for the changes in the myocardium (Yates and Dhala, 1975). The high concentration of isoperenol may be a causative factor for reversible damage in necrotic lesions to the myocardial membrane in experimental myocardial infarction (Kunfman et al., 1987; Senthil Kumar et al., 2001; Philhstrom et al., 2005; Manimegalai and Venkatalakshmi, 2012).

Naturally free radicals and reactive oxygen species have been implicated in cardiac diseases and metabolic disorders which result due to exposure of chemicals and environmental agents (Subhashini et al., 2001; Karthikeyan et al., 2007; Torabian et al., 2009; El-Sayed, 2011). Grimm et al. (1998) have reported that a toxic dosage of isoperenol caused characteristic myocardial damage that subsequently resulted in heart failure. A wide array of plants and its active principles, with minimal side effects, provide an alternate therapy for ischemic heart disease. Moreover, the plant kingdom represents a largely unexplored reservoir of biologically active compounds. Recently, the keen interest in medicinal plants for cardio protection has increased because of their numerous possible cardio protective mechanisms besides antioxidant activity (Ai and Bolling, 2002).

Cardiac marker enzymes like creatine kinase (CK-MB), aspartate transaminase (AST) and troponin I and T are proteins formed in cardiac tissue and found in blood, and an increased level of these enzymes are observed in myocardial infarction (Halliwell and Gutteridge, 1999; Trivedi et al., 2006; Velavan et al., 2009). Lactate dehydrogenase (LDH), AST and CK-MB were the original marker enzymes used to diagnose a heart attack. Currently, troponin I and T, as well as
membrane-bound creatine kinase (CK-MB) are the most commonly employed marker enzymes because of their relative specificity for cardiac tissue. Elevated level of troponin or CK-MB is indicative of cardiac injury (Priscilla and Prince, 2009; Jutila et al., 2011). By studying isoproterenol-induced myocardial infarction in rats is a widely employed experimental model and shows the biochemical alterations. It is possible to gain altered metabolic process in human myocardial infarction. The amount of these cellular enzymes present in blood reflects the alterations in plasma membrane integrity and permeability. Drug treatments such as naringin, silibinin and squalene evidenced by a decline in lactate dehydrogenase, glutamic oxalacetic transaminase and creatine kinase levels indicated their membrane stabilizing action (Rajadurai et al., 2006; Zhou et al., 2006). Phytomedicines having antioxidant properties may therefore, have protective role in cardiovascular diseases (Viswanatha et al., 2010). There is increasing trend towards the application of herbal medicines to treat the cardiovascular diseases (Nandave et al., 2007; Hina et al., 2010; Ojha et al., 2011). The present study demonstrated both important curative and preventive modes of cardio protective activity.

Lipids play an important role in cardiovascular diseases by modifying the composition, structure and stability of cellular membranes and lead to the development of hyperlipidemia. Increased oxidative stress and the generation of the free oxygen radicals can result in modification of LDL to oxidized LDL that could lead to atherosclerotic lesions. Also; inflammation occupies a very important central position in all phases of atherosclerosis, which is underlying cause of myocardial infarction (Libby, 2003).

In the current study, the cardio protective effects of ethanolic and pure extracts (without isoprenaline) of leaf and seed of Elaeocarpus serratus at low and high doses (200 and 400mg/kg b.w. p.o.) on isoprenaline-induced cardiac damage in rats. Administration of extracts showed a significant reduction in TC, TGL, LDL cholesterol and VLDL cholesterol and significant increase in high density lipoprotein (HDL) level when compared with isoprenaline-induced. Likewise, Juvekar et al. (2010) examined the cardio protective study of aqueous extract of Moringa oleifera stem bark of isoprenaline-induced cardiac damage in rats. Pretreatment with
*M. oleifera* at 500mg/kg showed significant decrease in HDL in ISO-induced animals. HDL is known to be involved in the transport of cholesterol from tissues to the liver for excretion into the bile and thus called “good cholesterol”. Thus the cholesterol lowering activity of *M. oleifera* could be mediated through increasing the activity of extra hepatic lipoprotein lipase which increased hydrolysis of triglycerides that result in the transfer of lipids and apolipoproteins to HDL and thereby facilitate their excretion.

Similarly, the ethanolic stem bark extract of *Mammea africana* produced significant decrease is the levels of total cholesterol, triglycerides, LDL and VLDL cholesterol of the extract-treated animal group which is a dose-dependent fashion. However, there was a significant increase in the levels of HDL cholesterol in a dose-dependent fashion when compared to control. Oxidation of LDL has been known to play a crucial role in atherogenesis or formation of atheroma (Okokon *et al.*, 2007). This finding corroboration with that of lowering of cholesterol levels in rats have been reported to be due to antioxidant activity of phytochemical constituents (Igarashi and Onhurma, 1995). Pretreatment with *Bixa orellana* ethanolic leaf extract and α-tocopherol in ISO-treated rats showed a significant reduction in the level of serum lipoproteins by offering protection against LDL oxidation thereby inhibiting the development of atherosclerosis (Paritha and Devi, 1997).

Similar trend was noticed by *Commiphora mukul* treatment reduced the levels of triglycerides and cholesterol in the isoproterenol-induced lipid peroxidation. The plant extract might improve the perfusion to sub-endocardium thereby, reducing the myocardial injury (Muthulakshmi *et al.*, 2012). Determination of LDH in heart is a useful parameter for assessing myocardial damage (Mair, 1997). Pretreatment with aqueous extract of *Sansevieria senegambica* on alloxan-treated rats showed no significant differences in the plasma total cholesterol levels of the animals. The plasma triglyceride and VLDL cholesterol levels of the test groups were not significantly different from the control, test control and reference groups. The plasma LDL cholesterol level of the plant extract treated was significantly lower than test control. The plasma HDL cholesterol level of extract-treated group was highest than test control (Catherine, 2010). The result correlates with the present study. In
E. serratus the crude leaf and seed extract administration increased the HDL cholesterol level and reduced the cardio vascular risk.

According to clinical data, increase in plasma HDL cholesterol concentration decreases cardio vascular risk (Assmann and Gotto, 2004; Rang et al., 2005). High HDL exerts a protective effect by decreasing the rate of entry of cholesterol into the cell via., LDL and increasing the rate of cholesterol release from the cell (Marcel et al., 1980) and inhibiting the oxidation of LDL as well as the atherogenic effects of oxidized LDL by virtue of its antioxidant property (Ademuyiwa et al., 2005; Brunzell et al., 2008). In addition Daher et al. (2006) found that aqueous extract of Urtica dioica (150mg/kg) could reduce TC and LDL/HDL cholesterol ratio. It was suggested that the extract may have a direct role in lipoprotein synthesis metabolism. Moringa oleifera stem bark of aqueous extract treatment showed the normalization of the activity of diagnostic marker enzymes (AST, CK and LDH, ALT) when compared with isoproterenol-treated rats indicating the antioxidant potential of plant extracts which protects heart from lipid peroxidative damage (Gunjal et al., 2010).

Also, flavonoids may directly scavenge some radical species and also help in uptake of oxidatively modified low density lipoprotein (LDL) through scavenger receptors. Scientific studies have revealed that quercetin an important flavonol suppressed the LDL oxidation (Burns et al., 2000; Edijala et al., 2005). Likewise, Meguro et al. (2001) has explained several mechanisms about the cholesterol lowering activity of plant sterol. It was reported that plant sterols which are structurally similar to cholesterol could displace cholesterol from mixed micelles, since they are more hydrophobic than cholesterol. This replacement causes a reduction of micellar cholesterol concentration and consequently lowers cholesterol absorption (El-Haouari et al., 2006).

Ability of Bixa orellana to reduce the levels of LDL, VLDL, triglycerides and increased HDL cholesterol has been justified by de Paula et al., 2009, which strengthened our claim in supporting cardio protective action of B. orellana ethanolic leaf extract. Conversely, decreased plasma HDL cholesterol is a risk factor for cardiovascular diseases (Brehm et al., 2004; Dobiásová, 2004; Lewis and Rader, 2005; Rang et al., 2005; Lichtennstein et al., 2006; Usoro et al., 2006; Martirosyan
Earlier, Nassiri et al. (2009) showed the effect of *Urtica dioica* ethanolic extract at 100 and 300mg/kg and lovastatin at 10mg/kg in reducing plasma TC and LDL cholesterol. Their results were statistically significant as compared with the untreated group. It seemed that the effects of *Urtica dioica* ethanolic leaf extract at 100mg/kg were greater than administration of 300mg/kg. This result was similar to previous findings by Avci et al. (2006). They found that ethanol extract of *U. dioica* at a dose of 100mg/kg greatly induced TC and LDL cholesterol as compared with aqueous extract.

Isoproterenol induction increases the biosynthesis of cholesterol with a concurrent decrease in its utilization. Free radicals are liberated in excess on induction with isoproterenol which might also be a reason for the accumulation of cholesterol in tissues. This may also lead to a decrease in the rate of ester hydrolysis of cholesterol and also reduces the efflux of cholesterol. Pretreatment with the *Cissus quadragularis* extract restored the level of cholesterol (Deepa and Varalakshmi, 2005). The increased phospholipids content in drug-induced rats may be due to greater degradation, due to the injury caused in the cardiac tissue. The phospholipids content was close to normal levels in the animals given treatment with *C. quadragularis*. Due to its membrane stabilizing activity, the plant extract might have induced myocytes to regenerate new phospholipids which was necessary to repair the damaged membrane (Upaganlawar et al., 2009). The plant extract treatment also maintained the HDL levels in serum and decreased the TG and cholesterol content, indicating that *C. quadragularis* could be used as a lipid lowering agent (Sharma et al., 2001).

In ISO-treated animals the lipid metabolism plays an important role in myocardial necrosis produced by ischemia. Vandana and Suresh (2009) reported the cardio protective activity of *Ocimum sanctum*, two effective doses (50 and 75mg/kg) were selected and combined with *Ginkgobiloba* 100mg/kg. The combination of two herbs with potent cardio protective and antioxidant activities was expected to have marked myocardial protective activity in ISO-induced cardiac necrosis. In previous studies *Ginkgo biloba* phytosome has shown promising cardio protective activity due to its antioxidant effects. The flavonoids of *Ginkgo biloba*, by scavenging free
radicals, inhibit lipid peroxidation and augment activities of endogenous antioxidants (Kleijnen and Knipschild, 1992; Naik et al., 2006; Panda and Naik, 2008). The cardio protective activity of Ocimum sanctum has been attributed largely to the antioxidant properties associated with its flavonoid and phenolic constituents, which are known to augment GSH and antioxidant enzyme levels and scavenge lipid peroxides (Uma Devi et al., 2001; Arya et al., 2006).

Isoproterenol is a well-known cardio toxic agent due to its ability to destruct myocardial cells. Due to this action, cytosolic enzymes such as LDH, AST, ALT and CK-MB are released into blood stream and serve as the diagnostic markers of myocardial tissue damage (Farvin et al., 2004; Gürgün et al., 2008). Enzymes are the best markers of tissue damage because of their specificity and catalytic activity to the tissue. The amount of these cellular enzymes present in blood reflects the alterations in plasma membrane integrity or permeability.

Myocardium contains an abundant concentration of diagnostic marker enzymes of myocardial infarction viz., CK-MB, LDH and alanine transaminases (AST, ALP, ALT) and once metabolically damaged, releases its content into the extra cellular fluid. Radhika et al., (2011) elucidated the cardio protective activity of Ethanolic extract of whole plant of Hybanthus Enneaspermus using isoproterenol-induced rats. Administration of extract (500mg/kg, p.o.) dose of H. enneaspermus reduced the oxidative stress by decreased lipid per oxidation and reduced glutathione (GSH) and also normalized the levels of cardiac marker enzymes such as CK-MB, LDH, SGOT, SGPT and cardiac specify protein troponin I in the blood of both normal and isoproterenol myocardial infarcted rats treated.

In the present findings, the treatment of (200 and 400mg/kg b.w.) leaf and seed Ethanolic extracts of E. serratus against to isoprenaline (ISO) demonstrated a decline in LDH, ALT, AST CK-MB level indicating their membrane stabilizing action. Correspondingly, the reduction in the levels could be due to its action maintaining membrane integrity thereby restricting the leakage of these enzymes. The increased level of these cellular enzymes in blood is due to leakage from heart as a result of ISO- induced necrosis (Trivedi et al., 2006 and Zhou et al., 2006).
Likewise, carotenoids have the ability to scavenge ROS and this antioxidant activity may contribute to the protection of membranes and lipoproteins from lipid peroxidation, thus, offering cardio protective action in myocardial infarcted rats (Stahl and Sies, 2003). *Bixa orellana* contains rich amounts of carotenoids which have greater oxygen-quenching potential and this might play a strong role in reducing the risk of cardiovascular disease (Di Mascio et al., 1989). Bixin present in *B. orellana* has the potential to inhibit lipid peroxidation and thus has the ability to prevent the development of cardiovascular disease offering cardio protection in rats (Silva et al., 2001). The present study confirms the results of several previous studies.

Equally, pretreatment with mangiferin, (from the leaves of *Mangifera indica*) (5,10 and 20mg/100gm b.w. daily) retained the activities of cardio marker enzymes to near normal levels in heart tissue as compared to isoproterenol- induced myocardial infarcted rats (Suchalatha and Shyamala Devi, 2004). Yoshikawa et al. (2002) mangiferin, a principal phenolic compound also has potent free radical scavenging activity and protective effect against altered changes in AST and ALT activities caused by toxicant. Agreeing to, pretreatment with ethanolic extract of *Momordica cymbalaria* at 250 and 500mg/kg prevented the elevation of serum marker enzymes, lactate dehydrogenase, transaminases, aspartate transaminase, alanine transaminase, alkaline phosphatase caused by isoproterenol (60mg/kg 2days)-induced myocardial infarction in rats (Waring et al., 2000).

Coordinating, Jahan et al. (2011) the cardio protective potential methanolic extract of bark of *Terminalia arjuna* in widely used ISO-induced model of myocardial infarction in rabbits. As a result of this myocardium destruction, cytosolic enzymes (LDH, AST, ALT and CK-MB) are released into blood and serve as diagnostic markers of myocardial tissue damage. Treatment of different groups of rabbits with extracts (200mg/kg b.w.) significantly blocked the ISO-induced secretion of all cardiac diagnostic marker enzymes (LDH, AST, ALT and CK-MB). The decline in enzymes levels could be due to potential of extracts for repairing and maintenance of the membrane due to antioxidant polyphenols, thereby preventing the secretion of enzymes. Pathophysiological changes including cell necrosis, contractile failure, ventricular arrhythmias and subcellular changes after ISO administration (85mg/kg)
are comparable to those taking place in human myocardial ischemia or infarction (Nandave et al., 2007; Panda and Niak, 2008; Ojha et al., 2011).

Matching with, Muruganandan et al. (2002) have reported that intraperitoneal administration of mangiferin (*Mangifera indica*) significantly reduce the activity of CK-MB and LDH in heart as well as ameliorates the oxidative stress. Similar to current findings Rajadurai et al. (2011) have shown significant decrease in cardiac markers such as CK-MB, LDH, AST and ALT in the heart of ISO-induced rats, which is consistent with earlier report (Kurian et al., 2005; Rajadurai and Stanely, 2006; Nigam, 2007).

These finding are in agreement with earlier reports (Karthikeyan et al., 2007) ISO-treated rabbits showed significant increase in the levels of diagnostic marker enzymes including CK-MB, LDH, AST, and ALT at the dose of 85mg/kg. The high levels of enzymes are an indicator of the severity of ISO-induced myocardial cell necrosis. The myocardial cell necrosis can be due to increase in lipid peroxidation. Ramadoss et al. (2012) investigated the ethanolic extract of whole plant of *Sida rhombifolia* at the dose of (100 and 200mg/kg p.o) produced significant reduction of CK-MB, ALT, AST and LDH enzymes in blood serum of isoproterenol- induced rats. The plant extract of *S. rhombifolia* has been reported to possess phenolic compounds and flavonoids which exhibit lipid peroxidation, antioxidant and free radical scavenging properties. The scavenging for oxygen free radicals, resulting in the preservation of cellular viability serving, secondarily, to preserve cardiac cells and thereby retaining near normal functioning of the cardiac cell thus preventing myocardial necrosis.

Pretreatment with *Daucus carota* (250 and 500mg/kg p.o) extract showed significant increase in lactate dehydrogenase level, when compared to ISO-treated groups (Prabhu et al., 2006). Alike, Radhika et al. (2012) observed that the ISO-induced rats showed myocardial necrosis which is confirmed by biochemical markers (AST, ALT, CK and LDH) and the pretreatment with *Cissus quadrangularis* showed normalization of marker enzymes due to antioxidant potential. Analogous, to treatment with the ethanolic extract of *Zingiber officinale* (200mg/kg) in ISO-treated
rats showed a near normal activity of the diagnostic marker enzymes LDH and CK, in the serum (Sheela and Shyamala Devi, 2000).

Similarly, Jennings et al. (1990) pretreatment of Withania somnifera at 50mg/kg dose on isoproterenol (85mg/kg)-induced myocardial rats showed a significant decrease in creatinine kinase and lactate dehydrogenase levels. These findings might be rational to understand the cardio protective effect due to the presence of multiple chemical constituents in the methanolic extract of Syzygium cumini seeds is probably related to its ability to strengthen the myocardial membrane by its membrane-stabilising action. However, this study warrants the investigation to isolate and identify the active principles and to elucidate the exact mechanism of action.

5.3.5. Evaluation of Anti-diabetic activity of E. serratus

Diabetes mellitus is a serious disease of disordered metabolism of carbohydrate, protein and fat which is caused by the complete or relative insufficiency of insulin secretion and insulin action (Balkau et al., 2000; Meetoo et al., 2007) or both (Kaleem et al., 2008). This disease is major degenerative ailment in the world today, affect at least 15 million people (Sharma and Kumar, 2010). Globally, the estimated incidence of diabetes and projection for the year 2030, as given by International Diabetics Federation (IDF) is 350 million against 191 million estimated in 2000 (Wild et al., 2004; Menaka et al., 2010; Whiting et al., 2011). Diabetes affects mainly the developing countries like India. Diabetes mellitus is epidemic in India as a result of societal influence and changing lifestyles. Diabetes has been known in India for centuries as ‘a disease of rich man’ but now spread among all masses (Gupta and Misra, 2007). Indeed, India presently has the largest number of diabetic patients in the world and has been infamously dubbed as the ‘diabetic capital of the world’ (Abate and Chandalia, 2007).

More over increased oxidative stress and generation of excessive free radicals in diabetic patients thought to be the etiology of chronic diabetic complications. The increased free radicals generation along with declined antioxidant defense systems may damage enzymes, cellular organelles and lipid peroxidation (Tepa et al., 2007;
Nabavi et al., 2009). Defects in carbohydrate machinery and consistent efforts of the physiological system to correct the imbalance in carbohydrate metabolism pose an over exertion of the endocrine system, which leads to control exacerbates the metabolic disturbances by altering carbohydrate metabolism enzymes and leads to primarily hyperglycemia (Valcheva-Kuzmanova et al., 2007). The long term hyperglycemia is an important factor in the development progression of secondary complications of micro and macro vascular complications, (Altan, 2003; Strojeck, 2003) which include eyes, nerves, blood vessels and cardiovascular (Shim et al., 2011).

Several drugs such as biguanides, sulfonylurea and thiazolidinediones are presently available to reduce hyperglycemia in diabetes mellitus (Jung et al., 2006 and Matsui et al., 2006). The use of these drugs is accompanying side effects (Donath et al., 2006 and Noor et al., 2008). The medicinal plants may provide the useful source of new oral hypoglycemic compounds for the development of pharmaceutical entities or as dietary adjunct to existing therapies (Kavishankar et al., 2011). Some of which are scientific scrutiny although world health organization had encouraged and recommended the use of plants as an alternation therapy for diabetes (Jia et al., 2009). if any medicinal plant can work as a potential antioxidant together with having anti-diabetic property then it could prevent or reduce diabetic complication more effectively than the conventionally used anti-diabetic drug (Naziroğlu and Butterworth, 2005; Kamalakannan and Prince, 2006). More than 1200 species of plants have been used empirically for their anti-diabetic activity (Marles and Farnsworth, 1994; Singh and Rajini, 2005; Dixit and Kar, 2010).

A great number of medicinal plants have been suggested as a rich as yet unexplored scientific source of potentially useful anti-diabetic drugs. Many have been used in the treatment of diabetes in the different part of the world. The present decade has witnessed a tremendous and intense resurgence in the interest and use of medicinal plant and medicinal plant products. The beneficial effects of these plant materials has been attributed to the combinations of secondary metabolites present in the plant (Briskin, 2000). The healing power of herbs has been recognized and botanic medicine has been one of the oldest practiced professions by mankind (Oduola et al.,
There are more than 200 compounds from plant sources that have been reported to show blood glucose lowering effect. A wide variety of chemical classes indicates that a variety of mechanisms of action are likely to be involved in lowering blood glucose levels (Prisilla et al., 2012). Animal models provide valuable clues in understanding the underlying pathological mechanisms of diabetes and are useful for the screening of drugs for the prevention and treatment of diabetes. Currently induced models have gained widespread acceptance for pathogenesis and drug screening research due to their rapid induction of diabetes.

5.3.5.1. Evaluation of In vitro Anti-diabetic Activity of Plant Extracts

Diabetes is a debilitating disease affecting millions of people worldwide. Since the disease has no known modern allopathic cure, it requires lifelong health. In fact modern medicine merely attempts to control the symptoms of diabetes like increased blood sugar level and tries to mitigate the various other complicated problems that can arise out of diabetes like increased cardiovascular risks, diabetic retinopathy, diabetic neuropathy and kidney failure leading to more diabetes related complications and an untimely death (Biswas et al. 2011). Hence, plants have been suggested as a rich, as yet unexplored source of potentially useful anti-diabetic drugs. However, only a few have been subjected to detailed scientific investigation due to a lack of mechanism-based available in vitro assays (Saxena and Vikram, 2004).

One anti-diabetic therapeutic approach is to reduce gastrointestinal glucose production and absorption through the inhibition of carbohydrate digesting enzymes such as $\alpha$-amylase and $\alpha$-glucosidase. Inhibition of $\alpha$-amylase and $\alpha$-glucosidase enzymes involved in digestion of carbohydrates can significantly decrease the post prandial increase of blood glucose after a mixed carbohydrate diet and therefore can be an important strategy in management of blood glucose (Ahamad et al. 2011). Several natural $\alpha$-glycosidase and $\alpha$-amylase inhibitors including acarbose, voglibose and miglitol are clinically used as a treatment, but their prices are high and clinical side effects occur (Scott and Spencer, 2000).

Acarbose, a microbial pseudo-tetrasaccharide, is an inhibitor of both $\alpha$-amylase and $\alpha$-glucosidase (Asano, 2009), and is widely used clinically as an oral
hypoglycaemic agent, in combinations with other anti-diabetic agents, to control postprandial hyperglycemia (Fujisawa et al., 2005; Van de Laar et al., 2005). However, the use of acarbose as an oral hypoglycaemic agent is reported to be associated with gastrointestinal side effects such as abdominal discomfort, flatulence, and diarrhea (Lebovitz, 1997; Inzucchi, 2002; Cheng and Fantus, 2005) which are allegedly caused by the excessive inhibition of pancreatic α-amylase by acarbose (Bischoff et al., 1985; Horii et al., 1987). In contrast to acarbose, plant derived α-amylase and α-glycosidase inhibitors are reported to have lower inhibitory effect against α-amylase activity and stronger inhibitory activity against α-glucosidase (Kwon et al., 2008), an indication that plant extracts and their constituents may be effective therapeutic agents for the management and control of postprandial hyperglycemia with less side effects than acarbose. α-amylase and α-glucosidase enzymes include: Pseudosacharides such as acarbose, miglitol and vogiblose (Asano, 2009), and phenolic phytochemicals (Kim et al., 2000; McDougall et al., 2005; Tadera et al., 2006; Matsui et al., 2007).

Interestingly, in the present investigation, indicated that the ethanolic extract of fruit of *E. serratus* had *in vitro* α-amylase and α-glucosidase inhibitory activity was dose-dependent manner. The plant extract exhibited weak α-glucosidase enzyme inhibition when compared to α-amylase. Similarly, Hannan et al. (2010) evaluated the α-amylase carbohydrate hydrolyzing enzyme activity of 70% ethanol, ethanol extract, petroleum ether, chloroform and ethyl acetate extracts of *Nepeta cataria*. Among them, inhibition of α-amylase by chloroform and ethyl acetate extracts appeared to have highest significant reducing activity (44.59±1.55 and 43.64±1.79%) at 1000 ug/ml.

Further, observations suggested that *in vitro* human urinary α-amylase and presumably human pancreatic α-amylase are inhibited by more polar constituents of *Clausena anisata* leaf (Shai et al., 2012). This was agreement with the results of related studies which reported α-amylase inhibitory activities in the more polar (aqueous, methanol, acetone, hexane) extracts of crude extract of *C. anisata* leaves could be attributed the presence of polyphenols, flavonoids and their glycosidase which are known to be soluble in polar solvents (Hara and Honda, 1990; Jung et al., 2006; Andrade-Cetto et al., 2008; Ortiz-Andrade et al., 2007). On the other hand,
C. anisata acetone extract competitively inhibited α-glucosidase. This observation suggested that α-glucosidase inhibitory components present in the C. anisata acetone extracts could resemble the normal substrates of this enzyme in structure (Vanable and Aschenbrenner, 2007).

Mohamed et al. (2012) demonstrated in vitro studies of 50% ethanolic extract of Orthosiphon stamineus and sinensetin on α-glucosidase and α-amylase. The IC$_{50}$ values showed the equal preference for both α-glycosidase and α-amylase enzymes. Several class chemicals also have been found in O. stamineus that are rich terpenoids, caffeic acid and derivates and chromene (Malterud et al., 1989; Tezuke et al., 2000; Olah et al., 2003). The compounds present in this extracts were mentioned by Tadera et al. (2006) and Kwon et al. (2008) as being effective inhibitors of α-glycosidase and α-amylase. Likewise, Kim et al. (2005) demonstrated that the triterpenoid acids showed a significant inhibitory effect on α-amylase. Moreover urosolic acid, pentacyclic triterpenoids and oleanolic acid derivatives exhibited the strongest α-amaylase suppressing activity and are responsible for a major part of the activity of the total hexane extract of many anti-diabetic plants.

In a parallel result, McCue and Shetty, (2004) illustrated that the amylase activity was inhibited in the presence of herbal extracts containing rosmarinic acid and the extent of amylase inhibition correlated with increased concentration of rosmarinic acid. The anti-amylase inhibitory activity may be due to the ability of phenolic compounds to interact with or inhibit proteins enzymes (Rohn et al., 2002). The same authors added that phenolic substances that are able to form quinines (such as caffeic acid, chlorogenic acid, gallic acid, etc) are more reactive than those phenolic that cannot form quinines and suggested that semiquinones formed may react with amino acid side chains and free thiol groups on the enzyme. Analogous to the present study, Ali et al. (2006) demonstrated several compounds isolated from many anti-diabetic plants such as flavanone glycoside, thiosugar kotalanol and luteolin (flavonoid) and proved to have inhibitory effect on α-glucosidase. In close agreement, Tepa et al. (2007) and Dudhgaonkar et al. (2009) attributed the anti-diabetic effect through inhibition of digestive enzyme α-glucosidase to the potential enzyme
inhibitors-3-Ogalloylpipecatechin and -3-O-gallylcatechin extracted from *Nepalese herb Pakhanbhed.*

Furthermore, the hypoglycemic potential of *Carpesium abrotanoides* was evaluated by the α-amylase and α-glucosidase inhibition assay. The optimal concentration of *C. abrotanoides* required for the 50% inhibition (IC$_{50}$) against α-glucosidase was 44.22μg/ml. Acarbose was used as positive control with IC$_{50}$ value of 2.5μg/ml (Mayur *et al.*, 2010). According to Gayathri Devi *et al.* (2012) appraised the acetone extract of both the fruits (68.9%) and leaves (89.6%) shows strong inhibitory activity against α-amylase and the aqueous extract of fruit (6.89%) and leaf (7.5%) of *T. bellirica* were found to exhibit highest α-glucosidase activity.

Additionally, *Scaphium scaphigerum, Litsea glutinosa, Hibiscus esculentus, Ocimum canum, Trigonella foenum-graecum, Plantago ovata* seeds and *Basella alba* showed the inhibitory percentage of 82.6, 41.0, 37.6, 32.8, 30.6, 27.0 and 25.0%, respectively whereas, *S. scaphigerum* was further investigated and found that the concentration for 50% inhibition of α-glucosidase activity (IC$_{50}$) was 0.17% (Palanuvej *et al.*, 2009). Equally, Dinesh Kumar *et al.* (2010) investigated that the petroleum ether extract of stem bark of *Mangifera indica* (Mangiferin) exhibited appreciable α-amylase and α-glucosidase inhibitory activity. In addition, mangiferin showed appreciable α-amylase inhibitory effect (IC$_{50}$=74.35±1.9μg/ml) and α-glucosidase inhibitory effect (IC$_{50}$=41.88± 3.9μg/ml) when compared with standard drug acarbose (IC$_{50}$ =83.33±1.2μg/ml). Our results are corroborative with that of Dinesh Kumar *et al.* 2011 who have reported that the ethanol extracts of *Mangifera indica, Azadirachta indica* and also petroleum ether extract of *Murraya koenjii* (at a concentrations 10-100μg/ml) showed maximum α-amylase inhibitory activity from 35.79 ± 0.33 to 62.49 ± 0.34%, 16.50 ± 1.23 to 66.66 ± 0.93% and 21.57 ± 1.46 to 60.78 ± 0.55% with an IC$_{50}$ = 37.86 ± 0.32, 62.99 ± 1.20 and 59.0±0.51μg/ml, respectively.

Kavitha Sama *et al.* (2012) screened the *in vitro* α-amylase and α-glucosidase activity of crude ethanol extract of *Cissus arnottiana* fruit. The plant expressed significant enzymes inhibitory activity (78.91, 81.25%) at the concentration of
10mg/ml. Likewise, the in α-glucosidase activity of ethanolic extract of whole plant of *Evolvulus alsinoides* Uma *et al.* (2012) demonstrated the percentage inhibition at 100, 80, 60, 40 and 20mg/ml concentrations of plant extracts showed a concentration-dependent reduction in percentage inhibition. The 100% concentration tested showed a maximum inhibition of nearly 63% and the standard acarbose showed the inhibitory activity of 74%. The plant extract produced a weak α-glucosidase enzyme inhibition when compared with α-amylase. The maximum inhibition was found to be 63% at a concentration of 100mg/ml.

Mai and Chuyen *et al.* (2006) showed that the aqueous extract of flower buds of *Cleistocalyx operculatus* had an inhibitory effect on α-glucosidase in vitro assay the *Psidium guajava* (guava) leaf extract inhibited the activities of α-amylase. Our experiment was in accordance with the results reported by Deguchi *et al.* (1998) who reported the in vitro results demonstrated that the bioactive components inhibited the rat-intestinal α-amylase *E. serratus* fruit. The inhibition of α-amylase by a polyphenolic extract of green tea has been reported (Hara and Honda, 1990; Grover *et al*., 2000). Polyphenolic compounds derived from red cabbage, strawberries and raspberries are also inhibitors of α-amylase and α-glucosidase (McDougal, 2005). Our present results suggest that polyphenolic compounds have a potentially important role in managing diabetes via the inhibition of α-amylase and α-glucosidase enzyme activities.

Similarly, Catherine *et al.* (2010) and Rani *et al.* (2011) showed that the methanolic extract of *Amaranthus cruentus* and *Moringa oleifera* investigated samples showed promising levels of α-amylase (10-45%) and α-glucosidase (13-80%) inhibition activities. According to Manikandan *et al.* (2013) the methanolic extract of leaves of *Psidium guava* efficiently inhibited both α-amylase (89.4%) and α-glucosidase (96.3%) enzymes in vitro in a dose-dependent manner. Similarly, the aqueous extracts from *Syzygium cumini* seeds and *Psidium guava* leaves showed a dose-dependent inhibitory effect on α-amylase activity (Karthic *et al*., 2008).

In conclusion, the present study has demonstrated that *E. serratus* ethanolic extract of fruit of exerted both α-amylase and α-glucosidase inhibitory action in vitro,
and significantly prevented a rise in postprandial blood glucose levels. Strong inhibition of $\alpha$-amylase and low inhibition of $\alpha$-glucosidase could be potentially used as an effective complementary therapy for postprandial hyperglycemia with minimal side effects.

5.3.5.2. Evaluation of In vivo Anti-diabetic Activity of Plant Extracts

The increase in number of diabetic patients has motivated the scientists to find new methods to cure diabetes. In spite of the presence of known anti-diabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease (Fatima et al., 2010). The untreated diabetic rats showed severe body weight loss. This characteristic weight loss in diabetic rats could be due to degradation and catabolism of fats and proteins (Mc Nurlan and Garlick, 1979). Thus, increased catabolic reactions leads to muscle wasting which may be the major cause for weight loss in diabetic rats (Rajkumar et al., 1991). Streptozotocin (STZ) is a commonly used chemical to generate diabetic animals in the laboratory for its ability to destroy insulin producing $\beta$-cells (Tjalve, 1983; Szkudelski, 2001). Glibenclamide is a sulfonylurea that decreases blood glucose levels in diabetic subjects by increasing insulin secretion from pancreatic beta cells decreasing blood glucagon concentrations and improving insulin action on target tissues (Ojewole et al., 2006).

In the present study, untreated diabetic rats showed severe body weight loss. This characteristic weight loss in diabetic rats could be due to degradation and catabolism of fats and proteins (Mc Nurlan and Garlick, 1979). Thus, increased catabolic reactions leads to muscle wasting which may be the major cause for weight loss in diabetic rats (Rajkumar et al., 1991). However, the administration of ethanolic extract of fruit of E. serratus increased the body weight of the STZ treated animals in a dose-dependent pattern which suggested the protective effect of the extract by preventing it from muscle wastage and other macromolecular degradations. Also, Rattima et al. (2007) investigated the hypoglycemic effect of Elaeocarpus grandiflorus water (leaves, twigs and fruits) extracts in alloxan-induced diabetic rats. The diabetic-induced animals significantly reduced body weight by the extract at 0.001g/kg. STZ-induced diabetes is characterized by a severe loss in body weight.
Likewise, Kim et al. (2006) reported a significant body weight gain in diabetic rats after oral administration of *Morus alba* extract in a dose-dependent manner. This result agrees with other investigators who noticed increase in body weight gain upon improvement of diabetes status (Craft and Failla, 1983; Schwechter et al., 2003). Similarly, Hamdy (2012) showed that the administration of aqueous extract of leaves of *Morus alba* (low and high dose) significantly increased the body weight at the end of the test period when compared with diabetic rats. Adekomi et al. (2011) identified that the ethanoilc extract of *Catharanthus roseus* leaf had a modulating effect on body weight in a dose-dependent pattern.

During fasting the body stimulates the release of the hormone glucagon, which in turn releases glucose into the blood through catabolic process. Normally the body produces and processes insulin to counteract the rise in glucose levels but in diabetes, glucose levels normally remain high. Streptozotocin, a glucose analogue (2-deoxy-2-(3-methyl-3-nitrosuuredio)-D-glucopyranose), is a potent diabetogenic agent and widely used for indicating diabetes in a variety of animals by the selective degeneration and necrosis of pancreatic cells (Merzouk et al., 2000; Elsner et al., 2000). Streptozotocin, an extract from *Streptomyces achromogenes*, has been used for inducing Diabetes mellitus by its toxic action to islet β-cells of pancreas. It breaks the nuclear strand of the islet cells and brings an increase in blood glucose levels (Takasu et al., 2000). Glibenclamide is often used as a standard anti-diabetic drug in streptozotocin-induced diabetic rats to compare the efficacy of variety of hypoglycemic compounds (Prakasam et al., 2002).

The current research appraised the anti-diabetic activity of ethanoilc extract of fruit of *Elaeocarpus serratus* using streptozotocin-induced diabetic rats. The low and high doses of the plant extracts (200 and 400mg/kg b.w.) significantly decreased the blood glucose level when compared to diabetic-control rats. Similarly, Sridhar et al. (2011) elucidated the anti-diabetic effect of methanolic extract leaves of *Muntingia calabura* (Elaeocarpaceae) in normal and alloxan-induced diabetic rats. The methanolic extract (500mg/kg b.w.) significantly lowered the blood glucose levels to comparable to standard anti-diabetic drug (Glipizide 5mg/kg b.w.) in both normal and diabetic rats. The extract (500mg/kg body weight) increased the glucose tolerance in
glucose loaded rats. The results suggest that methanolic extract of *M. calabura* leaf possessed significant anti-diabetic activity. In concomitant, Rattima *et al.* (2007) investigated the hypoglycemic effect of water extract of leaves, twigs and fruits *Elaeocarpus grandiflorus* by alloxan-induced diabetic rats. The results showed that hypoglycemic effect of the water extracts was a dose-dependent. The glucose lowering effect was also continuously observed at the highest dose.

Hyperglycemia is reported to increase oxidative stress through free radical formation (Ashok *et al*., 2010). Endogenous oxygen free radicals scavenging enzymes can respond to such conditions of oxidative stress in diabetes with a compensatory mechanism. Saha *et al.* (2011) showed that *Cucurbita maxima* significantly reduced the elevated fasting blood glucose level with respect to those of diabetic control animals. This was accordance to the studies reported in earlier cases (Jasmine and Daisy, 2007; Okokon *et al*., 2007). On similar note, Haque *et al.* (2012) evaluated the anti-diabetic activity of ethanolic leaf extract of *Centella asiatica* in alloxan-induced diabetic rats. The different test doses of the plant extract (250, 500 and 1000mg/kg) caused various degree of reduction of the blood glucose levels. Agreeing to the present study, Ojewole, (2003) and Dimo *et al.* (2007) speculated that blood glucose lowering effects of hexane extract of *Sclerocarya birrea* stem bark could be associated with the stimulation of insulin secretion from the pancreatic beta cells.

On the other hand, Gondwe *et al.* (2008) reported that *Sclerocarya birrea* decreased the blood glucose level in STZ-induced diabetic rats without affecting the plasma insulin levels. Hanan *et al.* (2010) revealed significant amelioration in blood glucose levels in post treatment of diabetic rats with crude ethanol, petroleum ether and chloroform extracts of *Nepeta cataria*. In line with the present study, Vats *et al.* (2004) found that *Ocimum sanctum* significantly improved beta cell function and enhanced insulin section leading to lowering blood glucose level. In this respect, Oliver and Baver, (1986) found that the bark and root aqueous extracts of *Mangifera indica* significantly lowered the blood glucose level.

Rezaeizadeh *et al.* (2011) showed that blood glucose levels fell significantly in both *Momordica charantia* and glibenclamide-treated diabetic rats. These findings are agreement with previous studies (Virdi *et al*., 2003; Chandra *et al*., 2007). The
glucose lowering activity of *Leonotis leonurus* leaf aqueous extract was compared with standard drug glibenclamide (Ratnasooriya *et al*., 2004). The presence of flavonoids and phenolics compounds in the extract may be responsible for the above observation. Uma *et al*. (2012) studied that the administration of ethanolic extract of *Evolvulus alsinoids* managed the post prandial blood glucose level. They also are useful for people taking sulfonylurea medication or metformin, who need an additional medication to keep their blood glucose levels within safe range. This earlier supported by Mc Cue *et al*., 2004; Subramanian *et al*., 2008).

Also, Christudas *et al*. (2012) showed that the administration of aqueous leaf extract of *Biophytum sensitivum* produced significant glucose lowering activity in hyperglycemic rabbits. The extract contained biflavones and flavonoids (Lin and Wang, 2000) and these may be responsible for the anti-diabetic effect (Andrade-cetto and Wiedenfeld, 2007; Park *et al*. 2007). Similarly, Maroo *et al*. (2002) have demonstrated a mechanism whereby *Enicostemma littorale* lowered blood sugar by the stimulation of insulin release from the pancreas in diabetic rats.

The acute and prolonged treatment of STZ-induced diabetic rats with various doses of the *Homelicum letestui* extract produced a significant reduction in blood glucose level of the rats in a manner comparable to that of the standard drug. The treatment also caused a significant increase in weight of the animals which is attributed to the extract hypoglycemic activity. This hypoglycemic effect of the extract is linked to the presence of flavonoids and terpenes in the extract (Okokon *et al*., 2006). These compounds have been implicated in the anti-diabetic activities of many plants (Shimizu *et al*., 1984; Ivorra *et al*., 1989; Reher *et al*., 1991).

Furthermore, the methanol extract of *Syzygium cumini* and glibenclamide caused highest significant reduction in the serum blood glucose level as compared to diabetic control (Farswan *et al*., 2009). The concentration of blood glucose level was increased in diabetic rats at the same time *Morus alba* treated groups there was a significant decrease in blood glucose levels as compared to diabetic rats (Hamdy, 2012). Additionally, Rahman *et al*. (2011) investigated that the *Tabernaemontana divaricata* methanolic flower extract (300mg/kg) more effective dose to reduce maximum blood glucose level at 10th hour of the treatment period.
New drugs are investigated in animals both for desired effects and for the undesired (toxic) effects. This is important because any hepatic and renal damage will alter the structure and function of these vital organs and have serious effects on overall metabolism. The liver is the most important organ in the metabolism of drugs and other substances. Liver cell destruction shows its effects mostly as important in the liver cell membrane permeability, which results in the leaking out of tissue content into the blood stream (Ozsoy-Sacan et al., 2006). Insulin generally has an anabolic effect on protein metabolism in that it stimulates protein synthesis and retards protein degradation (Murray, 2000). A previous report has shown that protein synthesis is decreased in all tissues due to decreased production of alkaline phosphatase in absolute or relative deficiency of insulin (Chatterjee and Shinde, 1994) that may be responsible for decreased level of plasma protein; albumin and globulin may be related with increased level of plasma insulin in diabetic treated with tetrahydrocurcumin and curcumin.

Alderson et al. (2004) demonstrated a significant increase of total protein excretion, albuminuria, glucosuria and urinary urea levels indicating impaired renal function. The reduction in serum total protein content in the present results may be related to reduction in albumin which is the most abundant blood plasma protein (70%) produced in liver. Non-enzymatic glycation of albumin was found the potential to alter its biological structure and function (Mendez et al., 2005). It could be observed that, diabetes caused a significant decrease in total protein, albumin and globulin levels in serum of diabetic rats.

In the present study, treating the diabetic rats with ethanolic extract of *E. serratus* fruits caused significant increase in total protein and total albumin levels compared with control diabetic rats. Likewise, Reda et al. (2010) treating diabetic rats with aqueous *Mangifera indica* leaves extract caused significant increase in total protein and albumin levels compared with untreated diabetic rats. Similarly, Sethi et al. (2004) demonstrated that the enhanced level of serum proteins post treatment of diabetic rats with aqueous extract of *Ocimum sanctum*. This attributed effect of insulin-like factors in the extract, since insulin is reported to increase protein synthesis. In concomitant with the present results (Otsuki and Williams, 1982) found
significant reduction in serum total protein concentrations in diabetic rats and this may be due to reduction in the three major phases in protein secretion, intracellular transport and discharge.

Measurement of the activities of various enzymes and non enzymatic indices in tissues and body fluids play a significant role. In several organs, cell membrane damaging is followed release a number of cytoplasmic enzymes to the blood, a phenomenon that provides the basic for clinical diagnosis. Abnormal levels of aspartate transaminase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in the serum of experimental animals are indicative of tissue damage by toxicants or disease conditions (Singh et al., 2000; Sroka and Cisowski, 2003). The changes in serum enzyme activities are normal in uncomplicated diabetes. However, when tissue damage caused by metabolic and circulatory alterations occur, their activities are increased.

The liver is regarded as the central metabolic organ in the body, with an important role in glucose and lipid homeostasis (Eidi and Eidi, 2009). AST, ALT and ALP are considered as liver toxicity markers (Badole and Bodhankar, 2010). The increase in the activities of plasma AST, ALT and ALP indicated that diabetes may be induced hepatic dysfunction. Therefore the increment of the activities of AST, ALT and ALP in plasma may be mainly due to the leakage of these enzymes from the liver cytosol to blood stream, which indicate the hepatotoxic effect of STZ (Eliza et al., 2009).

In current investigation, diabetic rats showed a significant elevation in the activities of serum AST, ALT and ALP as compared to normal rats. However, treatment with the ethanolic extract of fruit of *Elaeocarpus serratus* low and high doses significantly reduced the enzyme activities to the normal levels. The curative effect of ethanol extracts of *E. serratus* fruit could the easily the noticed through the normalization of all enzyme tested returned more or less to the level of normal control and standard drug treated group.

On a similar note, serum enzyme levels including ALP were significantly raised to high values in diabetic animals, reflecting active damage disorder
(Mohamed et al., 2009). Treatment with methanolic extract of *Cucurbita maxima*, like glibenclamide, caused significant reduction in the activities of the enzymes to normal level, showing the protective effect of the extract. Also, Hanan *et al.* (2010) noticed a significant improvement in liver function enzyme markers in treatment of diabetic rats with petroleum ether, chloroform and crude ethanol extracts of *Nepeta cataria*. It was shown that administration of the plant extracts reflected an improvement of cellular damage as determined. The above works consistent with previous studies that administration of some antioxidants (as zinc, selenium, vitamin C and E) to diabetic rats, normalized the elevated activities of liver function enzymes AST, ALT, ALP induced in response to diabetes mellitus (Abdel Mageed, 2005).

Diabetes resulted in a significant increase in liver enzymes (AST) and (ALT) suggested the possible necrotic injury of the liver (Van Hoof *et al*., 1994). Liver enzyme activities were decreased to the normal levels found in the negative control rats after treatment with 30, 50 and 70 mg aqueous *Mangifera indica* leaves extract. The significant decrease in total protein and albumin with diabetes is an indication of compromised liver excretory function and impairment of the liver function, which was improved by aqueous *M. indica* leaf treatment. The present study was on parallel with Martinez *et al.* (2000), Hassan *et al.* (2007), Rashad *et al.* (2008) and Reda *et al.* (2010) found that, serum total protein, AST and ALT levels were improved in lambs on dietary inclusion of mango leaves. Balamurugan *et al.*, (2012) reported the treatment of the diabetic rats with *Acorus calamus* methanol extract caused reduction in the activity of AST, ALT and ALP enzymes in plasma compared to the diabetic untreated group and consequently alleviated liver damage caused by STZ-induced diabetes. The mechanism of hepatoprotective ability of plant extracts may be attributed to numerous bioactive compounds such as terpenoids, flavonoids, sterols, essential oils, alkaloids and polysaccharides. Most of them (especially flavonoids, triterpenoids) showed a mechanism to improve the function of liver and hence normalization of liver enzymes (El Hilaly and Lyoussi, 2002; Li *et al*., 2004; Zheng *et al.* 2007; Gilani *et al*., 2009).

Also, the activities of AST, ALT and ALP are most sentive tests employed in the diagnosis of organ damage. The increased activity of serum enzymes (AST, ALT
and ALP) was noticed in STZ-induced diabetic rats (Kim et al., 2012). Administration of Chrysanthemi flos flower extract and gliclazide to diabetic rats showed decreased activities of serum AST, ALT and ALP. The above results were consistent with earlier report (Bopanna et al., 1997; Heo et al., 2007). Similarly, there was significant increase in liver AST, ALT and ALP due to exposure of alloxan-induced diabetic rats. Administration of ethanolic seed extract of Strychonous potatorum reduced the levels of liver AST, ALT and ALP to almost normal level (Dhasarathan and Theriappan, 2011).

In this context, several investigators reported the increases in AST, ALT in the liver and serum enzymes of streptozotocin-diabetic rats (Singh et al., 2001). The changes in levels of serum enzymes are directly related to changes in the metabolism. The increased protein catabolism accompanying gluconeogenesis in the diabetic state might be the reason for the elevated activities of these enzymes, which were brought back to near normal by tetrahydrocurcumin treatment. The observation of an increased serum activity of alkaline phosphatase in diabetes has been interpreted as a manifestation in serum of the increased phosphatase activity that may occur in tissues in the diabetic state (Belfiore et al., 1972).

In experimental diabetes, enzymes of glucose metabolism are markedly altered. Persistent hyperglycemia is a major contribution to such metabolic alterations that lead to pathogenesis of diabetic complications, especially, neuropathy and microvascular diseases. One of the key enzymes in the catabolism of glucose is hexokinase, which phosphorylates glucose and converts it into glucose-6-phosphate (Pari and Rajarajeshwari, 2009).

In the present study, the decreased activity of carbohydrate metabolizing enzymes like hexokinase (HK) and phosphor glucoisomerse (PGI) and increased activity of glucose 6 phosphate (G-6-P) and fructose 1,6 diphosphatase (F-1,6-DP) was observed in the diabetic rats as compared with normal control rats. These values were reversed by treatment with ethanolic extract of fruit of E. serratus (low and high doses) and the standard glibencalmide. Ethanolic extract of E. serratus fruits restored the level of these carbohydrate metabolizing enzymes to normalcy. Administration of streptozotocin resulted in an increased activity of hexokinase can cause increased
glycolysis and increased utilization of glucose for energy production. *E. serratus* extracts has been observed to reduce the level of glucose in the blood. The decreased concentration of blood glucose in STZ-treated rats with *E. serratus* extract may be due to increased glycolysis or liver hexokinase activity. Hexokinase, which brings about the first phosphorylation step of glucose metabolism, is reduced significantly in the diabetic group of rats (Nehal, and Baquer, 1989). In the present study the observed decrease in the activity of hexokinase in the diabetic rat liver might be due to the diminished consumption of glucose in the system and increased blood sugar level.

On similar note, Jayanthi *et al.* (2010) observed decreased activities of hexokinase and increased activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase in the liver of diabetic rats as compared with normal control rats, and these values were reversed by treatment with aqueous leaf extract of *Biophytum sensitivum*. Likewise, Vats *et al.* (2004) found that anti-hyperglycemic effect of *Ocimum sanctum* is at least partially dependent upon insulin release from the pancreas and significantly increased the activity of hexokinase towards normal levels. In addition it increases glycogen in muscle and liver by stimulating glycogen synthase, suggesting that the anti-hyperglycemic action is the result of increased glucose utilization at the level of liver and muscle.

The liver is an important organ that plays a vital role in glycolysis and gluconeogenesis pathways. Liver plays an important role in the maintenance of blood glucose level by regulating its metabolism. Glucose-6-phosphatase and fructose-1, 6-bisphosphatase are the key enzymes in homeostatic regulation of blood glucose level (Liu *et al.*, 1994; Massillon *et al.*, 1996; Singh and Kakker, 2009). In addition, glucose-6-phosphatase plays an important role in glucose release in liver and kidney through a mechanism involving gene expression or biochemical inhibition of its enzymatic activity (Pari and Srinivasan, 2010). The level of these hepatic gluconeogenic enzymes were increased significantly in diabetic rats. The increased activities of these two enzymes might be due to the activation or increased synthesis of the enzymes contributing to the increased glucose production during diabetes by the liver (Baquer *et al.*, 1998).
Increased glucose-6-phosphatase activity in diabetic rats provides hydrogen, which binds with NADP$^+$ in the form of NADPH and enhances the synthesis of fats from carbohydrates i.e. lipogenesis (Rajagopalan and Sasikala, 2008) and finally contributes to increased levels of glucose in blood. Increased hepatic glucose production in diabetes mellitus is associated with impaired suppression of the gluconeogenic enzyme fructose-1,6 bisphosphatase. Activation of gluconeogenic enzymes is due to the state of insulin deficiency, because under normal conditions, insulin functions as a suppression of gluconeogenic enzymes.

In support of the present findings, Ramya and Daniel (2012) elucidated that the leaves of Costus pictus aqueous extract and glibenclamide treated groups restored the level of these carbohydrate metabolizing enzymes, which could be due to increased insulin secretion stimulated by the treatment. Treatment with methanol extract of stem of Tinospora cordifolia in diabetic rats decreased the glucose-6-phosphatase activity nearly equal to normal (Rajalakshmi et al., 2009). Also, Shanmuga Sundaram and Subramanian (2012) evaluated that the reduced activities of both glucose-6-phosphatase and fructose-1,6-bisphosphatase in hepatic tissues of diabetic rats upon oral administration of ethanolic extract of tepals of Musa paradisiaca revealed the reduced endogenous glucose production. Hence, M. paradisiaca tepal extract might play a crucial role in maintaining the fasting blood glucose level. The regulation of gluconeogenic flux by the extract might be one of the possible mechanisms for its anti-hyperglycemic nature.

Correspondingly, in STZ-induced diabetic rats, administration of Acorus calamus rhizome methanolic extract at the dose of 200mg/kg to diabetic rats decreased the activities of glucose-6-phosphatase and fructose-1, 6 bisphosphatase and also increased the activity of glucose-6-phosphatase (Balamurugan et al., 2012). Hamdy, (2012) revealed the administration of Morus alba enhanced the reversal of high glucose-6-phosphatase activity in diabetic rat. The reduction of glucose-6-phosphatease can lead to a decrease in gluconeogenesis and blood glucose concentration (Dijik et al., 2001).

The present findings suggest that the plant extract is nontoxic, since no marked changes were observed in the normal rats fed with the extract. Thus, at normal
therapeutic doses, the extract was considered to be safe for long-term treatment in diabetic condition. The fruit ethanolic extract showed potent anti-diabetic activity such as body weight, blood glucose level, liver serum marker enzymes and carbohydrate metabolizing enzymes. These finding suggested that *E. serratus* ethanolic extract is useful in the control of diabetes mellitus.

In conclusion, *Elaeocarpus serratus* is proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit. The plant is blessed with immense potent activities in combating different types of diseases, the requirement is to explore it the most for its active constituents and further more regarding its mode of action and structural analysis so that a better and more advanced formulation can be prepared for the main stream administration of the drug. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent natural drugs of natural origin.