5. DISCUSSION

Cells and organs in the human body are constantly exposed to a variety of oxidizing agents and free radicals which are generated in vivo due to the various biochemical reactions occurring in the living tissues. In normal metabolism, the level of oxidants and antioxidants are maintained in a delicate balance by the antioxidant defense system, which is important for sustaining optimal physiological conditions (Temple, 2000). Overproduction of oxidants can cause oxidative stress and oxidative damage to lipid, protein and nucleic acid, which are associated with various chronic diseases (Bolton et al., 2000; Pratico and Delanty, 2000; Prior and Cao, 2000; Smith et al., 2000; Arteel, 2003; Upston et al., 2003; Kinnula and Crapo, 2004; Touyz, 2004; Guidi et al., 2006; Hyun et al., 2006; Singh and Jialal, 2006; Rackova et al., 2007; Sas et al., 2007; Tripathi et al., 2008).

Usually the aerobic organisms have their own inherited antioxidative defense mechanisms to protect against the oxidative damages. Sometimes these protective mechanisms are disrupted by various pathological processes, and antioxidant supplements are vital to combat oxidative damages and the risks for chronic diseases (Tanizawa et al., 1992; Sasaki et al., 1996; Saleem et al., 2002; Prior, 2003; Chen et al., 2005; Zhang et al., 2005). Recently, plant drugs containing radical scavengers are gaining importance in the treatment of diseases. Sources of natural antioxidants are primarily, plant phenolics that may occur in all parts of plants such as fruits, vegetables, nuts, seeds, leaves, roots and barks (Pratt and Hudson, 1990; Mathew and Abraham, 2006). Many plants exhibit efficient antioxidant activities owing to their phenolic constituents (Larson, 1988; Tripathi et al., 2005).

Recently, many medicinal plant species have been investigated in the search for novel antioxidants (Chu, 2000; Mantle et al., 2000; Koleva et al., 2002; Oke and Hamburger, 2002) but generally there is still a demand to find more information concerning the antioxidant potential of plant species. Cook et al. (1998) estimated the antioxidant activity of 17 wild edible plants used in traditional medicine. They observed
that *Balanites aegyptiaca*, *Bombax costatum*, *Boscia senegalensis*, *Entada africana*, *Gynandropsis gynandra*, *Hyphaene thebaica*, *Leptadenia hastate*, *Sesbania pachcarpa* and *Tapinanthus globiferus* possessed strong antioxidant activity. Plants like turmeric (*Curcuma domestica*), betel leaf (*Piper betel*), pandan leaf (*Panadanus odorus*), asam gelugur (*Garnicia atroviridis*), mengkudu (*Morinda citrifolia*), pegaga (*Centella asiatica*), ginger (*Zingiber officinale*), cassava shoot (*Manihot asculenta*), kesum (*Polygonum minus*) and selom (*Oenathera javanica*) (Jayamalar and Suhaila, 1998; Zin et al., 2002; Zainol et al., 2003; Noriham et al., 2004; Huda-Faujan et al., 2007) also exhibited good antioxidant activity.

Thus, research to find the new sources of natural antioxidants is important. In this sense, the possible therapeutic potential of antioxidants in *Elaeocarpus serratus* and *Elaeocarpus tuberculatus* crude extracts in controlling diseases associated with marked oxidative damage such as inflammation and ulcer have been evaluated.

5.1. Estimation of Percentage Yield of Plant Extracts

Solvent extraction has been the most frequently used technique for the isolation of plant antioxidant compounds. However, the extract yields and resulting antioxidant activities of the plant materials were strongly dependent on the nature of extracting solvent, due to the presence of different antioxidant compounds of varied chemical characteristics and polarities that may or may not be soluble in a particular solvent. In the present study, acetone, methanol and water were used as extraction solvents for the leaves, stem bark and fruits of *Elaeocarpus serratus* and *Elaeocarpus tuberculatus*. Generally, the acetone and methanol extracts of plant parts used showed maximum yield when compared to water extracts. The different antioxidant activities of phenolic extracts could be attributed to different extracting solvents, as the antioxidant activity depended on the type and polarity of the extracting solvent, the isolation procedures, the purity of active compounds, as well as the test system (Meyer et al., 1998). Interestingly, Sultana et al. (2009) showed that among the four solvent extracts [absolute ethanol, absolute methanol, aqueous ethanol (ethanol: water, 80:20 v/v) and aqueous methanol (methanol: water, 80:20 v/v)] used, the aqueous methanol and aqueous ethanol extracts had the
highest total phenolic content. This might be due to the fact that phenolics are often extracted in higher amounts in more polar solvents such as aqueous methanol/ethanol as compared with absolute methanol/ethanol (Anwar et al., 2006; Sultana et al., 2007). This was in agreement with the findings of Shon et al. (2004) who investigated that methanol and hot water were more efficient to extract antioxidant compounds from *Phellinus baumii*. Similarly, Peschel et al. (2006) analysed that the polar solvents were frequently employed for the recovery of polyphenols from a plant matrix. The most suitable of these solvents were (hot or cold) aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate. Methanol and ethanol have been extensively used to extract antioxidant compounds from various plants. Other studies have also demonstrated the efficacy of ethyl acetate to extract phenolic compounds from onion and citrus peel (Abdille et al., 2005; Peschel et al., 2006; Rehman, 2006; Li et al., 2006).

Bonoli et al. (2004) reported that maximum phenolic compounds were obtained from barley flour with mixtures of ethanol and acetone. Chatha et al. (2006) reported that maximum extract yield (g/100g) from rice bran was obtained with aqueous methanol. Similarly, aqueous methanol was found to be more effective in recovering highest amount of phenolic compounds from *Moringa oleifera* leaves (Siddhuraju and Becker, 2003). The differences in the extract yields from the tested plant materials in the previous analysis might be ascribed to the different availability of extractable components, resulting from the varied chemical composition of plants (Hsu et al., 2006). Nagore et al. (2010) extracted the dried herbal parts of *Cassia sophera* first with 95% ethanol and subjected for sequential fractionation with different solvents such as chloroform, ethyl acetate and eventually with ethanol. After fractionation, the percentage yield was found to be 16.14%, 3.1% and 8.35%, respectively. Singh et al. (2010) showed that of all the solvents (pet ether, chloroform, ethanol and water) used, the ethanol extract of *Elaeocarpus ganitrus* had a maximum extractable value of 2.4% and chloroform had a minimum value of 0.5%. All the above reports were on par with the present investigation. In the present study, the solvents acetone and methanol were more efficient in extracting the bioactive compounds than water.
5.2. Assessment of Antioxidant Phytoceuticals in Plant Extracts

5.2.1. Evaluation of Total Phenolic Content in Plant Extracts

A wide range of plant-derived phytochemicals assist in maintaining good health and combating diseases. The medicinal properties of plants have been investigated with recent scientific developments throughout the world, due to their potential antioxidant activities, no side effects and economic viability (Auudy et al., 2003). Recently, there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury (Pormouard et al., 2006). The role of medicinal plants in disease prevention or control has been attributed to the antioxidant properties of their constituents, usually associated with a wide range of amphipathic molecules, broadly termed secondary metabolites like polyphenolic compounds (Ivanova et al., 2005; Aliyu et al., 2009). Many studies reviewed that the secondary metabolites’ distribution might fluctuate between different plant organs (Pratt and Hudson, 1990; Luo et al., 2004; Mathew and Abraham, 2006; Lisiewka et al., 2006).

Naturally occurring phenolic compounds have free radical scavenging properties, due to their hydroxyl groups (Hatano et al., 1989; Shahidi and Wanasundara, 1992; Diplock, 1997). Phenolic compounds easily donate hydroxyl hydrogen and can terminate the free radical reaction chain by changing it to the stable compounds (Fessenden and Fessenden, 1994; Amarowicz et al., 2000). The hydroxyl group of phenols also plays an important role in preventing lipid peroxidation (Rice-Evans et al., 1995; Mayur et al., 2010). In addition to their roles as antioxidants, these compounds exhibit a wide spectrum of medicinal properties, such as anti-allergic, anti-inflammatory, anti-microbial, anti-thrombotic, cardio-protective and vasodilatory effects (Middleton et al., 2000; Balasundram et al., 2006).

The antioxidant activity of many compounds of botanical origin is proportional to the phenolic contents, suggesting a causative relationship between total phenolic contents and antioxidant activity (Duh et al., 1999; Lamien-Meda et al., 2008; Coulidiati et al., 2009; Ciz et al., 2010; Cespedes et al., 2010). A highly positive relationship between
total phenols and antioxidant activity appears to be the trend in many plant species (Oktay et al., 2003). Several studies had been conducted to evaluate the correlation between phenolic compounds and antioxidant activity. Somova et al. (2003) demonstrated the antioxidant potential of triterpenoids isolated from the leaves of Olea europaea, subspecies, africana and wild African olive leaves. Similarly, El-Ghorab et al. (2003) reported on the promising antioxidative activities of ethanol extract from the leaves of Eucalyptus camaldulensis var. brevirostris harvested from Egypt which contained gallic and ellagic acid as the major components. Phenolic compounds in holy basil extracts were one of the major causes of its pharmacological actions. Further, chemical analysis of Bombax ceiba root has revealed that it contained phenolic compounds (Seshadri et al., 1971; Puckhaber and Stipanovic, 2001; Reddy et al., 2003). Besides, some phenolic compounds such as alkanin, shikonin extracted from Alkanna tinctoria root extracts showed high antioxidant capacity on oil substrates (Assimopoulou et al., 2004).

Velioglu et al. (1998) reported a strong relationship between total phenolic content and total antioxidant activity in selected fruits, vegetables and grain products. Javanmardi et al. (2003) have found a significant correlation between the total antioxidant activity and total phenolic contents of Iranian Ocimum accessions. The antioxidant activity of Du-Zhong (Eucommia ulmoides) (Yen and Hsieh, 1998) and anise (Pimpenella anisum L.) seed (Gülçin et al., 2003) were found to correlate with the phenolic compounds. Sawadogo et al. (2006) also found a significant correlation between antioxidant activity and total phenolic content. Huda-Faujan et al. (2009) observed a positive correlation between total phenolic content and antioxidant capacity in linoleic acid emulsion in their study. In contrast, Kahkönen et al. (1999) and Conforti et al. (2008) found no correlation between antioxidant activity and phenolic content in plant extracts.

Looking at all this, the present investigation is an important step in developing new plant-based antioxidant therapeutic agent. Among the three solvents (acetone, methanol and water) used in the present study, the methanolic extract of stem bark, leaf and fruit of Elaeocarpus serratus and Elaeocarpus tuberculatus registered the maximum...
The total phenol content (390.84 ± 4.1; 313.73 ± 0.8; 256.52 ± 4.1; 70.05 ± 9.67; 62.51 ± 4.35 and 34.21 ± 0.83 mg/g tannic acid equivalent, respectively). At the same time, the water extracts of the studied plant parts contained comparatively lesser amount of total phenolics. Generally, the total phenol content of *E. serratus* was almost five times higher as compared to *E. tuberculatus*.

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±1.6 mg 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. Similarly, Kulkarni *et al.* (2007) showed that the multiple radical scavenging potential of *Achras sapota* (sapota) juice was due to its nutraceutical components, viz., phenolics, carotenoids and ascorbic acid. The results of Stanojević *et al.* (2009) showed that aqueous ethanolic and methanolic extracts of *Hieracium pilosella* had high phenolics and flavonoid contents. Yen and Hsieh (1998) reported that the total phenolic content in *Eucommia ulmoides* ranged from 8700 to 21000 mg GAE/dry weight. Total phenolic content of *Moringa oleifera* in three different climates (India, Nicaragua and Niger) ranged from 2940 - 4250 mg gallic acid equivalent/g dry weight (Siddhuraju and Becker, 2003).

Further, the investigations of Senevirathne *et al.* (2006) supported the present study. According to them, the total phenolic content of different fractions of *Ecklonia cava* were solvent-dependent. Aqueous fractions of *E. cava* showed higher amounts of phenolics while their counterparts showed lower phenolic content. Kumar *et al.* (2008) showed the presence of large amounts of phenolics and flavonoids in the methanolic fruit extract of *Citrullus colocynthis* by preliminary phytochemical screening. Subsequent quantification showed the presence of 0.74% (m/m) phenolics (calculated as gallic acid) and 0.13% (m/m) flavonoids calculated as catechin equivalents per 100 g of fresh mass. The results obtained by Adedapo *et al.* (2008) also supported the present study that the level of the phenolic compounds in the methanol extracts of the leaves and stem of *Calpurnia aurea* were considerable.
5.2.2. Evaluation of Total Flavonoid Content in Plant Extracts

Flavonoids represent the most diverse and widespread group of plant phenolics contributing a major role in the metabolism of higher plants. This biologically active group of secondary metabolites can be found in fruits and vegetables (Bravo, 1998; Wolski and Dyduch, 2000; Horbowicz, 2000 and 2003; Sembratowicz and Czech, 2005; Mysiak and Tendaj, 2006). Flavonoids are capable of both preventing and eliminating the effects of reactive oxygen species (Politycka and Wójcik-Wojtkowiak, 2001; Gow-Chin et al., 2002; Kaur and Kapoor, 2002; Podsędek and Anders, 2002; Troszyńska et al., 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 contain hydroxyls, which are responsible for the radical scavenging effect (Younes, 1981; Das and Pereira, 1990; Mensor et al., 2001; Hou et al., 2003).

Several authors have investigated the effects of natural flavonoids (Silva et al., 2000; Sousa et al., 2004; Damasceno et al., 2004; Menezes et al., 2007; Volpato et al., 2008). Flavonoids exhibit multidirectional pharmacological action and the effects they produce in humans and animals are to a considerable degree dependent on their solubility in body fluids (Shahidi and Naczk, 1995; Bravo, 1998; Małolepsza and Urbanek, 2000). Flavonoid compounds strengthen the walls of capillary vessels and improve blood circulation in the heart muscle. They have a spasmodic, diuretic, anti-aggregation effect on blood platelets, and also have anti-inflammatory, anti-ulcerative, anti-allergic and anti-hepatotoxic action. They are known to inhibit lipid peroxidation in biological systems (Ferrali et al., 1997; Brown et al., 1998; Sugihara et al., 1999). Flavonoids reduce oxidative stress-induced tissue damage (Song et al., 2004). They also possess antifungal and antiviral properties and have an inhibiting effect on the action of some enzymes in biological system. Moreover, the flavonoids are important detoxifiers, as they easily form complex connections (chelates) with heavy metals (Robak and Gryglewski, 1996; Blackburn, 1997; Kohlmünzer, 2000; Sembratowicz and Czech, 2005). The antioxidative properties of flavonoids are due to several different mechanisms such as, scavenging of free radicals, chelation of metal ions such as iron and copper and inhibition of enzymes...

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responsible for free radical generation (Rice-Evans et al., 1997; Benavente-Garcia, 1997; Kessler et al., 2003).

In the present work, *Elaeocarpus serratus* registered the maximum amount of total flavonoids in the methanolic extract of fruit (100.27 ± 1.2 mg rutin/g dry weight), followed by the acetone extract of leaf (93.21 ± 0.1 mg/g) and methanol extract of bark (90.23 ± 0.6 mg/g). In *Elaeocarpus tuberculatus*, the maximum amount of total flavonoids was registered in the acetone extract of leaf (96.67 ± 3.84 mg/g dry weight), followed by the acetone extract of stem bark and methanol extract of leaf (53.31 ± 6.25 and 51.97 ± 2.23 mg/g dry weight, respectively). Generally, the lowest content of total flavonoids was recorded in the water extract of leaf, stem bark and fruit of *Elaeocarpus serratus* and *Elaeocarpus tuberculatus*. Similarly, 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± 0.3 mg rutin equivalents/g of dry material. Singh et al. (2010) reported the presence of flavonoids in the methanol extract of *Elaeocarpus sphaericus* fruits. Further, citrus fruits are an important source of bioactive compounds including antioxidants such as ascorbic acid, flavonoids, phenolic compounds (Fernandez-Lopez et al., 2005; Jayaprakasha and Patil, 2007; Ebrahimzadeh et al., 2004).

Moreover some researchers reported that flavanones, flavones and flavonols were the three types of flavonoids found in citrus fruit (Calabro et al., 2004; Ebrahimzadeh et al., 2004; Fernandez-Lopez et al., 2005; Jayaprakasha and Patil, 2007). The peel contained the highest concentrations of flavonoids in the citrus fruit (Manthley and Grohmann, 1996 and 2001; Anagnostopoulou et al., 2006). Non-polar flavonoids have been isolated from bay leaves (Demo et al., 1998; Elmastaş et al., 2004). Phytochemical and pharmacological studies of *Bauhinia* species have demonstrated the presence of flavonoids (Silva and Cechinel-Filho, 2002).
Pourmorad et al. (2006) carried out a systematic record of the relative free radical scavenging activity in selected Iranian medicinal plant species namely, *Mellilotus officinalis* (Fabaceae), *Equisetum maximum* (Equisetaceae), *Plantago major* (Plantaginaceae), *Adiantum capillus-veneris* (Adiantaceae) and *Urtica dioica* (Urticaceae) and found the relationship of total flavonoid and phenol contents with antioxidant activity. Zakaria (2007) demonstrated the presence of flavonoids, saponins, triterpenes and steroids in *Muntingia calabura, Dicranopteris linearis, Melastoma malabathricum, Bauhinia purpurea* and *Corchorus capsularis*. Harisha et al. (2011) demonstrated the presence of alkaloids, tannins, glycosides and flavonoids in *Cissus rependa*. Presence of these phyto-constituents in *C. rependa* supported the claim that these compounds had anti-inflammatory properties. Similarly, the presence of flavonoids in *Cassia fistula* ((Yadava and Verma, 2003) might be responsible for the anti-inflammatory and antioxidant effects. Further, plant extracts with a high phenolic content also contained high flavonoid content (Maisuthisakul et al., 2007).

5.2.3. Evaluation of Tannin Content in Plant Extracts

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). Preliminary phytochemical studies showed the presence of tannins and flavonoids in ethanolic extract of *Ficus bengalensis* (Pratt, 1992; Dreosti, 2000). According to Verma et al. (2008) the antioxidant potential of ethanolic extract of *F. bengalensis* fruits could be attributed due to the presence of polyphenolic compounds. Similarly, Latte and Kolodziej (2004) reported that the tannins of *Pelargonium reniforme*, possessed more antioxidant potential than the flavonoids. They ascribed the marked antioxidant activities of the hydrolysable tannins to the presence of galloyl and hexahydroxydiphenoyl groups, and carbonyl (ester) functionalities in oxidatively modified hexahydroxydiphenoyl moieties.

Ayoola et al. (2008b) observed that the presence of flavonoids and tannins in leaves of *Carica papaya, Psidium guajava, Vernonia amygdalina* and stem bark of
*Magnifera indica* was responsible for the free radical scavenging effects. The chemical composition of *Blepharis linearifolia, Dicliptera verticillata, Dyschoriste perrottetii, Hygrophila auriculata, Lepidagathis anobrya, Nelsonia canescens* indicated the presence of tannins and flavonoids (Nacoulma, 1996) which were known to possess antioxidant activities (Badami *et al.*, 2003; Aderogba *et al.*, 2005; Motalleb *et al.*, 2005). Tannins were detected by Zakaria (2007) in the leaves of *Muntingia calabura, Dicranopteris linearis* and *Melastoma malabathricum*. The ability to scavenge free radicals indicated that these plants could be used as a new source of antioxidant agents, and the activity could be attributed to the synergistic effect of various bioactive compounds present in these extracts.

In the present investigation, the tannin content in the various solvent (acetone, methanol and water) extracts of leaf, stem bark and fruit of *Elaeocarpus serratus* was high as compared to that of *Elaeocarpus tuberculatus*. In *E. serratus*, the highest content of tannin was registered in the stem bark acetone extract (355.72 ± 1.8 mg/g dry weight), followed by the leaf acetone extract (271.21 ± 1.1 mg/g) and methanolic extract of fruit (207.90 ± 2.7 mg/g), respectively. In *E. tuberculatus*, the maximum amount of tannin was recorded in the acetone extract of stem bark (66.78 ± 1.64 mg/g dry weight), followed by the methanol extract of leaf and fruit (58.14 ± 0.50 and 29.86 ± 0.50 mg/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.* (2010) reported the presence of tannins in the chloroform and methanol extracts of *Elaeocarpus sphaericus* fruits.

Considering the high concentration of phenolics in the different solvent extracts of various parts of *E. serratus* and *E. tuberculatus*, these plants could represent a potential natural source of antioxidants and some of its pharmacological effects could be attributed to the presence of these valuable constituents.
5.3. Assessment of *In vitro* Antioxidant Activity of Plant Extracts

A great number of *in vitro* methods have been developed to measure the efficiency of natural antioxidants either as pure compounds or as plant crude extracts since it is relatively easier to evaluate antioxidant properties in an *in vitro* than *in vivo* system because of the fact that in an *in vivo* system a variety of the radical species with varying rates of reactivity are generated (Damayanti *et al*., 2010). *In vitro* methods can be divided into two major groups: 1) Hydrogen atom transfer reactions like β-carotene bleaching and 2) Electron transfer reactions like DPPH radical scavenging assay, Superoxide anion radical scavenging assay, Hydroxyl radical scavenging assay and Nitric oxide radical scavenging assay (Diplock, 1997; Diplock *et al*., 1998; Huang *et al*., 2005). These methods are popular due to their high speed and sensitivity. However, it is essential to use more than one method to evaluate antioxidant capacity of plant materials because of the complex nature of phytochemicals (Salazar *et al*., 2008; Chanda and Dave, 2009).

In the present study, evaluation of antioxidant activity of *Elaeocarpus* species (*Elaeocarpus serratus* and *Elaeocarpus tuberculatus*) by means of various *in vitro* assays (DPPH• scavenging assay, Hydroxyl radical (•OH) scavenging assay, Superoxide radical (O$_2$•−) scavenging assay, Nitric oxide radical (NO•) scavenging assay, Reducing power assay, ABTS**+ scavenging assay, β–carotene/linoleic acid peroxidation inhibition assay and Metal chelating assay) have been carried out to assess the antioxidant potential of both the test plants.

5.3.1. DPPH • Radical Scavenging Assay

The antioxidants can seize the free radical chain of oxidation and form stable free radicals, which would not initiate or propagate further oxidation (Schimada *et al*., 1992; Duh and Yen, 1997). 1,1-diphenyl-2-picryl hydrazyl (DPPH) stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of plant extracts spectrophotometrically (Chang *et al*., 2002; Koleva *et al*., 2002; Lee *et al*., 2003; Wang *et al*., 2004). DPPH radical scavenging by antioxidants has been attributed to their
hydrogen-donating ability (Chen and Ho, 1995; Mensor et al., 2001; Muchuweti et al., 2007). Possible mechanism of DPPH• scavenging was suggested to be through reduction (protonation) of this radical by antioxidant compound to a more stable DPPHH form. Because of its unpaired electron, DPPH has an absorption maxima at 517 nm and as it gets reduced in the presence of a free radical scavenger, the absorbance decreases stoichiometrically with respect to the number of electrons taken up (Koleva et al., 2002). Therefore, in radical scavenging assay the exposure of DPPH and antioxidant compounds cause the purple colour of DPPH change to yellow. More yellowish colour of DPPH showed more antioxidant activity of the extract (Sultana et al., 2007; Liu et al., 2008; Moein et al., 2008).

In the present study, the acetone, methanol and water extracts of leaf, stem bark and fruit of Elaeocarpus serratus and Elaeocarpus tuberculatus showed a dose-dependent scavenging of DPPH radicals. In E. serratus the values of 50% inhibition concentration was between 2.73 ± 1.09 μg/ml and 92.42 ± 2.29 μg/ml but in E. tuberculatus the IC50 values ranged from 3.92 ± 3.4 μg/ml to 58.14 ± 0.7 μg/ml. As confirmed from the present investigation, the leaf and stem bark extracts of various solvents exhibited remarkable DPPH radical scavenging activity. In general, the methanol and acetone extracts of stem bark had higher activity than that of the leaf and fruit.

Earlier, the methanol extract of fruit of Muntingia calabura (Elaeocarpaceae) exhibited potent DPPH• quenching capacity (Einbond et al., 2004). Similarly, Elmastaş et al. (2006) illustrated a significant decrease in the concentration of DPPH radicals due to the scavenging ability of both bay leaf (Laurus nobilis) extracts and standards. The ethanol extract of bay leaf showed a significantly stronger DPPH• scavenging activity than the water extract. BHA, BHT, and α-tocopherol were used as standards. In another study involving the screening of 78 other extracts from 20 Malian medicinal plants belonging to 14 families, Diallo et al. (2001) demonstrated with DPPH spray that 20% of the plants, including Cussonia barteri (Araliaceae), Glinus oppositofolius, Lannea velutina (Anacardiaceae) possessed potent antioxidant activity.
Further, Kansci et al. (2003) supported the present study. According to them, two plants, *Dorstenia psilurus* and *Dorstenia ciliata* used as both food ingredient and recipe in traditional medicine possessed strong anti-radical activity when evaluated with the DPPH˙ assay. Various parts of *Bombax ceiba* possessed strong antioxidant potential. Mangiferin, isolated from its leaves showed DPPH radical scavenging activity with an IC₅₀ value of 5.8 ± 0.96 μg/ml (Dar et al., 2005). Gum of *Bombax ceiba* also possessed good antioxidant potential in DPPH˙ and ABTS˙⁺ radical scavenging assay (Surveswaran et al., 2007). Recently, Vieira et al. (2009) reported the antioxidant activity of methanolic extract of flowers of *Bombax ceiba* against DPPH˙, hydroxyl radicals and lipid peroxidation. The banana is a potent secondary antioxidant which contains active components that bind to metal ions strongly (Yan et al., 2006). Banana though a weak primary antioxidant is however a powerful secondary antioxidant.

Najda et al. (2008) found that the aqueous extracts from the roots of caraway plants showed the ability to reduce the DPPH radical to diphenylpicrylhydrazine, whose amount increased with the increase in the concentration of the tested extracts. After 30 minutes, at the extract concentration of 80 μg/ml, the highest ability to scavenge the DPPH radical was shown by the extracts from the roots harvested in October - 47.32%. The effect of scavenging the DPPH radical by the extracts from the roots that had been harvested in the months June through September was considerably smaller and was at an average level of between 17.38% and 23.21% at the extract concentration of 80 μg/ml. The ability to reduce the DPPH radical by the extracts from the roots of caraway plants was several times greater in relation to the results obtained from the extracts of dried greens of dill (8-12%), parsley (3-6%) and the fruits of black pepper (6- 16%) and *Nigella sativa* (5-7%) (Gawlik-Dziki and Świeca, 2007).

Zakaria (2007) demonstrated the radical scavenging property of several plants, namely *Muntingia calabura, Dicranopteris linearis, Melastoma malabathricum, Bauhinia purpurea* and *Corchorus capsularis*. The aqueous extracts of the leaves of the selected plants assayed against the DPPH radical scavenging and xanthine/xanthine oxidase superoxide assays, were found to exhibit remarkable radical scavenging activities.
with the percentage of inhibition recorded for the former and latter assays ranged between 94 – 99% and 83 – 100%, respectively. All the above positive views are in accordance with the present investigation.

Similarly, the DPPH’ assay determined the ability of ethanolic extract of *Ficus bengalensis* to reduce DPPH radical to the corresponding hydrazine by converting the unpaired electrons to paired ones, which in fact is the action of the antioxidants. The dose-dependent inhibition of DPPH radical indicated that ethanolic extract of *F. bengalensis* caused reduction of DPPH radical in a stoichiometric manner (Sanchez-Moreno et al., 1999; Sanchez Moreno, 2002). Siriwardhana et al. (2003) have also reported higher DPPH scavenging activities for a water and methanol extracts of *Hizikia fusiformis* (a brown alga), while ethanol, chloroform and ethyl acetate extracts also indicated strong inhibition activities over 50%. Further, Suja et al. (2005) explained that the IC50 value of purified extracts of sesame cake was 5.49×10³ μg/ml.

The free radical scavenging activity of methanolic fruit extract of *Citrullus colocynthis* was evaluated by Kumar et al. (2008) using *in vitro* assay of DPPH’. The scavenging effect of the fruit extract on the DPPH radical was 88.0 ± 2.7%, at a concentration of 2500 μg/ml compared to the scavenging effects of ascorbic acid, BHA and α-tocopherol at 50 μg/ml of 89.5 ±1.1, 83.2 ± 1.1 and 67.5 ± 0.8%, respectively. The results of Souza et al. (2009) also supported the present investigation. They demonstrated that the free radical scavenging activity of the concentrated and resulting dried (spouted bed and spray dried) extracts of *Bauhinia forficata* were concentration-dependent. The maximum DPPH’ reduction promoted by the concentrated extract was approximately 76% at a concentration of 39.2 mg/ml, giving an IC50 = 16.2 mg/ml. A maximum DPPH’ inhibition of 69.2 and 70.4% was obtained for the spouted bed and spray dried extracts, respectively. These results were reached at concentrations of 55.5 mg/ml of the extract material, giving IC50 values of 12.2 and 12.9 mg/ml, respectively. The results further suggested that the slight decrease in the maximum scavenging activity of the dried extracts may be associated with the occurrence of oxidative reactions,
decomposition of thermolabile compounds or even losses of volatile substances induced by the heat.

Moreover, Sundararajan et al. (2006) demonstrated that the hydroalcoholic extract of *Cytisus scoparius* exhibited a significant dose-dependent inhibition of DPPH$^*$ activity, with an IC$_{50}$ value of 1.5 μg/ml. The IC$_{50}$ value of the extract was found to be lesser than the standard vitamin C (IC$_{50}$=3.0 μg/ml). The decrease in the concentration of DPPH radical due to scavenging ability of hydroalcoholic extract of *C. scoparius* and vitamin C was comparable to the reported value of Thabrew et al. (1998).

In support of the present investigation, Kulkarni et al. (2007) showed that sapota juice exhibited a significant and concentration-dependent scavenging of DPPH radicals at all the tested concentrations (10–100 μl/ml reaction mixture). The sapota juice effectively scavenged the DPPH radicals with an IC$_{50}$ value of 87.53 ± 4.5 μl/ml. The DPPH radical-scavenging potential of sapota juice could be attributed to the synergistic activity of its phenolics and ascorbic acid content. Further, Adedapo et al. (2008) showed that the DPPH radical scavenging abilities of the methanol extract of the leaves and stem of *Calpurnia aurea* were lower than those of ascorbic acid (100%) and BHT (98.3%). The extracts had the proton-donating ability and could serve as free radical scavengers, acting possibly as primary antioxidants.

Earlier, Stanojević et al. (2009) indicated that the water, ethanol and methanol extracts obtained from the whole plant of *Hieracium pilosella* had significant free radical scavenging activity on stable DPPH$^*$ and highly reactive hydroxyl radical. Nagore et al. (2010) estimated the antioxidant activity of *Cassia sophera* fractions using DPPH$^*$ assay. The results of the DPPH$^*$ assay revealed that the ethanol fraction was most capable of scavenging free radicals (IC$_{50}$ = 0.2 ± 0.1 mg / ml) in solution at pH 7.4 and might prevent initiation of free radical mediated chain reactions by preventing the abstraction of hydrogen from susceptible substrate in oxidative reactions.
5.3.2. Hydroxyl Radical (HO\textsuperscript{\dot{}}) Scavenging Assay

Hydroxyl radical is one of the potent reactive oxygen species in the biological system and it bears the shortest half-life compared with other ROS. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell (Halliwell and Gutteridge, 1988). In biochemical system, superoxide radical and hydrogen peroxide react together to form a singlet oxygen radical and hydroxyl radical and this can attack and destroy almost all known biochemicals (Chakraborti et al., 1990). The hydroxyl radical has the capacity to conjugate with nucleotides in DNA, cause strand breakage, and lead to carcinogenesis, mutagenesis and cytotoxicity (Ko et al., 1993; Kaneko et al., 1996; Schlesier, 2002). Hydroxyl radicals are the most reactive free radicals causing lipid peroxidation and enormous biological damage (Aurand et al., 1977; Canadanovic-Brunet et al., 2005).

The acetone, methanol and water extracts of leaf, stem bark and fruit of *Elaeocarpus serratus* and *Elaeocarpus tuberculatus* in the present study exhibited a dose-dependent scavenging activity against the HO\textsuperscript{\dot{}} species. The IC\textsubscript{50} values of water extract of fruit and acetone and methanol extracts of stem bark of *E. serratus* were 71.14 ± 1.23, 72.30 ± 1.96 and 75.99 ± 2.98 µg/ml, respectively. The IC\textsubscript{50} values of the *E. serratus* extracts were more than that of the standard equivalents indicating the weak antioxidant potential of the plant. *E.tuberculatus* extracts were comparably efficient in quenching the hydroxyl radicals than *E.serratus* extracts. The water extract of leaf and methanol, acetone and water extracts of stem bark exhibited moderate \textsuperscript{\dot{}}OH scavenging activity with IC\textsubscript{50} values of 18.20 ± 1.37, 18.75 ± 1.88, 19.65 ± 2.23 and 20.55 ± 1.42 µg/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 µg/ml of *E. ganitrus*.

Further, the results of Senevirathne et al. (2006) demonstrated that aqueous chloroform fraction and 70% methanol extract of *Ecklonia cava* exhibited higher hydroxyl radical scavenging activity (IC\textsubscript{50} = 0.023± 0.003 mg/ml) which was similar to that of BHT, but significantly higher than that of \(\alpha\)-tocopherol (IC\textsubscript{50} = 0.046 ± 0.002
mg/ml). Organic n-hexane fraction also showed strong activity (IC$_{50}$ = 0.025 ± 0.003mg/ml) which was almost similar to the activity of BHT but significantly higher than that of α-tocopherol. According to these results, hydrophilic phenolics are dominant in *Ecklonia cava* which attributed to the radical scavenging properties.

In accordance with the present study, Sundararajan *et al.* (2006) confirmed that the hydroalcoholic extract of *Cytisus scoparius* exhibited hydroxyl radical scavenging potential (IC$_{50}$ value of 27.0 μg/ml) which was lesser than that of the standard vitamin E (IC$_{50}$ value of 32.5 μg/ml). Furthermore, Singh *et al.* (2008) investigated the antioxidant activity of different fractions (R1, R2 and R3) obtained from pet ether extract of black pepper fruits (*Piper nigrum*). The fractions R2 and R3 in the doses of 1000 μg/ml inhibited 61.04 ± 5.11 % and 63.56 ± 4.17 % hydroxyl radicals generated by Fenton’s reaction, respectively. However the activity was lesser than that of the standard catechin which inhibited 70.95 ± 4.28 % hydroxyl radicals. The pet ether extract of *Piper nigrum* not only scavenged off the free radicals but also inhibited the generation of free radicals. It was already reported that naturally occurring phenolic compounds had free radical scavenging properties, due to their hydroxyl groups (Diplock, 1997). Further, phenolic compounds are effective hydrogen donors, which make them antioxidant (Rice-Evans *et al.*, 1995).

Hazra *et al.* (2008) studied the antioxidant potential of methanol extract of *Spondias pinnata* stem bark. Similar to the present investigation, the IC$_{50}$ value (112.18 ± 3.27 μg/ml) indicated that the plant extract was a better hydroxyl radical scavenger than the standard mannitol (IC$_{50}$ = 571.45 ± 20.12 μg/ml). *Cassia sophera* fractions exhibited a concentration-dependent scavenging activity against hydroxyl radical generated in Fenton reaction system. *Cassia sophera* treatment demonstrated scavenging of hydroxyl radicals ranging from 24.46 % to 95.46 % (Nagore *et al.*, 2010). Interestingly, Stanojević *et al.* (2009) investigated the antioxidant activity of the aqueous, ethanolic and methanolic extracts of *Hieracium pilosella* by the ability of the extracts to scavenge hydroxyl radicals. EC$_{50}$ values for aqueous, ethanolic and methanolic extracts were 0.279 ± 0.012, 0.283 ± 0.007 and 0.267 ± 0.005 mg/ml, respectively.
5.3.3. Superoxide radical (O$_2^{\cdot-}$) Scavenging Assay

Superoxide anion (O$_2^{\cdot-}$), the one-electron reduced form of molecular oxygen, is a precursor to active free radicals. Although superoxide is a relatively weak oxidant, it decomposes to form stronger reactive oxygen species, such as singlet oxygen and hydroxyl radicals, which initiates peroxidation of lipids and contribute to oxidative stress (Korycka-Dahl and Richardson 1978; Meyer and Isaken, 1995; Duh, 1998). Numerous biological reactions generate superoxide anions which are highly toxic species. During normal metabolic processes, human body generates more than 2 kg of O$_2^{\cdot-}$ per year (Evans and Halliwell, 1999). Superoxide anion radicals increase under stress conditions such as heavy exercise, certain drugs, infection and various disease states. Superoxide anion radicals exhibit powerful reactions with biological macromolecules and are also very harmful to cellular components thereby inducing tissue damage (Korycka-Dahl and Richardson, 1978; Halliwell and Gutteridge, 1981). In living organisms, superoxides are produced from molecular oxygen due to oxidative enzymes (Sainani et al., 1997) of body as well as via non-enzymatic reaction such as autoxidation by catecholamine (Hemmani and Parihar, 1998). Usually in a biological system, the toxic effect of superoxide anions are eliminated by superoxide dismutase (SOD) (Halliwell and Gutteridge, 1985; Wettasinghe and Shahidi, 2000).

In the present investigation, both the plants (Elaeocarpus serratus and Elaeocarpus tuberculatus) had strong superoxide radical scavenging activity. Inhibition of superoxide radicals was proportional to the amount of the extracts added. In E.serratus the scavenging capacity was comparably good in the methanol extract of stem bark and acetone, water and methanol extracts of leaf (IC$_{50}$ = 58.00 ± 0.66, 59.52 ± 0.79, 60.67 ± 0.52, 67.78 ± 0.37 µg/ml, respectively). This activity was comparable to the activity of gallic acid with an IC$_{50}$ value of 38.27 ± 0.28 µg/ml. 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 ± 0.67, 25.92 ± 1.40 and 29.17 ± 6.01 µg/ml, respectively) than the standard gallic acid (IC$_{50}$ = 38.27 ± 0.28 µg/ml). Hence, in the
Present investigation *Elaeocarpus serratus* and *Elaeocarpus tuberculatus* crude extracts had strong activities in superoxide anion scavenging.

In accordance with the present work Kulkarni et al. (2007) stated that the sapota juice showed a dose-dependent superoxide radical scavenging potential with an IC<sub>50</sub> value of 8.26 ± 1.4 µl/ml. Among the chemical components of sapota juice, the phenolics, *viz.* gallic acid, showed the highest superoxide radical scavenging potential (IC<sub>50</sub> = 12.32 ± 3.2 µl/ml), followed by catechin (IC<sub>50</sub> = 22.43 ± 0.6 µl/ml). The β-carotene showed negligible superoxide radical scavenging activity, whereas ascorbic acid induced the superoxide radicals. The induction of superoxide radicals by ascorbic acid has also been reported earlier (Paolini et al. 1999).

In fact, Senevirathne et al. (2006) found that in *Ecklonia cava* the highest superoxide anion scavenging activity was reported for the 70% methanol fraction (IC<sub>50</sub> = 0.051 ± 0.003mg /ml), which was significantly higher than that of α-tocopherol (IC<sub>50</sub> = 1.3 ± 0.03mg /ml). Next highest values (IC<sub>50</sub> = 0.367 ± 0.01 and 0.477 ± 0.04 mg/ml) were shown by aqueous ethyl acetate and aqueous n-hexane fractions, respectively. Those activities were slightly lower when compared with the activity of BHT but significantly higher than that of α-tocopherol. Rajapakshe et al. (2005) have reported IC<sub>50</sub> value of 20 µg /ml for fermented mussel sauce which is lower than the values obtained for 70% methanol crude extract of *E. cava* (IC<sub>50</sub> = 0.051 ± 0.003 mg /ml). Further, these values are significant when compared with values of Rajapakshe et al. (2005) up to second purified stage (IC<sub>50</sub> = 66 and 52 µg /ml in first and second steps of purification respectively). Moreover, present data was higher when compared with other values (IC<sub>50</sub> = 0.5 µg /ml) obtained for crude extract of potato peel (Singh and Rajini, 2004).

Hazra et al. (2008) the superoxide radical scavenging activities of methanol extract of *Spondias pinnata* stem bark and the reference compound (quercetin) increased markedly with increasing concentrations. The IC<sub>50</sub> values of the plant extract and quercetin on superoxide scavenging activity were 13.46 ± 0.66 µg /ml and 42.06 ± 1.35
μg/ml, respectively. The IC$_{50}$ value of the extract was less than that of the standard. At 20 μg/ml, the percentage inhibition of the plant extract was 55.2% whereas that of quercetin was 29.6%. Previously, Saenjum et al. (2010) supported the present study. They showed that *Caesalpinia sappan* extract exhibited high scavenging activity on superoxide anion with an EC$_{50}$ value of 7.73 ± 0.06 μg/ml, which was comparable to the activity of L-ascorbic acid and rutin (EC$_{50}$ value of 6.65±0.07 and 7.83±0.13 μg/ml, respectively). These results revealed that the *C. sappan* extract was an efficient scavenger of superoxide anion radical. According to the source of raw material and extraction process, the scavenging effects on superoxide anion was higher than those of Jun et al. (2008), who reported that 95% ethanolic extract of *Caesalpinia sappan* heartwood showed little scavenging effects on superoxide anion compared to L-ascorbic acid. In general, the results suggested that the plants’ extracts were more potent scavengers of superoxide radical than the standards.

### 5.3.4. Nitric Oxide (NO·) Scavenging Assay

Nitric oxide (NO$^-$) is a defense molecule with cytotoxic, microbiocidal and microbiostatic activities. It is an important chemical mediator generated by endothelial cells, macrophages, neurons etc., and is involved in the regulation of various physiological processes like neural signal transmission, immune response, cardiovascular dilation and blood pressure (Lata and Ahuja, 2003). Despite these possible beneficial effects of NO$^-$, its contribution to oxidative damage is increasingly becoming evident. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions (ONOO$^-$) which act as free radicals (Huie and Padmaja, 1993; Cotran *et al*., 1999; Sainani *et al*., 1997). Further, NO$^-$ is a potential strong oxidant that can decompose to produce 'OH and NO$_2$ causing toxic reactions with biomolecules, like protein, lipids and nucleic acids (Moncada *et al*., 1991; Radi *et al*., 1991a and 1991b; Yermilov *et al*., 1995; Awah *et al*., 2010).

Excess concentration of NO$^-$ is associated with several diseases (Moncada *et al*., 1991; Ialenti *et al*., 1993; Ross, 1993; Preethi *et al*., 2010). Sustained levels of production of this radical are directly toxic to tissues and contribute to the vascular collapse whereas,
chronic expression of nitric oxide radical is associated with various carcinomas and inflammatory processes including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis (Tylor et al., 1997; Middleton et al., 2000; Olszanecki et al., 2002). Sodium nitroprusside served as a chief source of free radicals. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthylethylene diamine was used as the marker for NO\(^\cdot\) scavenging activity (Mukherjee, 1989). In the present work, the chromophore formation was not complete in the presence of \textit{Elaeocarpus serratus} and \textit{Elaeocarpus tuberculatus} extracts which scavenged the NO\(^\cdot\) formed from the sodium nitroprusside and hence the absorbance decreased as the concentration of the extracts increased in a dose-dependent manner. The acetone extract of fruit of \textit{E.serratus} showed the lowest IC\(_{50}\) value = 64.12 ± 0.44 µg/ml, followed by acetone extract of leaf (IC\(_{50}\) value = 66.53 ± 0.37 µg/ml) indicating their potent antioxidant activity. Almost all the plant parts of \textit{E.tuberculatus} in the various solvent extracts showed strong activities on NO\(^\cdot\) scavenging with the IC\(_{50}\) value of extracts lesser than that of the standards BHA (43.37 ± 1.26 µg/ml) and Gallic acid (29.76 ± 0.81 µg/ml). The acetone, methanol and water extracts of the leaf and stem bark showed higher activities with IC\(_{50}\) = 19.81 ± 0.75, 22.08 ±1.17, 21.38 ±3.90, 20.91 ±1.52, 23.19 ±1.83 and 23.46 ± 0.55 µg/ml, respectively.

Similar to the present study, \textit{Spondias pinnata} extract caused a moderate dose-dependent inhibition of nitric oxide with an IC\(_{50}\) of 24.48 ± 2.31 µg/ml. Curcumin was used as a reference compound and 90.82 ± 4.75 µg/ml curcumin was needed for 50% inhibition. The IC\(_{50}\) value of the extract was less than that of the standard. At 70 µg/ml, the percentage inhibition of the plant extract was 61.2%, whereas that of curcumin was 44.1% which proved that the extract had more potent nitric oxide scavenging activity than the standard curcumin (Hazra et al., 2008). The antioxidant potential in herbal barks extract of five therapeutically important medicinal plants native to India was investigated by Kumari and Kakkar (2008). They are \textit{Crataeva nurvala} (Capparidaceae), \textit{Buchanania lanzan} (Anacardium), \textit{Aegle marmelos} (Rutaceae), \textit{Dalbergia sissoo} (Fabaceae) and \textit{Cedrela toona} (Meliaceae). All of them showed high NO\(^\cdot\) quenching capacity and the
highest was recorded in *Crataeva nurvala*. This was in agreement with the present findings.

In confirmation with the present research, Senevirathne *et al.* (2006) found that the organic ethyl acetate and aqueous chloroform fractions of *Ecklonia cava* showed strong NO$^\cdot$ scavenging activity ($IC_{50} = 0.33 \pm 0.05$ and $0.33 \pm 0.03$ mg/ml, respectively). The activity was significantly higher than that of BHT and $\alpha$-tocopherol ($IC_{50} = 1.59 \pm 0.2$ and $2.1 \pm 0.6$ mg/ml, respectively). All aqueous fractions also showed significantly higher activity than commercial antioxidants tested. Aqueous fractions of $n$-hexane and chloroform showed higher activity than their organic counterparts but organic ethyl acetate fraction showed higher activity than its aqueous counterpart. Suppression of NO$^\cdot$ released may be partially attributed to direct NO$^\cdot$ scavenging, as all fractions of *Ecklonia cava* decreased the amount of nitrite generated from the decomposition of sodium nitroprusside in vitro. Similarly, Mayur *et al.* (2010) studied the nitric oxide scavenging of methanolic extract of the aerial parts of *Carpesium abrotanoides* In this study, the methanolic extract of *C. abrotanoides* in sodium nitroprusside solution decreased the levels of nitrite, a stable oxidation product of NO$^\cdot$ liberated from sodium nitroprusside in a dose-dependent manner ($IC_{50} = 798.5 \mu g/ml$).

Nitric oxide radical inhibition study of Sundararajan *et al.* (2006) proved that the hydroalcoholic extract of aerial part of *Cytisus scoparius* is a potent scavenger of nitric oxide with an $IC_{50}$ value of 116.0 $\mu g/ml$ which was found to be lesser than the standard rutin ($IC_{50} = 160.0 \mu g/ml$). The $IC_{50}$ value of rutin is comparable to the reported value of Badami *et al.* (2003). According to Preethi *et al.* (2010) NO$^\cdot$ scavenging activity of methanol extract of fruit of *Muntingia calabura* was higher ($IC_{50} = 187 \pm 0.04 \mu g/ml$) compared to other extracts. The NO$^\cdot$ scavenging activity ranged in the following descending order: methanol ($IC_{50} = 187 \pm 0.6 \mu g /ml$) > butanol ($IC_{50} = 189 \pm 0.26 \mu g /ml$) > pet ether ($IC_{50} = 207 \pm 0.02 \mu g /ml$) > chloroform ($IC_{50} = 250 \pm 0.08 \mu g / ml$) > ethyl acetate extract ($IC_{50} = 497.2 \pm 0.08 \mu g /ml$).Verma *et al.* (2008) showed that the ethanolic extract of fruit of *Ficus bengalensis* competed with oxygen to react with nitric oxide and thus inhibited the generation of the NO anions. At a concentration of 14.81
μg/ml of extract, 50% of nitric oxide generated by incubation was scavenged which indicated its antioxidant property.

5.3.5. Reducing Power Assay

Reducing power assay is a convenient and rapid method for measuring the antioxidant potential (Oyaizu, 1986; Oktay et al., 2003; Chanda et al., 2011). Reducing power of a compound is related to electron transfer ability of that compound and therefore, the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Meir et al., 1995). Reducing power is evaluated by the transformation of Fe (III) to Fe (II) in the presence of the sample extracts (Gülçin et al., 2003). Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Yen and Chen, 1995; Jayprakash et al., 2001; Khanam et al., 2004).

In the present investigation, Elaeocarpus serratus and Elaeocarpus tuberculatus exhibited a dose-dependent increase in reducing power which in turn suggested the antioxidant potential of the plant extracts. The methanolic extract of leaf of E. serratus showed a maximum absorbance of 2.027 at 100 μg/ml concentration, followed by methanolic extract of stem bark (1.625 at 100 μg/ml concentration). This was comparable to the positive control BHA and gallic acid which showed higher reducing power of 1.728 and 1.691, respectively at the concentration of 100μg/ml. In the case of E. tuberculatus, the acetone extract of the bark at 80μg/ml reduced most of Fe^{3+} ions having a reducing power of 1.018 which was comparatively higher than 80μg/ml of BHA standard (0.908). Sundararajan et al. (2006) also confirmed that the reducing power increased with increase in the amount of plant extract. Similarly, Satish Kumar et al. (2008) showed that the reducing power of ethanolic extract of leaves of Elaeocarpus ganitrus ranged from 1.112 to 1.973 for 100 μg/ml to 400 μg/ml of extract. Further, the reducing ability of Bombax ceiba root was found to increase with rising concentrations of methanolic extract and 500 μg/ml of the extract was shown to have maximum reducing power. The reducing property of ethanolic extract of Ficus bengalensis implied that it
was capable of donating hydrogen atom in a dose-dependent manner (Verma et al., 2008).

Similar results were obtained by Nagore et al. (2010) who demonstrated that the reducing power of *Cassia sophera* was enhanced with the increasing concentration of samples. The reducing power of ethanol fraction of *Cassia sophera* was found to be concentration-dependent and showed significantly higher activity than that of the control. The reducing power of fractions was found to be in the order like ethanol fraction > ethanol extract > ethyl acetate fraction > chloroform fraction.

The results of the present findings are in line with the previous studies of Huda-Faujan et al. (2009). According to them, the reducing power of methanolic extracts of *Cosmos caudatus, Polygonum minus, Oenanthe javanica, Centella asiatica* and *Murraya koenigii* increased with the increase in extract concentration. The data showed that all the samples increased their reducing ability when the concentration of extracts was increased. This result was also similar to that reported by Gülçin et al. (2003) and Noriham et al. (2004), who demonstrated antioxidative activity on *Pimpinella anisum* seeds extracts and four types of Malaysian plants. At 200 ppm, BHA had the highest ability to reduce Fe (III) and had no significant difference with *P. minus*. The ability of reducing power of methanolic extract of *P. minus* showed almost similar with synthetic antioxidant, BHA at 600, 800, 1000 and 1200 ppm. At 1200 ppm, all methanolic plant extracts have higher ability than BHT to reduce Fe (III) to Fe (II). The ability to reduce Fe (III) may be attributed from hydrogen donation from phenolic compounds (Shimada et al., 1992) which is also related to presence of reductant agent (Duh, 1998). Guo et al. (2001) have reported that the aqueous and methanol extracts of stem and leaf of broccoli showed higher reducing power at the concentration of 4 mg/ml but lower reducing abilities than that of the fractions of *Ecklonia cava* (2mg/ml level showed highest reducing abilities).

In support of the present study, the results obtained by Hazra et al. (2008) has shown that at 0.1 mg/ml, the absorbances of the methanol extract of *Spondias pinnata* stem bark and reference compound BHT were 0.32 and 0.02 nm, respectively while at 0.4
mg/ml, the absorbances of both extract and BHT were almost the same. This result indicated that maximum activity was shown at a lower dose by the extract (0.1 mg/ml) than by BHT. Therefore the reducing power of the plant extract as compared with the standard BHT was found to be superior.

Senevirathne et al. (2006) showed the reducing capacities of different fractions (respective organic and aqueous fractions of n-hexane, chloroform and ethyl acetate) of 70% methanol extract of *Ecklonia cava* (a brown seaweed). The organic ethyl acetate fraction of *E. cava* showed the highest reducing ability of all other fractions tested. That activity was higher than that of BHT and α-tocopherol. Aqueous chloroform, n-hexane, ethyl acetate fractions and 70% methanol extract showed higher activities indicating that more hydrophilic phenolics are present in those fractions which affect those interesting values in reducing capacities. Also the reducing ability of each fraction was dose-dependent and significantly higher than the control. The presence of reductants in the fractions *Ecklonia cava* extract caused the reduction of the Fe$^{3+}$/ferricyanide complex to the ferrous form. Similarly, Kuda et al. (2005) have reported that crude fucoidan and crude alginate showed the reducing abilities at the concentration of 10mg/ml which were lower than that of *E. cava* reducing abilities. At the concentration of 0.8mg/ml chitosan, the highest absorbance value reached by a purified sample was 0.26 (Xing et al., 2005) while *E. cava* fractions were above 1.0 absorbance except for organic n-hexane fraction, indicating that crude extract of *Ecklonia cava* showed excellent activities in reducing power.

Similar to the present study, Sultana et al. (2009) showed that the aqueous organic solvent extracts of the tested plant materials (extracts of barks of *Azadirachta indica*, *Acacia nilotica*, *Eugenia jambolana*, *Terminalia arjuna*, leaves and roots of *Moringa oleifera*, fruits of *Ficus religiosa*, and leaves of *Aloe barbadensis*) depicted good reducing power. The reducing potential of antioxidant components is very much associated with their total phenolic content. The plant extracts with higher levels of total phenolics also exhibited greater reducing power (Siddhuraju and Becker, 2003; Cheng, et al., 2006; Sultana et al., 2007).
5.3.6. ABTS** Radical Scavenging Assay

Generation of the ABTS radical cation forms the basis of one of the spectrophotometric methods that have been applied to the measurement of the total antioxidant activity of solutions of pure substances (Rice-Evans et al., 1995; Miller et al., 1996), aqueous mixtures and beverages (Salah et al., 1995; Rice-Evans and Miller, 1995). The ABTS**, a protonated radical generated from oxidation of ABTS by potassium persulfate, is presented as an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants (scavengers of aqueous phase radicals) and of chain-breaking antioxidants (scavengers of lipid peroxyl radicals) (Leong and Shui, 2002; Mathew and Abraham, 2006). The decolorization of ABTS radical cation is an unambiguous way to measure the antioxidant activity of phenolic compounds.

Addition of *Elaeocarpus serratus* and *Elaeocarpus tuberculatus* extracts to the pre-formed ABTS** reduced it to ABTS in a concentration-dependent manner. The results were compared with those obtained using Trolox and the Trolox equivalent antioxidant capacity (TEAC) values demonstrated that the extracts were potent antioxidant. *E.serratus* extracts showed potential activity in ABTS** decolorization with the antioxidant capacities of the extracts ranging from 4840.7 ± 33.41 to 19796.6 ± 32.4 µmol TAA/g. The methanol extract of stem bark was a fast and effective scavenger of ABTS** radical which showed the highest value of 19796.6 ± 32.4 µmol TAA/g extract. Similar trend was noticed in *E. tuberculatus* extracts with maximum activity seen in the methanol extract of stem bark (19960.6 ± 28.9 µmol/g), closely followed by the acetone extract of stem bark (19507.4 ± 169.5 µmol/g). Generally the ABTS** scavenging activity was higher in *E.tuberculatus* than *E.serratus*. Decolorization of ABTS** in the present study reflected the capacity of the plant extracts to donate electrons or hydrogen atoms to inactivate this radical cation. These experimental results are in line with the following previous data. Using the Trolox assay, Cook et al. (1998) estimated the antioxidant activity of 17 wild edible plants of Niger Republic used for food and traditional medicine. They observed that *Balanites aegyptiaca, Bombax costatum, Boscia*
senegalensis, Entada africana, Gynandropsis gynandra, Hyphaene thebaica, Leptadenia hastate, Sesbania pachcarpa and Tapinanthis globiferus possessed strong antioxidant activity, while *Parinari macrophylla* had the lowest. The antioxidant capacity of holy basil was reported by Juliani and Simon (2002) using the ABTS** assay. The TEAC value was 297 μmol Trolox equivalents per gram of dry weight. Arts *et al.* (2004) found that some products of ABTS** scavenging reaction may have a higher antioxidant capacity and can continually react with ABTS**.

Satish Kumar *et al.* (2008) demonstrated the dose-dependent activity of ethanolic extract of leaf of *Elaeocarpus ganitrus* in ABTS** decolorization. 55.77 % inhibition was noted with 500 μg/ml of *E. ganitrus*. IC50 value was found to be 297.12 μg/ml *E. ganitrus*. Kumaraswamy and Satish (2008) evaluated the antioxidant activity of *Woodfordinia fructicosa* by ABTS** assay. Significant ABTS free radical scavenging activity was evident in methanol and water extracts of *Woodfordinia fructicosa* (IC50 = 6.4 and 7.15 μg/ml respectively). According to Adedapo *et al.* (2008) the methanol extract of the leaves and stems of *Calpurnia aurea* were fast and effective scavengers of the ABTS radical and this activity was comparable to that of BHT. Higher concentrations of the extracts were more effective in quenching free radicals in the system. Similarly, Hazra *et al.* (2008) evaluated the antioxidant capacity of bark extract of *Spondias pinnata* using an improved ABTS radical cation decolorization assay. The extract showed total antioxidant activity with a trolox equivalent antioxidant capacity value of 0.78 ± 0.02 mg Trolox /mg extract.

Saenjum *et al.* (2010) observed that *Caesalpinia sappan* extract exerted strong scavenging activity on ABTS** with the Trolox equivalent antioxidant capacity = 0.9159 ± 0.0055 gram Trolox/gram extract. Further, Mayur *et al.* (2010) evaluated the antioxidant activity of methanolic extract of the aerial parts of *Carpesium abrotanoides* using the ABTS** assay and found that the extract effectively scavenged the ABTS radical cation with IC50 = 15.6 μg / ml. It was observed that the ABTS** reducing property is directly proportional to the amount of phenolics. Therefore, the potent ABTS** reduction observed in this assay might be due to the phenolics compounds.
present in the extract. According to Adedapo et al. (2009) the methanol extracts of the leaves and stems of *Celtis africana* were fast and effective scavengers of the ABTS radical and this activity was comparable to that of BHT. At 0.02mg/ml, the extracts exhibited higher activity than BHT, but at 0.1 mg/ml the activity of the extracts were similar to that of BHT. The percentage inhibition was 98.8, 98.8, and 99.3% for the leaf and stem extracts and BHT respectively, at 0.1 mg/ml concentration.

5.3.7. β – Carotene / Linoleic Acid Peroxidation Inhibition Activity

Lipids, proteins and DNA are susceptible to attack by free radicals (Yu et al., 1992; Cotran et al., 1999). Lipid peroxidation is a free radical-mediated propagation of oxidative damage to polyunsaturated fatty acids involving several types of free radicals. Hydrogen abstraction is easier in unsaturated fatty acids than in their saturated counterparts, thus making them more susceptible to ROS attack. In biological systems, lipid peroxidation generates a number of degradation products, which has been found to be an important cause of cell membrane destruction and cell damage (Janero 1990; Box and Maccubbin, 1997; Yoshikawa et al., 1997). The termination of lipid peroxidation occurs in biological system through enzymatic means or by radical-scavenging activity by antioxidants (Heim et al. 2002). Antioxidants may offer resistance against oxidative stress by scavenging the free radicals, inhibiting lipid peroxidation.

In β-carotene bleaching assay, the various solvent extracts of leaf, stem bark and fruit of *Elaeocarpus serratus* and *Elaeocarpus tuberculatus* showed moderate to high antioxidant capacity. The methanolic extract of stem bark of *E. serratus* and acetone extract of fruit and leaf of *E. tuberculatus* showed considerably strong antioxidant response with an inhibition of $87.95 \pm 0.79$, $89.50 \pm 5.41$ and $87.91 \pm 3.92\%$, respectively which was comparable with that of the standard BHA and gallic acid ($91.37 \pm 2.2$ and $94.11 \pm 1.5\%$, respectively).

The antioxidant activity of spice extracts was retained even after boiling for 30 min at 100°C, indicating that the spice constituents were resistant to thermal denaturation (Shobana and Naidu, 2000; Shan et al., 2005). The antioxidant activity of these dietary
spices suggested that they possessed potential health benefits by inhibiting the lipid peroxidation (Jessie and Krishnakantha 2005). Some of the dietary constituents commonly used in Indian foods such as cloves (Syzygium aromaticum, Myrtaceae), licorice (Glycyrrhiza glabra, Fabaceae), mace (aril of Myristica fragrans, Myristicaceae) and greater cardamom (Amomum subulatum, Zingiberaceae), were selected as the test samples to find their effects on the inhibition of lipid peroxidation (Tapsell et al., 2006). The results showed that the spices had significant ability to inhibit lipid peroxidation due to their polyphenol content, strong reducing power and superoxide radical scavenging activity. Cloves showed the highest antioxidant activity probably due to the higher polyphenol content as compared to other spices (Tapsell et al., 2006).

Shobana and Naidu (2000) studied the bioactive effects of spices and herbs using water and alcoholic extract (1:1) of commonly used spices (garlic, ginger, onion, mint, cloves, cinnamon and pepper). Their results revealed a dose-dependent oxidation inhibition of fatty acid and linoleic acid in the presence of soybean lipoxygenase. Among the spices tested, cloves exhibited the highest effect while onion showed the least antioxidant activity. The relative antioxidant activities decreased in the order of cloves, cinnamon, pepper, ginger, garlic, mint and onion. Spice mix namely ginger, onion and garlic; onion and ginger; ginger and garlic showed cumulative inhibition of lipid peroxidation thus exhibiting their synergistic antioxidant activity.

Moreover, Jayaprakasha et al. (2001) suggested that the bleaching mechanism of β-carotene was a free radical mediated phenomenon resulting from the formation of hydroperoxides from linoleic acid. In the absence of antioxidant, β-carotene undergoes rapid discoloration indicated by the decrease in absorbance. The inhibition of the β-carotene activity by Pangi (Pangium edule) seed extract at 1mg/ml was 58% and this result was slightly lower than the guarana (Paullina cupana) seed extract (73%) (Majhenic et al., 2007). This may be due to the higher temperature (75-100°C) used in the guarana seed extraction, which increased the amount of extractable phenolic compounds as compared to the Pangi seed extraction at room temperature. The use of
mixed solvents (acetone/ethyl acetate, water, acetic acid) in the extraction of the Pangi seed gave higher inhibition of β-carotene bleaching activity.

Pandey and Tripathi (2010) demonstrated a significant and concentration-dependent inhibition of FeSO$_4$ induced lipid peroxidation by tuberosin, an active compound isolated from root-tubers of *Pueraria tuberose*. Tuberosin had 7.95 fold lower EC$_{50}$ value (98 μg/ml) as compared to the alcoholic extract of *Pueraria tuberose* (780 μg/ml). Tuberosin exhibited free radical trapping capacity in a chemical reaction system, however, variability in its potency towards various free radical species, could be because of the difference in the electron potential of these free radical species (Pasha et al., 2007). According to Souza et al. (2009) the inhibition of lipid peroxidation by concentrated, spouted bed and spray dried extracts of *Bauhinia forficata* was also concentration-dependent. The IC$_{50}$ values obtained for the concentrated, spouted bed and spray dried extracts were 22.5, 25.9, and 19.4 mg/ml, respectively. Thus, the *B. forficata* extracts were able to inhibit the lipid peroxidation, evidencing their strong antioxidant properties. Similarly, Verma et al. (2008) stated that the ethanolic extract of *Ficus bengalensis* showed a dose-dependent prevention towards generation of lipid peroxides.

Further, Chye and Sim (2009) analysed the antioxidative activity of phenolic and alkaloid extracts of *Pangium edule* (Pangi) seed, reflected by its ability to inhibit the bleaching of β-carotene. The phenolic extracts of the Pangi seed had higher antioxidant activity than the other extracts for all concentrations tested. This can be explained by the presence of greater phenolic compounds due to the breakdown of conjugated phenolics by the β-glycosidase at higher concentrations, which allowed it to react with β-carotene and inhibit the bleaching activity. Similarly, 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 80 μg/ml concentration, *R. flava* extract, BHA and α-tocopherol showed 73.3, 96.4 and 98.6% of inhibition, respectively, whereas in 160 μg/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.

Gülçin et al. (2004) studied the effects of various amounts of water extract of nettle (*Urtica dioica*) (from 50 to 250 μg) on peroxidation of linoleic acid emulsion. The antioxidant activity of the extract increased concentration dependently. Water extract of *U. dioica* (50, 100 and 250 μg) showed higher antioxidant activities than that of 100 μg concentration of α-tocopherol. After incubation, the percentage inhibition of peroxidation in linoleic acid emulsion was 39, 66 and 98%, respectively and greater than that of α-tocopherol (30%). *Urtica dioica* demonstrated a marked capacity for iron binding, which suggested that their action as peroxidation protector may be related to its iron binding capacity.

Dash et al. (2005) also showed that the inhibition of lipid peroxidation by different concentrations of methanol extract of *Heracleum nepalense* root, ethyl acetate fraction and vitamin E was in a dose-dependent manner. The methanol extract at 1000 μg/ml exhibited maximum inhibition (69.25 ± 1.21%) of lipid peroxidation. On the other hand, ethyl acetate fraction at 50 μg/ml concentration showed (72.38 ±1.9%) inhibition, nearly equal to the inhibition produced by vitamin E (73.42 ± 2.3%). The inhibition could be caused by the absence of ferryl-perferryl complex or by changing the ratio of Fe³⁺ / Fe²⁺ or by reducing the rate of conversion of ferrous to ferric or by chelating the iron itself or combination thereof (Braugghler et al., 1986).

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 (IC₅₀ = 110.4 ± 0.64 μg/ml) extract was able to inhibit lipid peroxidation efficiently. The ethyl acetate and petroleum ether extracts exhibited moderate lipid peroxidation inhibitory activity with IC₅₀ values of 190.2 ± 0.62 μg/ml and 240.2 ± 0.04 μg/ml, respectively. Chloroform and butanol extracts showed minimum activity (IC₅₀ = 490.23 ± 0.24 μg/ml and 540.1 ± 0.02 μg/ml,
respectively). The results of the present investigation are in agreement with the observations of the above previous works.

In contrast, Kulkarni et al. (2007) affirmed that sapota juice showed a very strong lipid peroxidation inhibitory activity at a very low concentration (10 – 40 μl /ml), with an IC\(_{50}\) of 23.23 ± 3.0 μl /ml). Further, the increase in the sapota juice concentration brought only marginal increase in its lipid peroxidation inhibitory potential. The potential of sapota juice in inhibiting lipid peroxidation could be attributed to its phenolics and carotenoids.

5.3.8. Metal Chelating Activity

Chelating agents may serve as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of metal ion (Gordon, 1990). The transition metal ion, Fe\(^{2+}\) possess the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals (Aboul- Enein et al., 2003). Metal chelating activity is significant since it reduces the concentration of the catalyzing transition metal in lipid peroxidation (Duh et al., 1999; Naidu et al., 2008).

In the present study, E. serratus leaf, stem bark and fruit extracts with acetone, methanol and water showed good chelating activity. The highest metal chelating activity was exhibited by the water extract of fruit (1079.1 ± 2.4 mg EDTA/g extract), followed by methanol extract of fruit and stem bark (1074.9 ± 2.4 and 1071.9 ± 3.5 mg EDTA/g extract, respectively). In E. tuberculatus the highest metal chelating activity was exhibited by the water extracts of leaf, stem bark and fruit (1187.1 ± 1.6, 1096.2 ± 2.5 and 955.6 ± 5.2 mg EDTA/g, respectively). Accordingly, it is suggested that the high ferrous ion chelating effects of these fractions would be somewhat beneficial to protect against oxidation damage. The results are in accordance with Gülçin et al. (2004).

Earlier, Preethi et al. (2010) demonstrated the concentration- dependent chelating effects of fruit extracts from Muntingia calabura (Elaeocarpaceae) on the Fe\(^{2+}\) - ferrozine
complex. The methanol extract displayed the highest chelating activity with an IC$_{50}$ value of 80.26 ± 0.08 μg/ml followed by the ethyl acetate (IC$_{50}$ = 81.4 ± 0.04 μg/ml), chloroform (IC$_{50}$ = 91.2 ± 0.64 μg/ml), butanol (IC$_{50}$ 290.2 ± 0.24 μg/ml) and pet ether (IC$_{50}$ = 480.6 ± 0.02 μg/ml) extracts.

Similar trend was noticed by Satish Kumar et al. (2008) in the ethanolic extract of leaf of *Elaeocarpus ganitrus*. 76.70 % inhibition was noted with 500 μg/ml of *E. ganitrus*. IC$_{50}$ value was found to be 211.73 μg/ml. Metal chelating agents reduce the concentration of catalyzing transition metal in lipid peroxidation by forming sigma bonds with metals, reducing the redox potential, thereby stabilizing the oxidized form of the metal ion (Elmastaş et al., 2006). Further, Hazra et al. (2008) found that the methanolic extract of *Spondias pinnata* was a potent iron chelator with IC$_{50}$ = 66.54 ± 0.84 μg/ml. According to them, the plant extract was not as good as the standard EDTA (IC$_{50}$ = 1.27 ± 0.05); but the decrease in concentration-dependent color formation in the presence of the extract indicated that it had iron chelating activity.

Similarly, Senevirathne et al. (2006) showed that among organic and aqueous fractions of *Ecklonia cava*, 70% methanol fraction exhibited the highest ferrous iron chelating ability (IC$_{50}$ = 436 ± 0.03 mg/ml). These abilities were significantly higher than that of BHT and α-tocopherol (IC$_{50}$ = 1.6 ± 0.02 and 1.72 ± 0.2 mg/ml, respectively). Second highest abilities showed in aqueous n-hexane fraction (IC$_{50}$ = 0.660 ± 0.02mg/ml), which was also significantly higher than the values of commercial antioxidants. Further aqueous ethyl acetate fraction also showed higher activities than that of BHT and α-tocopherol. As reference antioxidants, BHT and α-tocopherol showed relatively lower activity when compared to the abilities obtained from the organic and aqueous fraction of *E. cava*. The iron chelating properties of the antioxidant extract may be attributed to their endogenous chelating agents, mainly phenolics. Certain phenolic compounds have properly oriented functional groups, which can chelate metal ions. The stability of the metal-antioxidant complex is higher in six-membered than five-membered ring complexes (Thompson and Williams, 1976).
Metal chelating activity of methanol extract and aqueous n-hexane fraction of *E. cava* was equal or slightly higher than that of the chelating activity showed by 100ppm concentrations of borage crude extracts and its purified fractions but slightly lower than that of the values showed by evening primrose at same concentration (Wettasinghe and Shahidi, 2000). Further, Ebrahimzadeh *et al.* (2008) showed that there was a direct relation between chelatory activity and the content of active compounds, phenols and flavonoids in some extracts. According to them, the extracts of *Epilobium hirsutum* and *Melilotus arvensis* contained highest phenol and flavonoid contents and also showed the best chelating activity. In contrast, *Zea mays* with high phenol and flavonoid content showed very weak chelating activity but *Pistacia lentiscus* with low phenol and flavonoid content showed good chelating activity.

Hence the crude extracts of *Elaeocarpus serratus* and *Elaeocarpus tuberculatus* obtained by using different solvents possessed different levels of free radical scavenging property, which might serve as a significant indicator of its potential antioxidant activity.

### 5.4. Antimicrobial Activity of Plant Extracts

Since the discovery of penicillin (1929) and its use in chemotherapy, a great number of important antibiotics have been found (El-Bana, 2007). The success story of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains of microorganisms. There has been an increasing incidence of multiple resistance in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This situation, coupled with the undesirable side effects of certain antibiotics and the emergence of previously uncommon infections are a serious medical problem (Marchese and Shito, 2001; Poole, 2001). This has forced scientists to search for new antimicrobial substances from various sources like the medicinal plants (Maurer-Grimes *et al.*, 1996; Rabe and Van Staden, 1997; Afolayan, 2003).

Many studies indicated that in some medicinal plants there are many substances such as peptides, unsaturated long chain aldehydes, alkaloidal constituents, some
essential oils, phenols and flavonoids which are soluble in water, ethanol, chloroform, methanol and butanol. These plants then emerged as compounds with potentially significant therapeutic application against human pathogens (Cowan, 1999; Gupta et al., 2004; Edeoga et al., 2005; El-Astal et al., 2005).

Earlier studies have shown that monoterpenes linalool, eugenol and thymol were found to be major constituents of the aromatic plants. These compounds were previously known for its antimicrobial activity (Karapmar and Aktug, 1987; Consentino et al., 1999). Maridass (2010) attributed the thymol antimicrobial action to its phenolic character, which can cause membrane-disturbing activities. Phenolics and polyphenols present in the plants were known to be toxic to the microorganisms (Mason and Wasseman, 1987). Flavonoids have been reported to have both antibacterial and antifungal activities (Tsuchiya et al., 1996). Tannins from Dichrostachys cinerea root bark possessed antibacterial activities against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa (Banso and Adeyama, 2007). Previously, this was strongly supported by the study on the effects of different antimicrobial compounds in garlic (Conner, 1993). Garlic extracts possessed antibacterial property against Salmonella typhi, Escherichia coli, and Staphylococcus aureus (Johnson and Vaughan, 1969; Saleem and Al-Delaimy, 1982; Arora and Kaur, 1999). Recent studies have shown that essential oils of Origanum vulgare, Thymus vulgaris, Pimenta racemosa and Syzygium aromaticum were most active against E.coli (Smith-Palmer et al., 1998; Hammer et al., 1999; Dorman and Deans, 2000). In the present study, the phytochemical analysis of E. serratus and E. tuberculatus solvent extracts revealed the presence of phenols, flavonoids and tannins which were biologically active against microorganisms. The present findings are in agreement with the above previous studies.

Furthermore, alkaloids (Chakraborty and Brantner, 1999; Torres et al., 2002; Faizi et al., 2003; Shaheen et al., 2003; Raghavendra et al., 2008; Urgeova and Polivka, 2009), amino acids (Chowdhury et al., 2008), phenols (Fernandez et al., 1996), flavonoids (Brandao et al., 1997; Mendoza et al., 1997; Hernandez et al., 2000; Mandalari et al., 2007), tannins (Akiyama et al., 2001; Amarowicz et al., 2008; Doss et
al., 2009), saponins (Baharaminejad et al., 2007), terpenoids (Amaral et al., 1998; Funatogawa et al., 2004) of various plant extracts proved to be effective antimicrobials. Phytochemical studies of Acharyya et al. (2009) revealed that the crude methanol extracts of Albizia lebbeck, Terminalia chebula, Syzygium cumini, Solanum nigrum, Picrorhiza kurrooa, Butea monosperma, Saraca indica, Aegle marmelos and Withania somnifera contained phenolics and flavonoids and these compounds have previously been reported to possess antimicrobials. In fact, the phytochemical characteristics possessed by Vitex doniana and Cajanus cajan might be attributed to their antimicrobial properties. This finding agreed with similar study by Kilani (2006) and Arokiyaraj et al. (2009).

In the present study, the antibacterial activity of E. serratus and E. tuberculatus leaf, stem bark and fruit extracts were examined against four bacterial species namely, Shigella sonnei, Salmonella typhi, Staphylococcus aureus and Klebsiella pneumoniae using agar well diffusion method. Both the plant extracts showed a dose-dependent inhibition of microorganisms. Among the extraction medium, acetone and methanol extracts of leaf and stem bark of E. serratus and E. tuberculatus displayed maximum antibacterial activity against all the bacterial species studied. In E. serratus the water extracts of leaf, stem bark and fruit were found to be more susceptible to the bacterial species and showed no zone of inhibition except Shigella sonnei whereas, in E. tuberculatus the water extracts showed little or no zone of inhibition. The standard antibiotic gentamycin inhibited the growth of all the bacterial species effectively at a lower concentration of 50 μg/ml. Generally, the inhibition of all the bacterial species (except Klebsiella pneumoniae) by the standard antibiotic gentamycin (50 μg/ml) was higher than the various solvent extracts of the test plants.

In a similar study, Singh and Nath (1999) investigated the antibacterial activity of petroleum ether, benzene, chloroform, acetone and ethanol extracts of dried Elaeocarpus sphaericus fruit against 28 Gram-positive and Gram-negative bacteria. The acetone fraction showed marked antimicrobial activity against ten organisms. Benzene extract was active against Salmonella typhimurium and Morganella morganii, and ethanol
extract was active against *Plesiomonas shigelloides*, *Shigella flexnerii* and *Shigella sonnei*. The use of bearberry (*Arctostaphylos uva-ursi*) and cranberry juice (*Vaccinium macrocarpon*) to treat urinary tract infections and lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tea tree (*Melaleuca alternifolia*) were described as broad spectrum of antimicrobial activity (R’ios and Recio, 2005).

Further, Ajaiyeoba (2000) studied the antibacterial activities of hexane and methanol extracts of leaves and stems of *Gynandropsis gynandra* and *Buchholzia coriacea*. All the extracts exhibited appreciable antibacterial properties inhibiting the growth of all the bacteria at 200 mg/ml. The stem hexane, leaf hexane and stem methanol extracts of *B. coriacea* displayed overwhelming activities inhibiting the growth of *Bacillus cereus* and *B. subtilis* up to 25 cm in diameter. In most instances, activities were greater than the standard therapeutic agent, ampicillin. Various sensitivities were observed for the bacteria. However, *Escherichia coli* and *Pseudomonas aeruginosa* were insensitive to ampicillin and extracts of both plants inhibited the growth of these two microorganisms. These findings supported the present study.

Interestingly, according to Nwachukwu and Uzoeto (2010) the antimicrobial activities of acetone, ethanol, methanol, hot and cold water extracts of leaves of *Vitex doniana* and *Cajanus cajan* on *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Bacillus subtilis* and *Staphylococcus aureus* revealed inhibition of growth, though the susceptibility pattern to the extracts was not uniform. *S. typhi* was highly sensitive to acetone extract (19.71 mm) of *V. doniana*, followed by methanol extract (14.61 mm) on *E. coli*. Ethanol extract of *C. cajan* produced the least (1.08 mm) pattern of sensitivity. Results of the present investigation agreed with the report of Arokiyaraj *et al.* (2009). According to them, *Vitex doniana* and *Cajanus cajan* acetone, methanol, ethanol extracts generally produced a clear inhibitory effect on the bacteria. Further, Nwachukwu and Uzoeto (2010) demonstrated reduction in the number of viable bacteria at 30 minutes interval using different concentrations of acetone, ethanol, methanol, hot and cold water extracts of leaves of *Vitex doniana* and *Cajanus cajan*. High level of reduction was recorded as the concentration of extract increased comparable with standard antibiotics.
used. The growth inhibitory effect was concentration-dependent (Achi, 2006). This was important in considering dosage and rate at which the extract inhibited the growth of organism (Egwar, 1999).

Chye and Sim (2009) studied the antibacterial activity of phenolic and alkaloid extracts of *Pangium edule* seed against *Listeria monocytogene* and *Salmonella typhimurium*. The Pangi seed extracts showed inhibition zones ranging from 8.67 to 24.07mm against *Listeria monocytogene* and *Salmonella typhimurium*. The phenolics exhibited the strongest inhibitory action against both *L. monocytogene* and *S. typhimurium* with mean values of 24.07±0.17mm and 22.24±0.05mm, respectively. This may be due to greater availability of bioactive phenolic compounds in the acetone extract such as flavonoids, anthocyanins, catechin and flavan (Chirinos et al., 2007). However, the free phenolic acid extract showed the lowest inhibitory activity against *L.monocytogene* and *S. typhimurium*.

Further, free phenolic acid such as protocatechuic acid, vanillic acid, ferullic acid, anisic acid, p-coumaric acid and p-hydroxybenzoic acid was generally identified as weak antibacterial compounds against selected microorganisms. Some of the alkaloid compounds, such as quinine and indole have shown great antimicrobial effects against selected Gram-positive and Gram-negative bacteria (Tanaka et al., 2006). The crude phenolic extract showed higher inhibitory effect against *Listeria monocytogene* and *Salmonella typhimurium* as compared to other *Pangium edule* (Pangi) seed extracts. This was supported by Rauha et al. (2000). According to Rauha et al. (2000) the crude phenolic extract usually contained a substantial quantity of phenolic compounds such as phenolic acid derivatives, flavonoids, tannins, essential oils, gallic acid and terpenoids that effectively reacted as antimicrobial agent. Rodriguez-Vaquero et al. (2007) confirmed that phenolic compounds especially, gallic acid is the most active against bacteria.

In addition, the results of Ekwenye and Edeha (2010) revealed that the ethanol extracts of *Citrus sinensis* exhibited inhibitory activities that were found to be a little higher than aqueous extract on *Pseudomonas aeruginosa, Klebsiella pneumoniae, and*
Staphylococcus aureus except Escherichia coli on which the aqueous extract had no inhibitory activity. Although with a slight difference it can be therefore inferred that the active principles of the plant may be more soluble in ethanol as employed in ethnomedicine. Further, low concentration of diffusible water soluble active constituents or excessive heating which often affect biologically active substances such as flavonoids, essential oils and other heterogeneous phytoconstituents present in the extract (Scalbert, 1991) might also influence their respective activity. Voravuthikunchai et al. (2004) reported good antibacterial activity in Peltophorum pterocarpum and Punica granatum against Escherichia coli using aqueous and methanol extracts.

Similarly, Nair and Chanda (2007) screened the antibacterial activity of ten medicinal plants, namely Commiphora wightii, Hibiscus cannabinus, Anethum graveolens, Emblica officinalis, Ficus religiosa, Ficus racemosa, Ficus benghalensis, Ficus tisela, Mentha arvensis and Minusops elengi, against medically important bacterial strains, namely Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, Bacillus cereus, Alcaligenes faecalis and Salmonella typhimurium. The results revealed that the ethanol extracts were more potent than aqueous extracts of all the plants studied. Emblica officinalis showed stronger activity than the other plants against all the tested bacterial strains.

The leaf, flower, root and stem bark of Cassia alata showed a range of activity against several bacteria and protozoa (Khan et al., 1999). Somchit et al. (2003) also tested the whole plant parts of Cassia alata and showed activity in the leaves against Staphylococcus aureus. Similarly, Chhetri et al. (2008) studied the antimicrobial activity of the ethanolic extract of traditionally used medicinal plants of Nepal namely, Azadiracta indica, Colquhounia coccinea, Curcuma longa, Elsholtzia fructicosa, Eucalyptus globulus, Ocimum sanctum and Rhododendron setosum and Zanthoxylum aromatum. Rhododendron setosum, Eucalyptus globulus, Azadiracta indica and Elsholtzia fructicosa showed significant antimicrobial activity. The extract of Rhododendron setosum was effective against Escherichia coli (ZI = 2.5 cm). Essential oil of Eucalyptus globulus was effective against Staphylococcus aureus (ZI = 3.3 cm).
Similarly, the extract of *Azadiracta indica* and *Elsholtzia fruticosa* were active against *Klebsiella species* (zone of inhibition ZI = 2.5 cm). Earlier observations done by Shrinivasan *et al.* (2001) also showed the antifungal and antibacterial activity of *Azadiracta indica*.

In support of the present study, Saganuwan and Gulumbe (2006) observed that the *Escherichia coli* were sensitive to methanol, hexane, chloroform and aqueous extracts of leaves of *Cassia occidentalis* at a concentration range 900-1000 mg/ml. Similarly, Jain *et al.* (1998) observed that the metabolite rich fraction of leaves, pods and flowers were effective against *E. coli* (inhibition zone 22 mm). It may be possible that emodin isolated from the roots of *Cassia occidentalis* was also responsible for the antimicrobial activity (Ubbink-kok *et al.*., 1986). The antimicrobial efficacy of the *C. occidentalis* may result from damages and inactivation of enzymes due to their ability to induce leakage of these ions (Samy and Ignacimuthu, 2000; Jawetz *et al.*., 2004). Sodium ions and potassium ions have been known to affect osmotic balances in the cell and their leakage might cause cell lyses and eventual death. These ions are also known to activate enzymes, which are organic catalyst that mediate biochemical reactions (Conway, 2002).

In the present work, the stem bark extracts of *E.serratus* and *E.tuberculatus* possessed bactericidal potential against *Shigella sonnei, Salmonella typhi, Staphylococcus aureus and Klebsiella pneumoniae*. The acetone and methanol extracts of stem bark of *E.serratus* showed a broad spectrum of activity against all the investigated bacteria whereas, the water extract of stem bark was more susceptible to the bacterial species and showed no zone of inhibition except *Shigella sonnei*. A similar trend was noticed in *E. tuberculatus* extracts, but the water extract showed inhibition of bacterial species with wider zones of inhibition at higher concentrations (150 and 200 μg/ml).

Several researchers have studied the bactericidal potential of bark of many taxon. These are in agreement with the present study. Stem bark extract of *Pterocarpus santalinus* showed maximum activity against *Bacillus subtilis* (17.0 mm) (Manjunatha, 2006). The methanolic extract of stem bark of *Tetracarpidium conophorum* inhibited the
growth of *B. subtilis* (12.3 mm) (Ajaiyeoba and Fadare, 2006). According to Manjunatha (2006) the stem bark extract of *Pterocarpus santalinus* inhibited the growth of *S. aureus* (16.05 mm). Methanolic extracts of stem bark of *Vitex doniana* also possessed bactericidal potential against *S. aureus* (Kilani, 2006). Stem bark extract of *Holarrhena antidysenterica* possessed antibacterial potential against enteric pathogen *E. coli* (Ballal et al., 2001). Doughari et al. (2008) noticed that stem bark of *Cochlospermum planchoni* inhibited the growth of *P. aeruginosa* (26 mm). Sangetha et al. (2008) noticed that methanolic extracts from the stem of *Cassia fistula* and *Cassia surattensis* arrested the growth of *S. typhi* (19 mm). Sangetha et al. (2008) also noticed the bactericidal potential of *Cassia fistula* against the bacteria *Klebsiella pneumoniae, Proteus mirabilis* and *Micrococcus spp.* Tamokou et al. (2009) isolated xanthones, phsiccion, friedelin and friedelanol, of these, xanthones and phsiccion exhibited the antimicrobial activities against bacteria *S. typhi, K. pneumoniae, P. aeruginosa, and B. subtilis* and four yeast species *Candida albicans, Candida tropicalis, Candida parapsilosis* and *Cryptococcus neoformans*, respectively.

In the present findings, *Elaeocarpus serratus* and *Elaeocarpus tuberculatus* extracts displayed high antifungal activity against *Candida albicans*. The water extracts of leaf and acetone extract of fruit of *E. serratus* produced the maximum inhibition zone of 18±1.05 and 18±0.92 mm at 200 μg/ml. The lower concentrations of water extracts of stem bark and fruit were susceptible to *Candida albicans* and showed very little or no zone of inhibition. The acetone extract of leaf of *E. tuberculatus* suppressed the growth of *Candida* to the maximum (ZI = 17±1.23 mm) at 200 μg/ml. Generally, the lower concentrations of the extracts were susceptible to the fungal pathogen. The present findings were supported by Singh et al. (2010) who evaluated the petroleum ether, chloroform, ethanol and water extracts of dried fruits of *Elaeocarpus ganitrus* for antifungal activity on different fungal strains. The chloroform and ethanol extracts were found to be more active antifungals.

This has further been confirmed by Dulger et al. (2002). According to them *Candida albicans* was resistant to the action of the methanolic extract of *Lepista nuda*. 

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Antifungal activity of *Solanum surattense* against *Aspergillus fumigatus* has been reported by Dabur *et al.* (2004), additionally, chloroform and hexane extracts (300.0 μg/ml) of the same were found to be active against *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, while methanol extract inhibited the growth of *Aspergillus flavus*, *A. niger* and *Candida albicans*. Ajaiyeoba (2000) also supported the present study. According to him, the hexane and methanol extracts of leaves and stems of *Gynandropsis gynandra* and *Buchholzia coriaceae* displayed high antifungal activity. *B. coriaceae* stem hexane extract showed the highest activity inhibiting the growth of *Candida albicans* up to 21mm. This was followed by *G. gynandra* stem methanol extract. The least activity was recorded in the the leaf hexane extract of *G. gynandra*. The hexane extract of *B. coriaceae* stem inhibited the growth of *A. niger* (19.6mm), a most insensitive mold chemotherapeutically while tioconazole was inactive.

The high inhibitory potential of acetone and methanol extracts of leaf and stem bark of *Elaeocarpus serratus* and *Elaeocarpus tuberculatus* might be due to the high solubility of the phytoconstituents in the organic solvents. The phytoconstituents might be present in higher concentrations in the leaf and stem bark along with some new microbicidal agents reflecting its higher bactericidal potential. Presence of these phytoconstituents in the leaf and stem bark pointed towards the pharmacological activities of this plant and supported the claim of the traditional users.

### 5.5. Assessment of *In vivo* Pharmacological Activity of Plant Extracts

In living systems, free radicals are produced in the body as by-products of normal cellular metabolic activities such as prostaglandin synthesis, mitochondrial electron transport, endoplasmic reticulum enzyme activity, oxyhaemoglobin, auto-oxidation and phagocytosis as well as when exposed to environmental pollutants, drugs, pesticides and ionizing radiation producing severe damaging effects on body tissues (Bhatia and Jain, 2003). In addition, chemical mobilization of fat stores under various conditions such as lactation, exercise, fever, infection and even fasting, can result in increased radical activity and damage to the immune system. Normally a balance is maintained between
the oxidative defense system prevailing in the cells and tissues of human body, but when the balance is tilted more towards generation of free radicals then degenerative changes set in causing many degenerative diseases. Considerable evidence have accumulated to implicate cellular damage arising from reactive oxygen species, at least in part, in the etiology and pathophysiology of human diseases such as neurodegenerative disorders, inflammation, viral infections, autoimmune pathologies, and digestive system disorders such as gastrointestinal inflammation and ulcer (Topping, 1993; McLennan, 1993; Gustafsson *et al*., 1994; Edmonds, 2000; Cragg and Newman, 2001; Karantonis *et al*., 2002; Reppeto and Llesuy, 2002; Aruoma, 2003; Surh and Ferguson, 2003).

In *vivo*, cells have their own inherited antioxidative defense system, in the form of various enzymatic, as well as nonenzymatic pathways, for removing the ROS. Among enzymatic pathways $\text{O}_2^-\text{ are dismutated by superoxide dismutase to H}_2\text{O}_2$, catalase reduces $\text{H}_2\text{O}_2$ to water and molecular oxygen. Glutathione peroxidase catalyzes the reduction of $\text{H}_2\text{O}_2$ to water and organic peroxide to alcohols at the expense of reduced glutathione, while glutathione-S-transferase conjugates xenobiotics with GSH for excretion. Among the nonenzymatic substances, $\beta$-carotene, GSH, vitamin A, vitamin E, and vitamin C scavenge free radicals (Yoshikawa *et al*., 1997; Mates *et al*., 1999).

Enzymes are the best markers of tissue damage because of their specificity and catalytic activity to the tissue (Sivakumar *et al*., 2007). The release of cellular enzymes reflects non-specific alterations in the membrane integrity and permeability. Recently, there is a growing interest on the discovery of natural antioxidants, mainly for two reasons: (I) there is epidemiological and clinical evidence suggesting that consumption of vegetables and fruits reduces the risk of developing chronic diseases, (II) phytochemicals are generally safer than synthetic chemicals (Bafna and Balaraman, 2004; Yazdanparast and Ardestani, 2007; Yazdanparast *et al*., 2008).

Plant extracts with various phytochemicals such as phenolic acids, flavonoids, coumarin derivatives, etc., are known to combat oxidative stress in the human body by helping to maintain a balance between oxidants and antioxidants (Devasagayam and
Sainis, 2002; Tapsell et al., 2006; Filburn et al., 2007; Delgado et al., 2008; Kukongviriyapan et al., 2008). These phytochemicals especially phenolics, could be present actively in inflammatory processes, in fact, antioxidant/anti-inflammatory activity have been related intrinsically to these chemical substances (Takahashi and Shibamoto, 2008; Jensen et al., 2008).

Considerable work has been done on natural products for the presence of nontoxic antioxidants that could be used in chemotherapy. Recently, many natural antioxidants have been isolated from different plant materials (Packer and Ong, 1997; Gyamfi et al., 1999; Jovanovic and Simic, 2000; Li et al., 2007).

5.5.1. Toxicological Evaluation of Plant Extracts

In the present investigation, toxicological evaluation of ethanolic extracts of leaf and stem bark of *E. serratus* and *E. tuberculatus* showed that the plant extracts were quite safe even at a high dose of 5000 mg/kg b.w. per day p.o. and had no acute toxicity on albino mice. Similarly, Gregory et al. (2009) found that *Ficus arnottiana* extract was non-toxic even at relatively high concentrations. Further, the acute toxicity assessment by Küpeli et al. (2006) has revealed that all extracts of Seseli species were safe in the administered doses. Tandan et al. (1990) also reported that seselin, a coumarin from *Seseli indicum*, was safe in doses up to 6 g/kg (oral) in 72 hour mortality study. These results are similar to the present study.

5.5.2. Assessment of *In vitro* Anti-inflammatory Activity of Plant Extracts

Most of the investigators have reported that denaturation of protein is one of the causes of rheumatoid arthritis. Phenylbutazone, salicylic acid, flufenamic acid (anti-inflammatory drugs) etc., have shown a dose-dependent ability to thermally induced protein denaturation (Mizushima and Kobayashi, 1968). Production of auto-antigens in certain rheumatic 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 (Vallabh
Deshpande et al., 2009). Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a rich source of proteinases which carry in their lysosomal granules many neutral serine proteinases. It was previously reported that leucocyte proteinases play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors (Das and Chatterjee, 1995).

Interestingly, in the present investigation, the leaf and stem bark extracts of *E. serratus* and *E. tuberculatus* had remarkable ability on the inhibition of protein denaturation and proteinase inhibition. *E. serratus* leaf extract showed the maximum inhibition of protein denaturation (62.38%) and maximum anti-proteinase activity (56.62%) at the concentration of 400 μg/ml. Similarly, from the results of Vallabh Deshpande et al. (2009) it could be stated that *Abutilon indicum* was capable of controlling the production of auto-antigens due to *in vivo* denaturation of proteins in rheumatic diseases. *Abutilon indicum* also exhibited significant anti-proteinase activity justifying its usefulness in the treatment of inflammation associated diseases like arthritis. 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.

5.5.3. Assessment of *In vivo* Anti-inflammatory Activity of Plant Extracts

Inflammation is a completely natural process that is part of the body’s response to any injury that it undergoes. It is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses (Kumar et al., 2004). Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can easily be induced (Sosa et al., 2002). It also involves a complex array of enzyme activation, mediator release, and extravasations of fluid, cell migration, tissue breakdown
and repair (Vane and Bolting, 1995). It is characterized by four major features: Redness, Heat, Pain, and Loss of Function (Cotran et al., 1999; Guyton and Hall, 2000; Chaudhary, 2001). Further, the endogenous formation of free radicals can contribute to the inflammatory process (Hernandez 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). The anti-inflammatory effects can be elicited by a variety of chemical agents and that there is little correlation between their pharmacological activity and chemical structure (Sertié et al., 1990).

Several experimental models of paw edema used for inflammation studies have been described by many investigators earlier. Carrageenan-induced paw edema is widely used for determining the acute phase of inflammation (Di Rosa et al., 1971; Manueli et al., 1994; Ialenti et al., 1995). The development of edema in the paw of the rat after the injection of carrageenan is due to release of histamine, serotonin and prostaglandin like substances (Vinegar et al., 1969).

Importance of a medicinal plant lies in their biologically active principles. There are two types of plant chemicals, primary metabolites such as sugars, proteins, amino acids, chlorophylls, etc., and secondary metabolites, which include alkaloids, terpenoids, saponins and phenolic compounds. These chemicals exert a significant physiological effect on the mammalian system. Many medicines of plant origin had been used since long time without any adverse effects. Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs (Ahmad et al., 1992). Therefore, a systematic approach should be made to find out the efficacy of plants against inflammation.
In the present study, injection of the inflammatory agent, carrageenan in the rat hind paws was employed to evaluate the anti-inflammatory activity of ethanolic extracts of leaf and stem bark of *Elaeocarpus serratus* and *Elaeocarpus tuberculatus*. Both the plant extracts gave significant reduction (p< 0.05) of rat paw edema at all assessment times. High doses (400mg/kg p.o.) of ethanolic extracts of leaf and stem bark of *E.serratus* and *E.tuberculatus* exhibited profound anti-inflammatory effect as compared to the control group. The significant ameliorative activity of the extracts of *E.serratus* and *E.tuberculatus* and standard drug indomethacin observed in the present study may be due to inhibition of the mediators of inflammation. This study demonstrated the efficacy of *E.serratus* and *E.tuberculatus* extracts as an anti-inflammatory agent and also scientifically justified the use of this plant as an anti-edematous agent in folk medicine.

The present study was in line with the investigations of several researchers. According to the study of Tandan *et al.* (1990) a coumarin component of *Seseli indicum* seeds, seselin, was shown to possess a significant and dose-dependent anti-inflammatory activity. Seselin is known to be found either in the roots, stems, leaves or inflorescence of several species. Ammar *et al.* (1997) have revealed the anti-inflammatory activity of bioactive fractions isolated from seeds of *Trigonella foenum graecum*, roots of *Glycyrrhiza glabra* and fruits of *Coriandrum sativum*. Saundane *et al.* (2000) reported that the crude ethanolic extract of *Leucas aspera* exhibited good anti-inflammatory activity compared to petroleum ether and chloroform extracts. Srinivas *et al.* (2000) also reported that the dried leaves of *Leucas aspera* possessed significant anti-inflammatory activity against carrageenan-induced paw edema and cotton pellet-induced granuloma. Bhattacharya *et al.* (2005b) have reported the anti-inflammatory potential of methanol extract of *Stepenia glabra*. The extract depicted anti-inflammatory activity at the dose of 150 mg/kg body weight.

Further, Küpeli *et al.* (2006) showed that the ethyl acetate extracts of *Seseli gummiferum* subsp.*corymbosum*, *Seseli petraeum*, *Seseli resinosum* and *Seseli tortuosum* also exhibited notable inhibition, ranging between 24.5–29.7, 28.1–33.3, 17.4–27.5 and 27.9–31.3%, respectively, in carrageenan-induced hind paw edema model at 100 mg/kg.
dose without inducing any gastric damage, quite comparable to indomethacin (41.8–44.8% inhibition) as a reference sample. On the other hand, the methanol (80%) extracts of *Seseli* species did not show any anti-inflammatory activity. Garcia-Argaez *et al.* (2000) has also proven the anti-inflammatory effect of several coumarins, including seselin, using a TPA-induced ear edema model in mice and concluded that the anti-inflammatory activity of coumarins depend on their individual substitution on the aromatic ring rather than the coumarin skeleton itself. They have further suggested that the activity might possibly be due to their ability to inhibit lipid peroxidation and to act as a radical scavenger.

In a similar study, Sharma *et al.* (2010) pointed out that both ethanol and aqueous extracts of *Cordia dichotoma* seeds significantly inhibited carrageenan-induced rat paw edema at 250 and 500 mg/kg b.w. At the dose of 500 mg/kg b.w. the activity of the ethanol extracts was comparable than that elicited by diclofenac sodium. Diclofenac sodium was a cyclooxygenase inhibitor, and could be said to inhibit the cyclooxygenase enzyme but lipoxygenase inhibitors also possessed significant anti-inflammatory activity against carrageenan-induced paw edema (Rowley and Benditt, 1956). Therefore, the inhibition of carrageenan-induced paw edema by the crude extract could also be due to its inhibitory activity on the lipoxygenase enzyme. The chloroform extract of *Trichodesma indicum* root has been evaluated by Perianayagam *et al.* (2006) for anti-inflammatory activity against edema produced by carrageenan, dextran, histamine and serotonin and against formation of granulation tissues by cotton pellet in rats. The results demonstrated the efficacy of *Trichodesma indicum* as an anti-inflammatory agent and also scientifically justified the use of this plant as a non-specific anti-inflammatory agent in folk medicine. The previous findings of Varier (1993) also supported the above work.

The aqueous extract of leaves of *Gynandropsis pentaphylla* possessed a marked anti-inflammatory activity and hence may pose itself as very good anti-inflammatory drug (Mule *et al.*, 2008). Abreu *et al.* (2006) observed that the administration of *Pedilanthus tithymaloide* tincture at doses of 500, 750 and 1000 mg/kg, caused 83%, 94% and 92% inhibition of the rat paw edema, respectively in carrageenan-induced paw
inflammation. This test model basically reflected the action of prostaglandins involved in the inflammation process induced by carrageenan (Guillén et al., 1997; Mujumdar and Misar, 2004).

Similar to the present study, the results of Subhan et al. (2007) revealed that the administration of both methanolic and aqueous rhizome extracts of Valeriana wallichii inhibited the edema even in the first hour. Maximum effect was observed at the end of the third hour. The significantly high anti-inflammatory activity of both methanolic and aqueous extracts might be due to the inhibition of mediators of inflammation such as histamine, serotonin released during the first phase of inflammation and prostaglandins and bradykinnins which were released during the second phase of inflammation (Vinegar et al., 1969; Crunkhon and Meacock, 1971; Hernández-Pérez and Rabanal-Gallego, 2002). The anti-inflammatory activity of both the extracts of Valeriana wallichii rhizomes could be attributed to the high amount of flavonoids (Falodun et al., 2003; Emim et al., 1994) and tannins (Starec et al., 1988) present in the plant. The presence of flavonoids in Cassia fistula ((Yadava and Verma, 2003) might be responsible for the anti-inflammatory and antioxidant effects. Alkaloid and flavonoid constituents have already been reported in Cassia fistula bark (Gupta et al., 1989).

Ratheesh and Helen (2007) also supported the present findings. According to them, methanolic extract of Ruta graveolens with a concentration of 20 mg/kg b.w. and ethanolic extract with a concentration of 50 mg/kg b.w. showed maximum (90.9%) inhibition as compared to the reference drug voveran, which showed only 72.72% inhibition on carrageenan-induced rat paw edema. Aqueous extract of R graveolens with two different doses 20 mg/kg b.w and 50 mg/kg showed only 18.2% and 36.3% inhibition, respectively. It was lower as compared to the reference drug. Paschapur et al. (2009) showed that the ethanolic extract of Borassus flabellifer male inflorescences exhibited significant anti-inflammatory activity in rats. The extract at the test doses 150 and 300 mg/kg b.w. reduced the edema induced by carrageenan by 33.75 and 41.26%, respectively at 3 hours whereas, the standard drug showed 47.35% of inhibition as compared to the control group.
In view of the anti-inflammatory action of *E. serratus* and *E. tuberculatus*, earlier the pharmacological screening of root bark extracts of *Securidaca longipedunculata* had revealed that the root bark possessed potent anti-inflammatory effect in the topical and systemic models of acute inflammation (Okoli *et al.*, 2005). These extracts might have inhibited the release of pro-inflammatory mediators of acute inflammation such as histamine and prostaglandin. Interestingly, the extracts caused gastrointestinal irritation in rats typical of anti-inflammatory prostaglandin inhibitors such as the non-steroidal anti-inflammatory drugs (Rang and Dale, 1988). The phytochemical constituents isolated from the root bark of *Securidaca longipedunculata* such as flavonoids are known to possess anti-inflammatory activity. Anti-inflammatory constituents such as oleanic acid, beta-sitosterol (Yang *et al.*, 2002), salicylic acid and benzoic acid (Yang *et al.*, 2001) have also been reported in *Securidaca longipedunculata* to varying extents which contributed to the anti-inflammatory activity.

Antioxidant enzymes have the capacity to lower the free radical burden and neutralize excess free radicals created by stress conditions. Oxygen handling cells have antioxidant 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 mainly acts by quenching of superoxide and active oxygen free radical, produced in different aerobic metabolism (Mac Millan-Crow *et al.*, 1998). GSH has an important function in maintaining cellular redox status (Rennenberg, 1980).

Earlier, Wang and Ballington (2007) supported that the *Vaccinium stamineum* fruit extract had high activities of antioxidant enzymes including glutathione-peroxidase (GSH-POD), glutathione reductase (GR), superoxide dismutase (SOD) and reduced glutathione (GSH). Antioxidant capacities were highly correlated to antioxidant enzymes activities. The results of Osama *et al.* (2009) also supported the present findings. According to them, there was a significant increase in the antioxidant defense enzymes
SOD and GSH activity with the administration of *Rosemarinus officinalis* extract. In contrary, the lower concentration of these enzymes (SOD and GSH) allowed activation of the initial signaling events leading to inflammations (Kang *et al*.*, 2008). The antioxidant defense enzymes (SOD and GSH) and natural antioxidant crude rosemary ethanolic extract prevented inflammation mediators which were associated with inflammation (Dinarello, 2000). In view of the above findings, the present study supported that the administration of ethanolic extracts of leaf and stem bark of *E. serratus* and *E. tuberculatus* showed significant increase in the activities of antioxidant enzymes (SOD, CAT, GPx, GST). However, the level of LPO and NO was significantly decreased.

**5.5.4. Assessment of In vivo Anti-ulcer Activity of Plant Extracts**

Ulcer is defined as the erosion in the lining of the stomach or duodenum and is caused by the disruptions of the gastric mucosal defense and repair systems (Grossman, 1981; Del Valle *et al*.*, 2003; Ojewole, 2004). Ulcer in the stomach is called gastric ulcer and in the duodenum is called duodenal ulcer and together peptic ulcer. In clinical practice, peptic ulcer is one of the most prevalent gastrointestinal disorders, commonly occurs in developed countries. Oxidative stress and ROS have been shown to play an important role in the pathogenesis of various diseases including gastric ulcer (Banarjee, 1990; Das *et al*.*, 1997; Goel *et al*.*, 2001; Miura *et al*.*, 2002). Thus, much attention has been recently focused on ROS contents (Ames *et al*.*, 1993) which cause lipid peroxidation in membranes (Takeuchi *et al*.*, 1991; Ames *et al*.*, 1993). Treatments available for ulcer are generally non-specific and are usually aimed at reducing the production of gastric acid and re-enforcing gastric mucosal protection. The drugs used in the treatment of ulcer include receptor blockers, proton pump inhibitors, drugs affecting the mucosal barrier and act on the central nervous system (Manonmani *et al*.*, 1995). Even though a range of drugs are available for the treatment of ulcer, many of these do not fulfill all the requirements and have side effects (Anoop and Jegadeesan, 2003; Dharmani *et al*.*, 2005).

Antioxidants have been reported to play a significant role in the protection of gastric muosa against various necrotic agents (Salim, 1992; Trivedi and Rawal, 2001).
Many researchers have proved that antioxidants may play an important role not only by protecting against gastric mucosal injury, but also by inhibiting progression of gastric ulcer (Singh et al., 2002). Recently, there has been much interest in natural medicines derived from the traditional knowledge of plant pharmacological properties. Large number of medicinal plants have been shown to possess gastroprotective activity (Borrelli and Izzo, 2000; Dharmani and Palit, 2006; Kath and Gupta, 2006; Malairajan et al., 2007; Siti Fatimah Zahra et al., 2009).

In the present study, the gastroprotective effects of ethanolic extracts of leaf and stem bark of *Elaeocarpus serratus* and *Elaeocarpus tuberculatus* at low and high doses (200 and 400 mg/kg b.w. p.o.) on ethanol-induced gastric damage in Wistar albino rats were studied. Ethanol-induced gastric lesion formation might be due to stasis in gastric blood flow which contributed to the development of the haemorrhage and necrotic aspects of tissue injury (Soll, 1990; Surendra, 1999). Ethanol produced necrotic lesions in the gastric mucosa by its direct toxic effect, reducing the secretion of bicarbonates and production of mucus (Marhuenda et al., 1993). Alcohol rapidly penetrated the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intracellular membrane permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This led to cell death and exfoliation in the surface epithelium (Soll, 1990; Surendra, 1999). The disturbances in gastric secretion, damage to gastric mucosa, alterations in permeability, gastric mucus depletion and free radical production have been reported as the pathogenic mechanism of ethanol (Salim, 1990).

Studies suggested that the ethanol damage to the gastrointestinal mucosa started with microvascular injury, namely disruption of the vascular endothelium resulting in increased vascular permeability, edema formation and epithelial lifting (Szabo et al., 1995). Exposure to ethanol increased the extension of cellular damage in a dose-dependent way (Mutoh et al., 1990). Absolute alcohol extensively damaged the gastric mucosa leading to increased neutrophil infiltration into the gastric mucosa (Coskun et al., 1996; Elliot and Wallace, 1998; Takeuchi et al., 1998; Nishida et al., 1998). Oxygen free
radicals derived from infiltrated neutrophils in ulcerated gastric tissues have an inhibitory effect on gastric ulcer healing in rats (Suzuki et al., 1998; Fujita et al., 1998; Cheng and Koo, 2000). Suppression of neutrophil infiltration during inflammation was found to enhance gastric ulcer healing (Tsukimi et al., 1996). It has been also demonstrated that nitric oxide is important in the regulation of acid and alkaline secretion (Takeuchi et al., 1997, 1998; Khattab et al., 2001), gastric mucosal blood flow and gastric mucus secretion (Brown et al., 1993).

In the present study, the gastric lesions were significantly reduced by the administration of the ethanolic extracts of leaf and stem bark of *Elaeocarpus serratus* and *Elaeocarpus tuberculatus* at 200 and 400 mg/kg b.w. compared with the ethanol-treated group. All doses of *Elaeocarpus* species showed a greater gastroprotective effect in comparison to the standard reference anti-ulcer drug omeprazole. Omeprazole is a proton pump inhibitor and exhibited anti-secretory and protective effect against ulcers (Del Valle et al., 2003; Li et al., 2004; Rao et al., 2004).

Considering the anti-ulcer activity, several ethanobotanical enquiries of various medicinal plants supported the present investigation. Results of Abdulla et al. (2010) revealed protection of gastric mucosa and inhibition of leucocytes infiltration of gastric wall in rats pretreated with *Centella asiatica* extract. Similarly, Kobayashi et al. (2001) reported that teprenone exerted a protective effect against mucosal lesions through inhibition of neutrophils infiltration in the ulcerated gastric tissue. The gastroprotective effect exerted by *Centella asiatica* extract (Guo et al., 2004), *Morus alba* extract (Chung et al., 2003; Sohn et al., 2004; Choi et al., 2005) and turmeric extract (Swarnakar et al., 2005) could be attributed to its anti-inflammatory activity (Guo et al., 2004). This anti-inflammatory activity could also be a key factor in the prevention of gastric ulcer as reported by Swarnakar et al. (2005).

Similar results were also obtained by Borrelli and Izzo (2000) who reported the use of plant flavonoids from *Bauhinia racemosa* as anti-ulcer remedies and are used for the prevention and treatment of peptic ulcer. Akthar and Ahmad (1995) reported the anti-
ulcerogenic activity of the methanolic extract of flower buds of *B. racemosa* in aspirin-induced gastric ulcers in rats. Their effects were studied on the volume of gastric juice secreted; acid output, peptic activity, mucin activity and curative ratio were recorded. *B. racemosa* (flower buds) decreased the ulcer index significantly, and showed some decrease in the ulcer index.

Gregory *et al.* (2009) have shown that *Ficus arnottiana* leaf methanolic extract possessed gastroprotective activity, as evidenced by its significant inhibition in the formation of ulcers induced by ethanol. The protective effect was confirmed by histological examination showing prevention of mucosal lesions and sub-mucosal edema. The anti-ulcer activity of methanolic extract was probably due to the antioxidant activity of flavonoids of the extract. Similarly, Jainu and Shyamala Devi (2004) demonstrated that methanolic extract of *Solanum nigrum* berries inhibited the increase in area of gastric mucosal lesions in aspirin-induced ulceration in rats. The protective effects were observed at oral doses of 500 mg/kg body weight of methanolic extract of *Solanum nigrum* berries and a recovery of 70.12% was observed within 7 days. It offered gastroprotection against aspirin-induced ulcer by significantly blocking lipid peroxidation. Prashanth *et al.* (2001) have reported that *Solanum nigrum* exhibited significant hydroxyl radical scavenging potential, thus suggesting its probable mechanism of cytoprotection. Scavenging activity of this extract also reduced lipid peroxidation. Moreover, flavonoids have been reported for their anti-ulcerogenic activity and gastric protection already (Alarcón de la Lastra *et al.*, 1994; Parmar and Parmar, 1998). Gülçin *et al.* (2004) showed that water extract of nettle (*Urtica dioica*) had effective antiulcer activity against ethanol-induced ulcerogenesis in rats. Preventive effects of 50, 100 and 200 mg/kg water extract of nettle were in a dose-dependent manner.

Desai (1975) reported the application of plucked stem bark of *Bauhinia racemosa* in case of wound and mouth ulcer. Sánchez Perera *et al.* (2001) have evaluated the anti-ulcer effect of *Rhizophora mangle* bark on the gastric ulcer induced by using ethanol-hydrochloric acid in rats which caused the mucosal damage in it. They have used the drug cimetidine as standard. The effect of these agents on the quality and quantity of the
gastric mucus were also determined by giving oral dosage of the aqueous extract of the sample at different doses. Oral doses at 500 mg/kg body weight gave the highest level of gastric protection. Mucus content increased, accompanied by a proportional increase in proteins. The wound healing capacity of *Excoecaria agallocha* during ulcer was due to several mechanisms, such as coating the wound, forming complexes with proteins of cell wall, chelating free radicals and reactive oxygen species, stimulating the contraction of the wound and increasing the formation of new capillaries and fibroblasts (Fernandez *et al.*, 2002).

Similar to the present study, Borikar *et al.* (2009) demonstrated a significant reduction in the ulcer number on administration of aqueous (200mg/kg body weight) and alcoholic extracts (100 and 200mg/kg body weight) of *Bauhinia racemosa*, when compared with control group. There was also significant reduction in the ulcer score when administered alcoholic extracts at 100 and 200mg/kg body weight of *Bauhinia racemosa*, when compared with control group. So it was considered that the plant *Bauhinia racemosa* had significantly decreased the number of ulcers in paracetamol-induced gastric ulcer in rats. This may due to the presence of flavonoids which may reduce the gastric secretion and peptic activity and prevent the formation of gastric ulcer. Surprisingly, Thirunavukkarasu *et al.* (2009) demonstrated that pretreatment with hot water and cold water extracts of *Excoecaria agallocha* bark, at doses (62.5 and 125 mg/kg body weight) decreased the ulcerated area. The volume and acidity of the gastric juice also decreased in the pretreated rats. *E. agallocha* had tannins or polyphenolic compounds which were responsible for its anti-ulcer property.

In line with the present investigation, the methanolic extract of fruits of *Terminalia chebula* showed protection against characteristic lesions produced by ethanol administration. This anti-ulcer effect of *Terminalia chebula* extract may be due to both reductions in gastric acid secretion and gastric cytoprotection (Raju *et al.*, 2009). Further, Ubaka *et al.* (2010) showed that the aqueous extract of leaves of *Aspilia africana* exhibited a significant and dose-dependent anti-ulcer activity in ethanol, indomethacin and aspirin-induced ulcer models. Percentage ulcer inhibitions of extract at 100 mg/kg for
ethanol, indomethacin and aspirin induced ulcers were 73.0, 60.9 and 87.6%, respectively. Ulcer protections in all the models by the extract were dose-dependent. The ulcer inhibitory effects of the extract were comparable with those of standard drugs especially in the drug-induced ulcers. Ulcer protection may be attributed to the presence of phytochemical constituents like flavonoids, tannins and saponins in the extract which have been shown to produce anti-ulcerogenic and anti-gastric activity (Carlo et al., 1994; Aguwa and Ukwe, 1997).

Recently, it has been also shown that ROS possess an important role in the pathogenesis of mucosal damages caused by indomethacin, ethanol and other agents (Das et al., 1997; Elliot and Wallace, 1998; Smith and Kvietsy, 1988; Miura et al., 2002). Superoxides produced by peroxidases in the tissues might damage the membranes and stomach tissues by increasing the lipid peroxidation (Takeuchi et al., 1991; Miura et al., 2002). Similarly, the results of the present study showed that there was a significant increase in the LPO level in rat stomach tissues administrated with ethanol. However, significant decreases in the LPO level were observed by the administration of all doses (200 and 400 mg/kg) of ethanolic extracts of leaf and stem bark of *Elaeocarpus serratus* and *Elaeocarpus tuberculatus* and omeprazole (10 mg/kg body weight).

On the other hand, organisms have enzymatic and non-enzymatic defenses, including SOD, GSH, CAT, GPx and GR against to the lipid peroxidation in tissues, caused by ROS (Mates et al., 1999). Previously, it has been shown that the administration of NSAIDs and ethanol decreased the levels of GSH, SOD and GPx in tissues (Takeuchi et al., 1991; El-Missiry et al., 2001; Bafna and Balaraman, 2004). Similarly, in the present finding, the levels of SOD, CAT, GPx and GST in rat stomach tissues were significantly reduced by administration of ethanol. Contrarily, the administration of ethanolic extracts of leaf and stem bark of *Elaeocarpus serratus* and *Elaeocarpus tuberculatus* at 200 and 400 mg/kg and omeprazole (10 mg/kg) resulted in a significant increase in the SOD, CAT, GPx, GST levels. However, according to present results, the levels of LPO and NO were found to be increased in ethanol-treated rat tissues as compared with tissues of healthy rats. The activities of these enzymes were lowered by
the administration of all doses of ethanolic extracts of leaf and stem bark of *Elaeocarpus serratus* and *Elaeocarpus tuberculatus*, which can be attributed to the decrease in the tissue H\textsubscript{2}O\textsubscript{2} and OH\textsuperscript{−} levels. This increase might be due to an increase in the mucosal H\textsubscript{2}O\textsubscript{2} and OH\textsuperscript{−} level, occurred by inhibition of peroxidases (Banarjee, 1990).

Further SOD and CAT enzymes are highly specific in their catalytic mode of actions and it decreased the gastric mucosal damaging effect (Halliwall and Gutteridge, 1985). Although the increase in catalase is necessary for effective antioxidant activity, the changes in CAT levels were significant (Mizui *et al*., 1987). Hence the antioxidant activity of methanolic extract of *Solanum nigrum* berries may be one of the important defensive factors involved in its ulceroprotective effect (Jainu and Shyamala Devi, 2004). The role of GSH as an endogenous gastric antioxidant in mucosal protection, however, remains controversial since recent evidence has indicated an inverse correlation between gastric mucosal GSH levels and mucosal protection (Mutoh *et al*., 1991). GST and GPx are essential for maintaining a constant ratio of reduced glutathione to oxidized glutathione in the cell. The *E. serratus* and *E. tuberculatus* extracts maintained the activity of GPx almost at near normalcy by its ability to increase the level of reduced GSH, and to decrease lipid peroxidation. Previous studies have shown that the increase in GST activity when rats were fed on *Solanum nigrum* diet (Moundipa and Domangang, 1991). The enhancement in GST activity was observed in the gastric mucosal tissues of animals pretreated with ethanolic extracts of leaf and stem bark of *E. serratus* and *E. tuberculatus* compared to ulcerated tissues.

Similarly, Berenguer *et al.* (2006) showed that pretreatment with the aqueous bark extract of *Rhizophora mangle* had a beneficial effect on NSAID-induced ulcers in rats as evidenced by the reduction in the ulcerated area and the histological appearance of the gastric mucosa. These results may be attributed to the polyphenolic compounds found in *Rhizophora mangle*, as it has been suggested that phenols stimulate Prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) formation based on their action as cosubstrates for the peroxidase reaction (Alanko *et al*., 1999). SOD activity decreased with diclofenac but augmented significantly in the *Rhizophora mangle* and omeprazole-treated groups. This enzyme,
which owed its antioxidant properties to its elevated capacity to scavenge $\text{O}_2^{\cdot-}$, played an important role in protecting the gastrointestinal mucosa. It has been shown that the subcutaneous administration of SOD and catalase significantly reduced the gastric damage induced by ischemia-reperfusion (Yoshikawa et al., 1989) or indomethacin treatment (Yoshikawa et al., 1993; Naito et al., 1998).

Interestingly, the gastroprotective effect of some drugs, such as flavonoids, also has been related with their capacity to modulate this enzymatic activity against ulcers induced by ethanol (La Casa et al., 2000). GPx enzyme played a marked role in the removal of $\text{H}_2\text{O}_2$ and lipid hydroperoxides in gastric mucosal cells and its antioxidant capacity was similar to that of SOD or vitamin E (Richard et al., 1997). GPx inhibition resulted in $\text{H}_2\text{O}_2$ accumulation and subsequent lipid oxidation and could be related to the gastric damage induced by indomethacin (Yoshikawa et al., 1993; Naito et al., 1998). The increase in SOD and GPx levels observed in the groups pretreated with ethanolic extracts of leaf and stem bark of *E.* *serratus* and *E.* *tuberculatus* clearly pointed to an antioxidant mechanism underlying its gastroprotective action and, on the other hand, the ability to prevent lipid peroxidation *in vitro* reinforced its potential use as a therapeutic drug for free radical pathologies. The increased values of SOD and GPx in omeprazole-treated animals also pointed to an antioxidant mechanism of proton pump inhibitors, which was supported by a recent study on ethanol–HCl induced ulcers in rats (Natale et al., 2004). Tannins might prevent ulcer development due to their protein precipitating and vasoconstricting effects (Aguwa and Nwako, 1988). Their astringent action could help precipitating microproteins on the ulcer site, thereby forming an impervious layer over the lining that hindered gut secretions and protected the underlying mucosa from toxins and other irritants (Nwafor et al., 1996, 2000; Al-Rehaily et al., 2002). This propensity to bind to proteins also explained the fact that polyphenols inhibited enzymes tested *in vitro*.

Bhattacharya et al. (2007) studied the healing activity of the ethanol extracts of *Piper betel*, *Emblica officinalis*, *Terminalia bellerica*, and *Terminalia chebula* against the indomethacin-induced stomach ulceration and compared it with that of misoprostol.
relative healing activities of the extracts were \( P. \text{betel} > E. \text{officinalis} > T. \text{bellerica} \sim T. \text{chebula} \). Therefore it was established that the chosen plant extracts especially ethanol extract of \( P. \text{betel} \) and \( E. \text{officinalis} \) could heal indomethacin-induced stomach ulceration in rats by their antioxidant action and ability to form mucus. Comparison of their efficacy with that of misoprostol further confirmed the findings.

Tissue damage is always associated with lipid peroxidation, loss of DNA content and impairment of protein synthesis (Marotta et al., 1999) due to excess generation of free radicals. These free radicals also damage the cellular antioxidant enzymes such as CAT, SOD and others, leading to aggravated tissue damage during stomach ulceration (El-Missiry et al., 2001). The present findings revealed that the ethanol-induced stomach ulceration was accompanied with a severe oxidative stress in the gastric tissues causing damages to key biomolecules such as lipids, proteins and DNA. The gastric activities of SOD and CAT were also decreased notably following ethanol intake. Treatment with the standard reference drug omeprazole could bring these parameters towards normal levels, than observed in natural recovery. Suppression of most of the biochemical adverse effects by omeprazole might decrease the ulcer progression and promote healing of gastric lesions induced by acute intake of ethanol. In conclusion, all doses (200 and 400 mg/kg b.w.) of ethanolic extracts of leaf and stem bark of \( E. \text{serratus} \) and \( E. \text{tuberculatus} \) showed a significant gastroprotective effect in the ethanol-induced ulcer model. The gastroprotective effect could be attributed to its reducing effect against oxidative damage.

From the present study it can be concluded that the preliminary phytochemical examination of \( E. \text{serratus} \) and \( E. \text{tuberculatus} \) indicated the presence of phenols, flavonoids and tannin which might be responsible for the antioxidant activity.

On the basis of the results of this study, acetone, methanol and water extracts of leaf, stem bark and fruit of \( E. \text{serratus} \) and \( E. \text{tuberculatus} \) possessed potent antioxidant and free radical scavenging properties. The capability of the crude extracts to scavenge
the different free radicals in different systems (DPPH’ scavenging assay, Hydroxyl radical (‘OH) scavenging assay, Superoxide radical (O$_2^{..}$) scavenging assay, Nitric oxide radical (NO’) scavenging assay, Reducing power assay, ABTS$^{..+}$ scavenging assay, β–carotene/linoleic acid peroxidation inhibition assay and Metal chelating assay), indicated that they might be useful therapeutic agents for treating free radical-related pathological damages.

The crude extracts of leaf and stem bark of *E.serratus* and *E.tuberculatus* had a remarkable anti-inflammatory activity against carrageenan-induced paw edema in rats. These activities might be due to their content of antioxidant phytoceuticals namely, phenols, flavonoids and tannin. This study demonstrated the efficacy of *E.serratus and E.tuberculatus* as an anti-inflammatory agent and also scientifically justified the use of these plants as an anti-edematous agent in folk medicine.

The gastroprotective effect of *E. serratus and E.tuberculatus* in the experimental ulcers induced by ethanol could be related with its antioxidant properties, which increased the activity of the antioxidant enzymes SOD, CAT, GPx, GST and on the other side decreased the levels of lipid peroxidation and nitric oxide. Further, the present study suggested that due to both antioxidant and gastroprotective properties, the solvent extracts of the leaf and stem bark of *E.serratus and E. tuberculatus* might represent an attractive therapeutic option for protecting against induced gastric ulcers.

Based on the foregoing preclinical efficacy and safety data, the *E.serratus* and *E.tuberculatus* extracts could be considered as safe and effective intervention for ulcer and inflammatory diseases. The bioguided isolation of the active constituents of *E.serratus and E.tuberculatus* is being conducted, aiming the formulation of a safer and efficient drug.