4. RESULTS

Two medicinal plant species of Elaeocarpaceae namely, *Elaeocarpus serratus* L. and *Elaeocarpus tuberculatus* Roxb. selected on the basis of ethnobotanical information compiled from folk medicine, have been evaluated for their *in vitro* antioxidant phytoceuticals, antioxidative, anti-inflammatory, antimicrobial and *in vivo* pharmacological activities.

In the present study, the effect of various solvents such as acetone, methanol and water on the per cent yield of leaf, stem bark and fruit of *Elaeocarpus serratus* and *Elaeocarpus tuberculatus* were investigated. Difference between extraction solvents were monitored *via* quantification of antioxidant phytoceuticals such as total phenols, flavonoids and tannin. The extracts obtained by different solvents were screened for their potent antioxidant activity using eight complementary test systems. The extracts of the medicinal plants were tested for their potential antimicrobial activity against human pathogens. Various *in vitro* anti-inflammatory pharmacological models such as, inhibition of protein denaturation and proteinase inhibitory action were studied. Further, the *in vivo* anti-inflammatory and anti-ulcerogenic activities of the plant extracts were assessed.

4.1. Estimation of Percentage Yield of *Elaeocarpus serratus* and *Elaeocarpus tuberculatus*

In the present study, leaf, stem bark and fruit of *E. serratus* and *E. tuberculatus* were shade dried and powdered materials were extracted in the Soxhlet apparatus successively with different solvents in the increasing order of polarity [Acetone (56.5°C), Methanol (64.7°C), Ethanol (78.5°C) and Water (99.98°C)]. Obtained extracts were dried, weighed and the per cent yield was calculated as depicted in Table 1. The maximum per cent yield was registered in the acetone extract of leaf of *E. serratus* and *E. tuberculatus* (27.67% and 22.01%, respectively). The methanol extract of stem bark of *E. serratus* and *E. tuberculatus* registered an yield percentage of 16.0% and 12.30%,
respectively whereas, the acetone extract of fruit of *E.serratus* and *E.tuberculatus* registered an yield per cent of 10.0 % and 14.20%, respectively. Generally, the acetone and methanol extracts of plant parts contained more constituents than the water extracts.

### 4.2. Quantitative Determination of Antioxidant Phytoceuticals in *E.serratus* and *E. tuberculatus*

The results in Tables 2 and 3 shows the estimated antioxidant phytoceuticals such as total phenols, total flavonoids and tannin in the acetone, methanol and water extracts of leaf, stem bark and fruit of *E. serratus* and *E. tuberculatus*.

#### 4.2.1. Estimation of Total Phenolic Content of *E.serratus* and *E. tuberculatus*

Table 2 shows the total phenolic content in the different solvent extracts (acetone, methanol and water) of *E.serratus* leaf, stem bark and fruit. The total phenols were estimated using Folin-Ciocalteau method and expressed as tannic acid equivalents. In the stem bark, the highest total phenolic content was confined to the methanolic extract (390.84 ± 4.1 mg /g), followed by the acetone extract (389.42 ± 6.7 mg /g). The next maximum was shown in the leaf methanolic and acetone extracts (313.73 ± 0.8 mg /g and 302.21 ± 6.2 mg /g, respectively). The total phenolic content of the methanolic extract of fruit was found to be 256.52 ± 4.1mg /g. In general, among the three solvent systems used, the methanolic extracts of leaf, stem bark and fruit offered the maximum total phenolic content. At the same time the water extracts of the studied plant parts contained comparatively lesser amount of phenols.

The total phenolic content of the different solvent extracts of leaf, stem bark and fruit of *E.tuberculatus* is depicted in Table 3. In *E.tuberculatus*, the methanol extracts of the different plant parts registered high total phenolic content like *E.serratus* extracts, but the highest value was recorded in the acetone extract of stem bark (70.05 ± 9.67 mg/g). Generally the total phenolic content of *E. serratus* extracts was almost five times higher than *E. tuberculatus* extracts.
4.2.2. Estimation of Total Flavonoid Content of *E.serratus* and *E. tuberculatus*

The obtained results (Tables 2 and 3) show the total flavonoid content of acetone, methanol and water extracts of leaf, stem bark and fruit of *E.serratus* and *E.tuberculatus* in terms of mg rutin equivalent / g dry weight.

In *E. serratus*, the highest and lowest total flavonoid content was registered in the methanolic and water extracts of fruit (100.27 ± 1.2 and 13.16 ± 0.5 mg rutin/g dry weight, respectively). Generally, the total flavonoid content in the different plant parts ranged in the following ascending order: methanol extract of leaf (79.90 ±1.2 mg /g) < acetone extract of stem bark (79.92 ±0.8 mg /g) < methanol extract of stem bark (90.23 ± 0.6 mg /g) < acetone extract of leaf (93.21± 0.1 mg /g). Further, the lowest total flavonoid content was registered in the water extracts of all the plant parts used (Table 2).

The estimation of total flavonoid content in *E. tuberculatus* revealed that the acetone extract of leaf rendered the highest total flavonoid content (96.67 ± 3.84 mg rutin/g dry weight), followed by the acetone extract of stem bark (53.31 ± 6.25 mg/g dry weight) and methanolic extract of leaf (51.97 ± 2.23 mg/g dry weight). But in the fruit, methanolic extract had the maximum total flavonoid content of 16.44 ±0.77 mg/g. Generally, the water extracts of all the plant parts contained lower values especially, the water extract of fruit had the lowest concentration of total flavonoids (6.40 ± 1.46 mg/g) (Table 3).

4.2.2. Estimation of Tannin Content of *E.serratus* and *E. tuberculatus*

Tables 2 and 3 show the tannin content of acetone, methanol and water extracts of leaf, stem bark and fruit of *E.serratus* and *E.tuberculatus* in terms of mg tannic acid equivalent / g dry weight.

From the Table 2, the highest tannin content was recorded in the acetone extract of stem bark (355.72 ± 1.8 mg/g), followed by the acetone extract of leaf (271.21 ± 1.1mg/g) and methanolic extract of fruit (207.90 ± 2.7 mg/g) of *E.serratus*. Of all the
parts used in the present study, the water extract of fruit showed the minimum tannin content (33.80 ± 1.6 mg/g).

In *E. tuberculatus* (Table 3) the tannin content in the acetone extract of stem bark was at a maximum level of 66.78 ± 1.64 mg/g, followed by the methanol extract of leaf and fruit (58.14 ± 0.50 and 29.86 ± 0.50 mg/g, respectively). The lowest value was observed in the water extract of fruit (8.52 ± 0.19 mg/g).

**4.3. Assessment of *In vitro* Antioxidant Activity of *E.serratus* and *E. tuberculatus***

The crude extracts of *E.serratus* and *E.tuberculatus* obtained by using different solvents were subjected to screening for their possible antioxidant activity. The *in vitro* antioxidant activities of the test plants’ extracts were measured in different systems of assay such as 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. In the present study, the plant extracts were found to have different levels of antioxidant activity in the different systems used.

**4.3.1. DPPH’ Radical Scavenging Activity of *E.serratus* and *E. tuberculatus***

The DPPH’ scavenging assay of the various solvent (acetone, methanol and water) extracts of leaf, stem bark and fruit of *E.serratus* and *E.tuberculatus* were studied. The DPPH’ test is based on the exchange of hydrogen atoms between the antioxidants and the stable DPPH free radicals. The IC$_{50}$ value is negatively related to antioxidant activity as it expressed the amount of antioxidant needed to decrease the radical concentration by 50%. The lower IC$_{50}$ value indicated higher antioxidant activity of tested sample. Practically, the reaction brings about the reduction of DPPH radicals to the corresponding hydrazine which is manifested by a colour change from violet to yellow when monitored spectrometrically. The dose-dependent response of DPPH radical
scavenging activity of various solvent extracts was studied and the IC$_{50}$ (µg/ml) values of the extracts are shown in Figs. 1 and 2.

Generally in $E.\text{serratus}$, the values of 50% inhibition concentration ranged between 2.73 ± 1.09 and 92.42 ± 2.29 µg/ml (Fig.1). All the plant parts used in the present study with their solvents showed higher antioxidant activity. The methanol extract of leaf showed the maximum DPPH$^-$ scavenging activity with IC$_{50}$ value of 2.73 ± 1.09 µg/ml, followed by the acetone extract of stem bark (IC$_{50}$ = 3.30 ± 1.10 µg/ml) and methanol extract of stem bark (IC$_{50}$ = 3.96 ± 1.48 µg/ml). The methanol extract of fruit showed an IC$_{50}$ value of 5.34 ± 0.90 µg/ml for the inhibition of DPPH$^-$ . These results indicated that the leaf and stem bark extracts displayed remarkable DPPH radical quenching property. The IC$_{50}$ values of BHA and gallic acid were 1.07 ± 0.4 and 0.63±0.3µg/ml, respectively. Generally, the DPPH$^-$ scavenging activity was lesser in the plant extracts than the standard BHA and gallic acid. The DPPH$^-$ quenching ability of the methanol extract of leaf was closer to that of the standard BHA.

The results depicted in Fig.2 indicate that the free radical reduction activity of the analyzed samples of $E.\text{tuberculatus}$ were concentration-dependent. The acetone extract of stem bark quenched the DPPH radical effectively (IC$_{50}$ = 3.92 ± 3.4 µg/ml) though it showed lesser activity than the standard BHA and gallic acid. The water extract of fruit showed the weakest DPPH radical quenching activity (IC$_{50}$ = 58.14 ±0.7 µg/ml). In general, the effectiveness of acetone and methanol extracts of leaf and stem bark as DPPH radical scavengers (IC$_{50}$) ranged in the following ascending order: methanol extract of leaf (6.32 ± 2.1 µg/ml) < acetone extracts of leaf (6.14 ±1.6 µg/ml) < methanol extract of stem bark (5.44 ± 1.9 µg/ml) < acetone extract of stem bark (3.92 ± 3.4 µg/ml). The methanolic fruit extract exhibited noticeable antioxidant activity (8.37 ± 1.9µg/ml). It was observed that acetone and methanol extracts of stem bark had higher activity than that of the leaf and fruit. In general, the $E.\text{serratus}$ extracts were more efficient in quenching the DPPH radical than $E.\text{tuberculatus}$ extracts.
4.3.2. Hydroxyl Radical (\(\cdot\)OH) Scavenging Activity of \textit{E.serratus} and \textit{E. tuberculatus}

Hydroxyl radical (\(\cdot\)OH) is the most reactive among reactive oxygen species. The results of antioxidant activity of solvent extracts of plants were compared with BHA and gallic acid standard equivalents (Figs. 3 and 4).

The scavenging activity of \textit{E.serratus} leaf, stem bark and fruit in various solvent systems on \(\cdot\)OH is presented in Fig. 3. The different solvent extracts of leaf, stem bark and fruit displayed a dose-dependent scavenging activity against the \(\cdot\)OH species, of which the water extract of fruit, acetone and methanol extracts of stem bark, acetone and water extracts of leaf showed IC\(_{50}\) values of 71.14 ± 1.23, 72.30 ± 1.96, 75.99 ± 2.98, 82.39 ± 2.71 and 89.73 ± 2.37 µg/ml, respectively. The methanol extracts of fruit and leaf and water extract of stem bark possessed weak antioxidant activity with IC\(_{50}\) values of 309.21 ± 3.79, 189.97± 4.53 and 274.72 ± 1.99 µg/ml, respectively. Generally, the IC\(_{50}\) values of the extracts were more than that of the standard equivalents BHA (IC\(_{50}\) = 7.94 ± 1.09 µg/ml) and gallic acid (IC\(_{50}\) = 2.7 ± 1.33 µg/ml).

Fig. 4 displays the \(\cdot\)OH scavenging activity of the different solvent extracts of leaf, stem bark and fruit of \textit{E.tuberculatus}. The hydroxyl radical scavenging activity of the water extract of leaf, methanol, acetone and water extracts of stem bark with IC\(_{50}\) values of 18.20 ± 1.37, 18.75 ± 1.88, 19.65 ± 2.23 and 20.55 ± 1.42 µg/ml, respectively was comparatively lesser than that of BHA (IC\(_{50}\) = 7.94 ± 1.09 µg/ml) and gallic acid (IC\(_{50}\) = 2.7 ± 1.33 µg/ml) standards. The \textit{E.tuberculatus} extracts were comparably effective in scavenging the \(\cdot\)OH radicals than \textit{E.serratus} extracts.

4.3.3. Superoxide Radical (O\(_2\)\(^\cdot\)) Scavenging Activity of \textit{E.serratus} and \textit{E.tuberculatus}

The superoxide anion radical (O\(_2\)\(^\cdot\)) is a highly toxic species which is generated by numerous biological and photochemical reactions. Fig.5 shows the superoxide radical scavenging activity of \textit{E.serratus} (leaf, stem bark and fruit) in different solvent systems. The scavenging capacity was comparably good in the methanol extract of stem bark (IC\(_{50}\) = 58.00 ± 0.66 µg/ml) and acetone, water and methanol extracts of leaf (IC\(_{50}\) = 59.52 ±
0.79, IC$_{50}$ = 60.67 ± 0.52 and 67.78 ± 0.37 µg/ml, respectively). The acetone extract of fruit (IC$_{50}$ = 148.28 ± 0.31 µg/ml) and the water extract of stem bark (IC$_{50}$ = 139.24 ± 1.29 µg/ml) showed comparatively weak activities than that of BHA (IC$_{50}$ = 14.57 ± 0.16 µg/ml) and gallic acid (IC$_{50}$ = 38.27 ± 0.28 µg/ml) standard equivalents.

The IC$_{50}$ values of superoxide radical scavenging activity of *E.tuberculatus* are depicted in Fig.6. The highest superoxide anion radical scavenging activity was reported in the acetone extract of leaf (IC$_{50}$ = 21.81 ± 0.67 µg/ml), which was higher than that of the gallic acid standard having an IC$_{50}$ value of 38.27 ± 0.28 µg/ml. The next highest IC$_{50}$ values of 25.92 ± 1.40, 29.17 ± 6.01 and 37.97 ± 0.38 µg/ml were recorded in the methanol extracts of leaf and fruit and water extract of stem bark, respectively. Generally the dose-dependent superoxide radical scavenging potential was higher in *E.tuberculatus* than *E.serratus*.

### 4.3.4. Nitric oxide (NO$^\cdot$) Radical Scavenging Activity of *E.serratus* and *E. tuberculatus*

Excess concentration of NO$^\cdot$ is associated with several diseases. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions, which act as free radicals. In the present study, the extracts compete with oxygen to react with nitric oxide and thus inhibit the generation of the anions (Figs. 7 and 8).

Fig.7 illustrates the dose-dependent scavenging of nitric oxide by different solvent extracts of leaf, stem bark and fruit of *E.serratus*. BHA and gallic acid were used as reference compounds. The acetone extract of fruit showed the lowest IC$_{50}$ value of 64.12 ± 0.44 µg/ml, followed by the acetone extract of leaf (IC$_{50}$ value = 66.53 ± 0.37 µg/ml), indicating their potent antioxidant activity. In the present study, the lowest nitric oxide radical scavenging activity was noted in the methanol extract of leaf (IC$_{50}$ = 117.48 ± 0.64 µg/ml). Generally, the IC$_{50}$ values of the extracts were found to be higher than that of the standards BHA (43.37 ± 1.26 µg/ml) and gallic acid (29.76 ± 0.81 µg/ml).

The scavenging of nitric oxide by different solvent extracts of leaf, stem bark and fruit of *E.tuberculatus* is shown in Fig.8. The various solvent extracts of the different
plant parts of *E. tuberculatus* scavenged 50% of nitric oxide generated by incubation. Generally, the IC50 values of the extracts were found to be lesser than that of the standard BHA (43.37 ± 1.26 µg/ml) and gallic acid (29.76 ± 0.81 µg/ml). NO\(^\cdot\) scavenging activity was higher in all the tested samples except, the water extract of fruit (IC50 = 52.25 ± 0.12 µg/ml). Interestingly, nearly all the extracts of *E. tuberculatus* showed higher NO\(^\cdot\) scavenging ability than *E. serratus* extracts.

### 4.3.5. Reducing Power Assay of *E. serratus* and *E. tuberculatus*

For the determination of antioxidant property, the reducing power of the solvent extracts of *E. serratus* and *E. tuberculatus* were determined. The results revealed that the plant extracts contained molecules that were potential electron donors and also reacted with free radicals, converting them to more stable products and terminated the radical chain. Higher absorbance of the reaction mixture indicated the higher reducing power.

Fig. 9 shows the reductive capabilities of acetone, methanol and water extracts of leaf, stem bark and fruit of *E. serratus* compared to gallic acid and BHA standard equivalents. The methanolic extract of leaf showed a dose-dependent reduction ability in reducing power assay with a maximum absorbance of 2.027 at a concentration of 100 µg/ml, followed by the methanolic extract of stem bark (1.625), comparable to BHA and gallic acid standards which gave maximum absorbance of 1.728 and 1.691 respectively, at a concentration of 100 µg/ml. The methanolic extract of leaf showed comparatively higher reducing ability than the standards used.

Fig. 10 shows the reductive capability of plant extracts compared to gallic acid and BHA standard equivalents. The reducing power of the extracts increased with the quantity of the sample. The reducing power of the acetone extract of stem bark (1.169) at 100 µg/ml, was comparable to that of the standards whereas, at 80µg/ml the extract reduced most of the Fe\(^{3+}\) ions and had a reducing power of 1.018 which was comparatively higher than 80µg/ml of BHA standard (0.908). Generally, all other extracts of plant parts used showed lesser reducing activity compared to the standards.
except the methanolic extracts of *E. serratus* leaf, stem bark and fruit which showed higher activities than acetone extract of *E.tuberculatus* stem bark.

### 4.3.6. ABTS•⁺ Radical Scavenging Activity of *E.serratus* and *E. tuberculatus*

Proton radical scavenging is an important attribute of antioxidants. ABTS•⁺, a protonated radical has characteristic absorbance maxima at 734nm which decreases with the scavenging of proton radicals. Table 4 depicts the potential activity of the different solvent extracts of leaf, stem bark and fruit of *E.serratus* in ABTS•⁺ decolorization. In the ABTS•⁺ assay, the antioxidant capacities of the extracts ranged from 4840.7 ± 33.41 to 19796.6 ± 32.4 µmol TAA/g. The methanol extract of stem bark which showed the highest value of 19796.6 ± 32.4 µmol TAA/g extract was a fast and effective scavenger of ABTS cation radical. The scavenging of ABTS•⁺ by other extracts namely, methanol extract of leaf (19324.2 ± 21.01 µmol/g), acetone extract of stem bark (19391.7 ± 44.2 µmol/g) and water extract of fruit (19390.7 ± 33.4 µmol/g) was found to be higher indicating high antioxidant activity. The lowest ABTS•⁺ scavenging activity was noted in the water extract of stem bark (4840.7 ± 33.41µmol TAA/g), followed by the acetone extract of fruit (8967.8 ± 229.6 µmol TAA/g).

The ability of the different solvent extracts of leaf, stem bark and fruit of *E.tuberculatus* to effectively scavenge the ABTS radical cation is displayed in Table 5. *E. tuberculatus* exhibited potent ABTS•⁺ scavenging activity with maximum activity seen in the methanol extract of stem bark (19960.6 ± 28.9 µmol TAA/g), followed by the acetone extract of stem bark (19507.4 ± 169.5 µmol TAA/g), water extract of fruit (19227.7 ± 213.2 µmol TAA/g) and methanol extract of leaf (19054.2 ± 116.9 µmol TAA/g). The ABTS•⁺ scavenging was comparatively higher in *E.tuberculatus* than *E.serratus* solvent extracts.
4.3.7. β-carotene / Linoleic Acid Peroxidation Inhibition Activity of *E.serratus* and *E. tuberculatus*

Antioxidant activities of different solvent extracts of leaf, stem bark and fruit of *E.serratus* and *E.tuberculatus* evaluated against inhibition of β-carotene/linoleic acid peroxidation are presented in Tables 6 and 7. The total antioxidative activity, reflected by the ability of the *E. serratus* leaf, stem bark and fruit solvent extracts to inhibit the bleaching of β-carotene was measured and compared with that of BHA and gallic acid standard control (Table 6). The maximum inhibition of peroxidation was seen in the methanolic extract of stem bark (87.95 ± 0.79%). However the percentage inhibition of peroxidation by the standards BHA (91.37 ± 2.2%) and gallic acid (94.11 ± 1.5%) always seemed to be higher than that of the plant extracts. This could be due to moderate inhibition of β-carotene bleaching activity.

In this assay, all the plant extracts of *E. tuberculatus* showed moderate antioxidant capacity. The highest inhibitory activity was observed in the acetone extract of fruit (89.50 ± 5.41%), followed by the acetone extract of leaf (87.91 ± 3.92%). The inhibition percentage of the standards BHA and gallic acid were 91.37 ± 2.2% and 94.11 ± 1.5%, respectively and greater than that of the plant extracts used (Table 7). In general, the inhibition of β-carotene/linoleic acid peroxidation by the *E. tuberculatus* extracts was higher than *E. serratus* extracts.

4.3.8. Metal Chelating Activity of *E.serratus* and *E. tuberculatus*

Tables 8 and 9 show the chelating activity of the various solvent extracts of leaf, stem bark and fruit of *E.serratus* and *E. tuberculatus*. The data obtained from Table 8 revealed that *E.serratus* most actively interfered with the formation of free radicals. *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 ± 2.4mg EDTA/g extract), followed by the methanol extract of fruit and stem bark (1074.9 ± 2.4mg EDTA/g and 1071.9 ± 3.5 mg EDTA/g,
respectively). The lowest chelating activity was noticed in the acetone extract of fruit (993.3 ± 1.3 mg EDTA/g extract).

In *E. tuberculatus* the highest metal chelating activity was exhibited by the water extracts of leaf, stem bark and fruit (1187.1 ± 1.6 mg EDTA/g, 1096.2 ± 2.5 mg EDTA/g and 955.6 ± 5.2 mg EDTA/g, respectively). The acetone and methanol extracts of the studied plant parts showed comparatively lesser chelating activity than the water extracts (Table 9). Generally, the *E. serratus* extracts exhibited remarkable metal chelating activity than *E. tuberculatus* extracts.

4.4. Assessment of Antimicrobial Activity of *E.serratus* and *E. tuberculatus*

The antimicrobial activity of *E. serratus* and *E. tuberculatus* leaf, stem bark and fruit extracts were examined against five pathogens [four bacterial species (Shigella sonnei, Salmonella typhi, Staphylococcus aureus and Klebsiella pneumoniae) and one fungal species (Candida albicans)] causing illness in human beings. The extraction was carried out using acetone, methanol and water. Results were recorded as presence or absence of zones of inhibition around the well (Tables 10 and 11 Plates 6 to 10).

The antimicrobial activity of different extraction solvents (acetone, methanol and water) of *E. serratus* leaf, stem bark and fruit is presented in Table 10 and Plates 6 to 10. The extracts exhibited a dose-dependent inhibition of test bacteria and fungi. Among the extraction medium, the water extract of leaf, stem bark and fruit was found to be more susceptible to the bacterial species and showed no zone of inhibition, except *Shigella sonnei*. In *Shigella sonnei*, the higher concentrations (150 and 200 μg/ml) of water extract of the plant parts showed minimum inhibition of bacteria. The acetone and methanol extracts of leaf and stem bark displayed maximum antibacterial activity against all the bacterial species studied as revealed by wider zones of inhibition. The bacterium *Salmonella typhi* was highly inhibited by the methanolic extract of leaf (zone of inhibition (ZI) = 18±0.41mm), followed by *Staphylococcus aureus* (ZI=17±0.99mm), *Shigella sonnei* (ZI=16.5±1.87mm) and *Klebsiella pneumoniae* (ZI=16±1.87mm) at 200 μg/ml, respectively. The fruit extracts had comparatively lesser inhibitory effect on the
bacterial species in the present study. The standard antibiotic gentamycin inhibited the growth of all the bacterial species effectively at a lower concentration of 50 μg/ml. The inhibition of all the bacterial species (except *Klebsiella pneumoniae*) by the various solvent extracts of the plant parts was generally lower than that of the standard gentamycin.

The antifungal activity of the *E. serratus* solvent extracts on *Candida albicans* as revealed by the inhibition of fungal growth through the susceptibility pattern was not uniform. The water extract of leaf and acetone extract of fruit produced the maximum inhibition zone of 18±1.05 and 18±0.92 mm, respectively at 200 μg/ml, followed by the methanolic extract of fruit (17±1.62 mm at 200 μg/ml and 16±1.04 mm at 150 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. On the whole, the various solvent extracts of the plant parts at higher concentrations (150 and 200 μg/ml) inhibited the fungal growth more effectively than the standard amphotericin which showed an inhibition zone of 11±0.02 mm at a concentration of 100 units per disc.

Acetone, methanol and water extracts of leaf, stem bark and fruit of *E. tuberculatus* showed antimicrobial activity (Table 11 and Plates 11 to 15). Generally, the results summarized that the acetone and methanol extracts of leaf and stem bark showed a broad spectra of activity against all the investigated bacteria. The diameter of growth inhibition ranged from 9 to 20 mm. The highest zone of inhibition was observed against *Shigella sonnei* (20±0.62 mm) at 200 μg/ml. The acetone extract of leaf on *Klebsiella pneumoniae*, methanolic extract of leaf on *Staphylococcus aureus*, acetone extract of stem bark on *Salmonella typhi* at 200 μg/ml exhibited good activity in the range of 19±1.01, 18.5±1.59 and 17±0.15 mm, respectively. Normally, the water extracts possessed minimum activity especially, the water extract of fruit had little or no effect on all the bacterial species investigated. Moreover, 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 plant parts. The acetone and methanol extracts of leaf and stem bark at high concentrations (150 and 200 μg/ml) showed zones
of inhibition ranging from 11.5 to 19 mm which was comparable to gentamycin (ZI=12±0.02 mm at 50 μg/ml).

In the present findings, the higher concentrations (150 μg/ml and 200 μg/ml) of acetone, methanol and water extracts of leaf, stem bark and fruit of E.tuberculatus were found to be active against the fungus with zones of inhibition ranging from 11 to 17mm. The acetone extract of leaf 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 various solvent extracts of the plant parts at higher concentrations (150 and 200 μg/ml) inhibited the fungal growth more effectively than the standard amphotericin which showed an inhibition zone of 11±0.02 mm at a concentration of 100 units per disc.

4.5. Assessment of In vivo Pharmacological Activity of E.serratus and E. tuberculatus

4.5.1. Toxicological Evaluation of E.serratus and E. tuberculatus

A single dose (1000, 2000, 3000, 4000, and 5000 mg/kg b.w.(p.o.) of ethanolic extracts of leaf and stem bark of E. serratus and E. tuberculatus administered to Albino rats showed no death up to 72 hours. At these doses, there were no abnormal clinical signs which include changes in skin colour and behavioral changes like alertness, grooming, restlessness, tremors, convulsions and writhing at any time during the observation period. The effect of plant extracts on touch response, torch response, pain response, righting reflex, gripping strength, pinna reflex, corneal reflex, pupils, urination, salivation and lacrimation were also found to be normal. These results indicated that the plant extracts were quite safe even at a high dose of 5000 mg/kg b.w.p.o. and had no acute toxicity (Table 12).
4.5.2. Assessment of *In vitro* Anti-inflammatory Activity of *E.serratus* and *E. tuberculatus*

4.5.2.1. Inhibition of Protein Denaturation and Proteinase Inhibitory Action

Denaturation of protein is a well documented cause of inflammation. As a part of the present investigation, the anti-inflammatory activity of the ethanolic extracts of leaf and stem bark of *E.serratus* and *E.tuberculatus* at two different dose levels (200 and 400μg/ml) were evaluated *in vitro* on the inhibition of protein denaturation and proteinase inhibition and depicted in Table 13. *E.serratus* and *E.tuberculatus* leaf and stem bark extracts have shown a dose-dependent ability to thermally induced protein denaturation. The inhibition of protein denaturation ranged between 27.06% and 62.38%. *E.serratus* leaf extract showed the maximum inhibition of protein denaturation (62.38%) at a concentration of 400 μg/ml. Similarly, the *E.tuberculatus* leaf extract showed the maximum inhibition of protein denaturation (49.96%) at 400 μg/ml concentration. The standard indomethacin showed 75.45% inhibition of protein denaturation at a dose level of 100 μg/ml.

The ethanolic extracts of leaf and stem bark *E.serratus* and *E.tuberculatus* at two different dose levels (200 and 400 μg/ml) also exhibited remarkable anti-proteinase activity as shown in Table 13. *E.serratus* leaf extract showed maximum inhibition of proteinase (56.62%) at a concentration of 400 μg/ml whereas, *E.tuberculatus* leaf extract showed maximum inhibition of 45.38% at 400 μg/ml. On comparison with the crude plant extracts, the standard indomethacin showed maximum inhibition of 72.41% at 100 μg/ml concentration.

4.5.3. Assessment of *In vivo* Anti-inflammatory Activity of *E.serratus* and *E. tuberculatus*

4.5.3.1. Carrageenan-induced Paw Edema Assay

The anti-inflammatory activity of ethanolic extracts of leaf and stem bark of *E.serratus* and *E. tuberculatus* at low and high doses (200 and 400 mg/kg b.w.) were evaluated by carrageenan-induced paw edema method in Wistar Albino rats. The paw
edema volume of rats is given in Table 14 and Plate 16 and the inhibition percentage is given in Fig.11.

High dose (400mg/kg b.w.) 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 standard reference drug indomethacin at 10mg/kg b.w. (Group III) showed an inhibition of 80%. Maximum inhibition of paw edema and high anti-inflammatory activity were observed at the end of 90 minutes following the administration of high dose (400mg/kg b.w.) of *E.serratus* leaf (66.15%), stem bark (47.69%) and *E.tuberculatus* leaf (63.07%) and stem bark (45.38%). The administration of low dose (200mg/kg b.w.) of plant extracts reduced the inflammation as compared to the induced group (Group II) after 120 minutes. Both the plant extracts gave significant reduction (p< 0.05) of rat paw edema at all assessment times. Generally the paw edema increased up to 90 mins or 120 mins and gradually subsided thereafter.

4.5.3.2. Histopathological Evaluation

Histopathological evaluation (Plates 17a and 17b) of the hind paw of rats treated with carrageenan (Group II) revealed the presence of non-specific inflammation indicated by hypertrophy and hyperplasia of the synovium. However, a preventive effect against inflammation was noticed in animals treated with the standard reference drug indomethacin (Group III) where the synovium showed no obvious abnormality as compared with the control (Group I). The synovium of animals treated with high dose (400 mg/kg b.w.p.o.) of ethanolic extracts of leaf of *E.serratus* and *E.tuberculatus* (Group V and Group IX) showed no obvious abnormality. Generally a preventive effect against inflammation was noticed in animals treated with high dose of leaf extracts of *E.serratus* and *E.tuberculatus*.

Careful evaluation of the pictomicrographs revealed that the synovium of animals treated with low dose of *E.serratus* leaf extract, high dose of *E.serratus* stem bark extract and low dose of *E.tuberculatus* stem bark extract (Groups IV, VII, X) showed hypertrophy and hyperplasia. The synovium showed infiltration by macrophages
indicating the presence of very mild non specific inflammation in animals treated with low dose of *E.serratus* stem bark extract, low dose of *E.tuberculatus* leaf extract and high dose of *E.tuberculatus* stem bark extract (Groups VI, VIII and XI).

**4.5.3.3. Evaluation of Biochemical Parameters in Blood Serum**

Table 15 shows the activities of antioxidant enzymes (SOD, CAT, GPx, GST) and LPO and NO activities in the blood serum of normal group (Group I) and experimental groups (Group II to Group XI) of rats. SOD, CAT, GPx and GST levels were significantly reduced (p< 0.05) in the carrageenan-induced animals (Group II) when compared with the control animals (Group I). The Groups (Group IV to XI) administered with the ethanolic extracts of leaf and stem bark of *E.serratus* and *E.tuberculatus* at doses of 200 and 400 mg/kg b.w.p.o. respectively, showed significant increase (p<0.05) in SOD, CAT, GPx and GST enzyme activities when compared to the induced group animals (Group II).

However in serum diagnosis, the LPO and NO activities were significantly increased (p < 0.05) in Group II animals (carrageenan-induced) when compared to control group (Group I) whereas, the activities were significantly decreased (p < 0.05) in the Group IV to Group XI when compared to the carrageenan-treated animals (Group II).

**4.5.4. Anti-ulcer Effect of *Elaeocarpus* Extracts on Ethanol-induced Gastric Damage**

The gastroprotective effect of ethanolic extracts of leaf and stem bark of *E. serratus* and *E.tuberculatus* at low dose (200 mg/kg b.w.p.o.) and high dose (400 mg/kg b.w.p.o.) on ethanol-induced gastric damage (ulcer index) was macroscopically determined in rats (Table 16 and Plates 18a and 18b).

**4.5.4.1. Ulcer index**

In control animals (Group I) oral administration of absolute ethanol (1 ml/kg) produced characteristic lesions in the glandular portion of the rat’s stomach which appeared as elongated bands of thick, black and dark red lesions. There were very
remarkable hyperaemias in the stomachs of ethanol-administered rats. From the results obtained (Table 16), it was inferred that there was a significant decrease (p < 0.05) in ulcer index in a dose-dependent manner in the Group V, VII, IX, and XI as compared with the ethanol-induced group (Group I). The oral administration of ethanolic extract of plant samples showed significant reduction (p < 0.05) in the ulcer index in Group V (15.36 ± 2.87), Group VII (17.04 ± 0.89), Group IX (18.75 ± 2.053) and Group XI (22.35 ± 2.67), as compared with the ethanol-treated Group II (49.2 ± 3.96). The highest reduction in ulcer index was noticed in Group V.

4.5.4.2. Ulcer Protection Percentage

The pretreatment of animals with ethanolic extracts of leaf and stem bark of *E. serratus* and *E. tuberculatus* provided more or less 40% to 68% ulcer protection as compared with the ulcer control animals. Although omeprazole the reference drug used in the present study at a dose of 10mg/kg b.w. provided the animals with the highest ulcer protection (75.42%), the effect of ethanolic leaf extract of *E. serratus* (68.78%) at high dose (400 mg/kg b.w.) was comparable with the reference drug (Table 16).

4.5.4.3. Biochemical Investigation of Stomach Tissues

In order to explore the effects of antioxidant defenses on the ulceration process in the stomach, the antioxidant levels (SOD, CAT, GPx, GST, LPO and NO) in the stomach tissues of normal group (Group I) and experimental groups (Group II to Group XI) of rats were evaluated and the results are presented in Table 17. This table shows a highly significant reduction (p < 0.05) in the antioxidant status (SOD, CAT, GPx and GST) in the rats administered with ethanol (Group II) as compared with control animals (Group I). In contrast to the ethanol-treated group, both the doses (200 mg/kg b.w. and 400 mg/kg b.w.) of the ethanolic leaf and stem bark extracts of *E. serratus* and *E. tuberculatus* (Group IV to Group XI) significantly increased (p<0.05) the levels of SOD, CAT, GPx and GST enzymes to near normalcy as in Group III treated with the reference drug omeprazole (10 mg/kg b.w.p.o.).
However, the lipid peroxidation and nitric oxide levels were found to be markedly elevated (p<0.05) in the ethanol-induced rats (Group II) as compared to the control rats (Group I). The activities of LPO and NO increased by ethanol, were significantly reduced (p<0.05) to near normalcy comparable with the standard group (Group III), by the administration of high and low doses (200mg/kg b.w. and 400 mg/kg b.w.) of ethanolic extracts of leaf and stem bark of *E. serratus* and *E. tuberculatus* (Group IV to Group XI). Generally the plant pretreatments showed impressive antioxidant enzyme augmenting abilities.