6. Summary

Nature has been a potential source of many therapeutic agents for thousands of years and an impressive number of modern drugs have been derived from plants. Even today, the World Health Organization estimates that up to 80 per cent of people still rely mainly on traditional remedies such as herbs for their medicines. Medicinal plants constitute one of the main sources of new pharmaceuticals and health care products. Plant-derived dietary supplements, phytochemicals and pro-vitamins assist in maintaining good health and combating diseases. Major phytochemicals, e.g., phenolic acids, flavonoids, tannin, coumarin derivatives, etc., are known to combat oxidative stress in the human body by helping to maintain a balance between oxidants and antioxidants. The role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents. Thus, there is renewing interest in phytomedicine during the last decade and nowadays many medicinal plant species are being screened for their pharmacological activities. The present work therefore, attempts to report the in vitro antioxidant phytoceuticals (phenols, flavonoids and tannin), antioxidative, anti-inflammatory, antimicrobial, in vivo anti-inflammatory and anti-ulcerogenic activities of Elaeocarpus serratus L. and Elaeocarpus tuberculatus Roxb. (Elaeocarpaceae).

The per cent yield of the plant parts (leaf, stem bark and fruit) of *E. serratus* and *E. tuberculatus* with the different solvents (acetone, methanol and water) was calculated. The highest per cent yield of both the plants was registered in the acetone extract of leaf (27.67 and 22.01%, respectively). Generally, the acetone and methanol extracts of the plant parts contained relatively higher amounts of phytoceuticals than the water extracts.

The content of the antioxidant phytoceuticals namely, total phenols, total flavonoids and tannin in the solvent (acetone, methanol and water) extracts of leaf, stem bark and fruit of *E. serratus* and *E. tuberculatus* were assessed. The total phenolic content was expressed as tannic acid equivalents. In *E. serratus*, high amount of total phenols was recorded in the methanol and acetone extracts of stem bark and leaf. A similar trend was noticed in *E. tuberculatus* extracts. Generally, the total phenolic content of *E. serratus*
was almost five times higher than *E. tuberculatus*. In *E. serratus*, the maximum amount of total flavonoids was present in the methanol extract of fruit (100.27 ± 1.2 mg rutin/g dry weight) and minimum amount was registered in the water extract of fruit (13.16 ± 0.5 mg/g). In *E. tuberculatus*, the maximum total flavonoid content was noticed in the acetone extract of the leaf (96.67 ± 3.84 mg/g) and the minimum was observed in the water extract of fruit (6.40 ± 1.46 mg/g). The tannin content of *E. serratus* ranged between 33.80 ± 1.6 and 355.72 ± 1.8 mg tannic acid / g dry weight. The maximum amount of tannin was present in the acetone extract of stem bark. Similarly, in *E. tuberculatus* the maximum amount of tannin was found in the acetone extract of stem bark (66.78 ± 1.64 mg/g). Generally, the water extracts of both the plants contained lower amount of tannin.

The leaf, stem bark and fruit extracts of *E. serratus* and *E. tuberculatus* obtained by different solvents (acetone, methanol and water) were subjected to screening for their possible antioxidant activity using eight complementary test systems namely, 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.

*E. serratus* and *E. tuberculatus* exhibited a dose-dependent DPPH$^-$ scavenging activity. All the plant parts of *E. serratus* generally registered high DPPH$^-$ quenching ability. The DPPH$^-$ quenching ability of the methanol extract of leaf of *E. serratus* ($IC_{50} = 2.73 ± 1.09 \mu g/ml$) was closer to that of the standard BHA ($IC_{50} = 1.07 ± 0.4 \mu g/ml$). The acetone extract of stem bark of *E. tuberculatus* quenched the maximum amount of DPPH$^-$ radicals ($IC_{50} = 3.92 ± 3.4 \mu g/ml$) though it showed lesser activity than the standards BHA and gallic acid. The acetone and methanol extracts of stem bark of *E. tuberculatus* displayed remarkable DPPH$^-$ quenching property than that of the leaves and fruits.

*E. serratus* and *E. tuberculatus* extracts also displayed a dose-dependent scavenging activity against the ·OH species. The water extract of fruit and acetone and
methanol extracts of stem bark of *E. serratus* showed moderate antioxidant activity (*IC*₅₀ = 71.14 ± 1.23, 72.30 ± 1.96 and 75.99 ± 2.98 μg/ml, respectively). On the other hand, *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 ⋅OH scavenging activity with *IC*₅₀ values of 18.20 ± 1.37, 18.75 ± 1.88, 19.65 ± 2.23 and 20.55 ± 1.42 μg/ml, respectively.

The superoxide radicals generated *in vitro* are effectively scavenged in a dose-dependent manner by the extracts of *E. serratus* and *E. tuberculatus*. The methanol extract of *E. serratus* stem bark and acetone and water extracts of leaf showed good O₂⋅ scavenging activity (*IC*₅₀ = 58.00 ± 0.66, 59.52 ± 0.79 and 60.67 ± 0.52 μg/ml, respectively). *E. tuberculatus* extracts showed very good potency compared with *E. serratus* extracts against superoxide radicals. The acetone extract of leaf (*IC*₅₀ = 21.81 ± 0.67 μg/ml) was more potent than gallic acid standard (*IC*₅₀ = 38.27 ± 0.28 μg/ml) in O₂⋅ scavenging.

*E. serratus* extracts showed a moderate dose-dependent NO⋅ scavenging ability. The lowest *IC*₅₀ value (64.12 ± 0.44 μg/ml) was observed in the acetone extract of fruit. Generally the standards BHA and gallic acid were more potent NO⋅ scavengers than *E. serratus* extracts. In contrast, nearly all the extracts of *E. tuberculatus* showed higher activities than the commercial antioxidants used with *IC*₅₀ values ranging between 19.81 ± 0.75 μg/ml and 52.25 ± 0.12 μg/ml. The *IC*₅₀ values of BHA and gallic acid were 43.37 ± 1.26 μg/ml and 29.76 ± 0.81 μg/ml, respectively.

The reductive capabilities of *E. serratus* and *E. tuberculatus* extracts were also dose-dependent. In *E. serratus* the methanol extract of leaf with an absorbance of 2.027 at 100 μg/ml showed higher reducing power than the standards BHA and gallic acid (absorbance = 1.728 and 1.691 at 100 μg/ml, respectively). In *E. tuberculatus* the acetone extract of stem bark was more potent in reducing the Fe³⁺ ions than the other extracts having a reducing power of 1.169 at 100 μg/ml concentration.
Both the plant species exhibited potent ABTS$^{•+}$ scavenging activity. In *E.serratus* the antioxidant capacities of the extracts ranged from 4840.7 ± 33.4 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. *E.tuberculatus* extracts showed higher ABTS$^{•+}$ scavenging activity as compared to *E.serratus* extracts. In *E.tuberculatus*, the maximum activity was seen in the methanol extract of stem bark (19960.6 ± 28.9 μmol TAA/g).

*E.serratus* and *E.tuberculatus* extracts possessed greater antioxidative activity by prohibiting the bleaching of β-carotene and inhibiting linoleic acid oxidation. The methanolic extract of stem bark of *E.serratus* showed the maximum inhibition of linoleic acid peroxidation (87.95 ± 0.79 %) followed by the acetone extract of stem bark (81.00 ± 0.77%). The inhibitory percentage of acetone extract of fruit of *E.tuberculatus* was 89.50 ± 5.41 % which was closer to that of the standard BHA (91.37 ± 2.2%).

*E.serratus* and *E.tuberculatus* extracts registered a marked metal chelating ability. In *E.serratus*, the highest chelating activity was displayed by the water extract of fruit (1079.1 ± 2.4 mg EDTA/g) followed by methanol extracts of fruit and stem bark. Among the extracts of *E.tuberculatus*, the highest chelating activity was exhibited by the water extract of leaf (1187.1 ± 1.6 mg EDTA/g).

The antimicrobial activity of *E.serratus* and *E. tuberculatus* leaf, stem bark and fruit extracts was examined against four bacterial species (*Shigella sonnei, Salmonella typhi, Staphylococcus aureus and Klebsiella pneumoniae*) and a fungal species *Candida albicans* using agar well diffusion method. Both the plant extracts showed a dose-dependent inhibition of microorganisms. The 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. The antifungal activity of *E.serratus* leaf (water extract) and fruit (acetone extract) were maximum as revealed by wider zones of inhibition (18±1.05 and 18±0.92 mm, respectively at 200 μg/ml) than the standard amphotericin. The *E. tuberculatus* extracts showed a concentration-dependent activity.
against *Candida albicans*. The acetone extract of leaf, stem bark and fruit suppressed the fungal growth to the maximum extent (ZI = 17±1.23, 16±0.91 and 16.5±0.85 mm, respectively at 200 µg/ml) which was higher than that of the standard amphotericin (ZI = 11±0.02 mm at 100 units / disc). Generally, the lower concentrations of the extracts were susceptible to the fungal pathogen.

The *in vivo* toxicological evaluation of ethanolic extracts of *E. serratus* and *E. tuberculatus* leaf and stem bark revealed 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 on animal model. 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. The inhibition of protein denaturation ranged between 27.06% and 62.38%. The ethanolic extract of leaf of *E. serratus* and *E. tuberculatus* showed maximum inhibition (62.38% and 49.96%, respectively) of protein denaturation at 400 µg/ml concentration whereas, the standard indomethacin showed 75.45% at 100 µg/ml. Similarly, the ethanolic leaf extract of *E. serratus* and *E. tuberculatus* exhibited maximum inhibition (56.62% and 45.38%, respectively) of proteinase at 400 µg/ml concentration. The standard indomethacin showed maximum inhibition (72.41%) of proteinase at 100 µg/ml concentration.

High dose (400mg/kg b.w. 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 (Group I) in the carrageenan-induced paw edema in Wistar albino rats. Both the plant extracts gave significant reduction (p<0.05) of rat paw edema at all assessment times. Histopathological studies further confirmed the anti-inflammatory action of the plant extracts. Further, the biochemical studies of the blood serum of the experimental animals revealed that the administration of ethanolic extracts of leaf and stem bark of *E. serratus* and *E. tuberculatus* at doses of 200 mg/kg and 400 mg/kg b.w. showed significant increase (p<0.05) in the level of antioxidant enzymes (SOD, CAT,
GPx and GST) when compared to the induced group (Group II). However, the LPO and NO activity was significantly decreased (p<0.05).

The plant extracts offered remarkable gastroprotection against ethanol-induced ulcer. The oral administration of plant extract samples showed significant reduction (p<0.05) in the ulcer index, especially in Group V pretreated with high dose (400mg/kg b.w.) of leaf extract of *E. serratus* (15.36 ± 2.87) as compared with the ethanol–treated Group II (49.2 ± 3.96). 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 to the ulcer control animals. Assessment of antioxidant enzyme levels (SOD, CAT, GPx and GST) in the stomach tissues revealed that both the doses (200 mg/ kg and 400 mg/kg b.w.) of plant extracts significantly increased (p<0.05) the levels of the enzymes to near normalcy as compared to Group III pretreated with standard reference drug omeprazole (10 mg/kg b.w). However, a decreasing trend was noted for the levels of LPO and NO. The lipid peroxidation and nitric oxide levels were reduced significantly (p<0.05) with the administration of high and low doses of the plant extracts. Generally the plant pretreatments showed impressive antioxidant enzyme augmenting abilities.

The present study registered remarkable antioxidant, antimicrobial, anti-inflammatory and anti-ulcerogenic activities for the methanolic and ethanolic extracts of *Elaeocarpus serratus* and *Elaeocarpus tuberculatus*. 

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