Summary

Eupatorium is one of the important genus belongs to the family Asteraceae, is a flowering plant containing 1200 species. Among them, *E. glandulosum*, *E. odoratum* and *E. triplinerve* have been selected for the present study. The leaves were screened for pharmacognostical, phytochemical, and anticancer properties. Besides, tissue culture studies were also carried out to standardize the protocol for mass production of callus and micropropagation.

The leaf powder of selected three plants were screened for physicochemical parameters such as loss on drying, total ash, acid insoluble ash, water soluble ash and solubility percentage in ethanol and water. The loss on drying and solubility percentage was high in *E. odoratum* and *E. glandulosum*.

Different solvents like petroleum ether, benzene, chloroform, acetone, ethanol and water were used for the extraction of leaf powder. Among the solvents used, the polar solvents showed maximum extractive value in *E. odoratum*.

The fluorescence behaviour of the powdered plant materials were studied by treating them with various solvents and observed under normal and UV light. Among the three plants, *E. odoratum* showed distinct variation under UV light.

Qualitative phytochemical studies were carried out in the leaf powder of selected plants to identify the secondary metabolites. Ethanol and water extracts of all the three plants confirmed the presence of alkaloids, glycosides, flavonoids, phenols, tannins and saponins. Fats and fixed oils were absent in *E. glandulosum* and *E. triplinerve* but reported in *E.odoratum*. The GCMS analysis confirmed 21 compounds in *E. odoratum* and 20 compounds in *E. glandusloum* and *E. triplinerve*.

Quantitaitve estimation of quercetin content was analysed using HPTLC. Among the three plants, the ethanol extracts of *E. glandulosum* showed maximum quercetin content.

Heavy metals like lead, chromium, nickel and cadmium were analysed in leaves and soil samples using Atomic Absorption Spectrophotometer. The results confirmed the presence of heavy metals within the permissible level prescribed by WHO.

Antimicrobial properties of the plants were studied by testing the leaf extracts (ethanol and water) against bacterial pathogens such as *Staphylococcus aureus, Bacillus subtilis, Eschericia coli, Bacillus cereus, Klebsiella pneumonia, Streptococcus pyogens* and fungal pathogens such as *Microsporum canis, Candida tropicalis, Candida albicans, Trichophyton rubrum, Aspergillus niger* and *Aspergillus flavus***.** Chloramphenicol and flucanozole were used as positive control against bacteria and fungi respectively. Among the three plants, the ethanol extract of *E. glandulosum* and *E. odoratum* showed good antimicrobial activity.

The antioxidant potential of the leaves of selected plants was tested by Hydrogen peroxide and DPPH scavenging method. The antioxidant activity of plant extracts was increased with increasing concentration. Among the three plants, the ethanol extract of *E. odoratum* and *E. glandulosum* showed good scavenging activity.

RAPD analysis was carried out in the selected plants to identify the molecular variations among the species. Among the three species, *E. glandulosum* showed highest polymorphism.

Cytotoxic studies were carried out using the leaf extracts of selected three plants against human colon carcinoma (HT-29) cell line by MTT, SRB and LDH assays. The ethanol extract of *E. glandulosum* inhibit the

growth and decrease the viability of the cell. The inhibitory effect was directly proportional to the concentration of the plant extracts.

Tissue culture technique has been adopted to develop a protocol for mass propagation of callus and shoots. Leaf, node and intermodal explants of selected plants were cultured in MS basal medium supplemented with various concentrations and combinations of auxin and cytokinin. All the leaf explants cultured in medium with 2,4-D showed best response in callus induction and proliferation. Among the three plants, *E. glandulosum* produced maximum amount of callus at the concentration of 2mg/l of 2, 4-D.

Multiple shoots were induced from nodal explants of *E. glandulosum* under various concentrations of IAA and BA. Effect of growth regulators and seasonal variation on shoot induction and proliferation has been standardized. Explant collected during the month of June-August and cultured in medium with 0.5mg/l of IAA and 3 mg/l of BA were considered as the most conducive environment for mass propagation.

In vitro grown shoots were rooted with IAA and IBA. Among the two growth hormones, IBA induced more number of roots at the concentration of 3 mg/l. After rooting, the rooted plantlets were successfully transferred to the field with 100% survival.

The leaf callus of selected plants was screened for phytoconstituents using GCMS analysis. The result revealed 16 compounds in *E. glandulosum*, 12 in *E. odoratum* and 17 in *E. triplinerve.* The leaves from hardened plants of *E. glandulosum* showed more number of phytochemical compounds (26) than leaves collected from the wild (20 compounds).

Quantitaive estimation of a flavonoid quercetin have been analysed in *in vitro* grown materials by HPTLC finger printing technique. Among the three plants studied, callus obtained from *E. glandulosum* showed high amount of quercetin. But the hardened leaves of *E. glandulosum* showed maximum quercetin content than callus.

Conclusion

The result of pharmacognostical, phytochemical, antimicrobial, antioxidant and anticancer activities confirmed the importance of the selected *Eupatorium* species namely *E. glandulosum, E. odoratum* and *E. triplinerve*. Among that, *E. glandulosum* performed well in all the experiments. It also possess high amount of flavonoid quercetin with good anticancer activity. Hence, a protocol has been developed for mass production of callus and micropropagation of plantlets using tissue culture techniques. From the current observation, it is suggested that tissue culture techniques can be adopted for conservation and sustainable utilization of the plant in future. This technique can also be used for mass production of callus and extraction of secondary metabolites at the industrial level.