SUMMARY AND CONCLUSIONS
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Traditional medicine has been recognized as a part of primary health care programmes in many countries and there is a need to evaluate scientifically the crude extracts of plants for their medicinal and pharmacodynamic properties, clinical usefulness and toxicological potential. Realizing this fact, three medicinal plants namely \textit{A. radiata}, \textit{C. epigaeus} and \textit{S. tenuifolius} were selected based on their ethnobotanical information to scientifically evaluate their anti-\textit{Staphylococcus aureus} and antioxidant activities.

\textit{In vitro} anti-\textit{Staphylococcus aureus} (ATCC MSSA, MRSA and MSSA) activity was evaluated by agar well diffusion and MIC methods for hexane, chloroform ethyl acetate, ethanol and aqueous extracts from selected plants and the results revealed that ethanol and hexane extracts of \textit{A. radiata} are potent among all the screened plant extracts and further to know their possible mechanism of action against all selected \textit{S. aureus} strains ethanol and hexane extracts of \textit{A. radiata} were analyzed by employing methods like Time kill assay, Ions leakage and Protein leakage methods. The results reveal that the possible mechanism of action of ethanol and hexane extracts of \textit{A. radiata} on \textit{S. aureus} may be due to disruption of membrane structure and functions by causing leakage of proteins, potassium and phosphate ions.

Hexane, chloroform, ethyl acetate, ethanol and aqueous extracts of \textit{Actiniopteris radiata}, \textit{Corallocarpus epigaeus} and \textit{Senecio tenuifolius} were subjected to preliminary screening for their antioxidant potential by employing various electron transport based \textit{in vitro} methods (DPPH, hydroxyl and superoxide anion radicals scavenging activities, reducing power and metal chelating activity). Ethanol extract of \textit{Actiniopteris radiata} was found to possess highest free radical scavenging activity when compared to the other extracts.

Based on the \textit{in vitro} results, the potent plant extracts selected was screened for qualitative (flavonoids, phenols, sterols, tannins, saponins and alkaloids) and quantitative (phenols and flavonoids) analysis of secondary metabolites. The phytochemical analysis revealed that ethanol extract of \textit{A. radiata} contain high amount of phenols and flavonoids.
which contribute majorly for antioxidant activity. A fair correlation was observed between phytochemical analysis and antioxidant activity.

In the present study, EEAR showed potent in vitro antioxidant activity when compared with the other extracts. The phytochemical analysis also supported the in vitro antioxidant activity. Based on the in vitro antioxidant activity and phytochemical analysis, EEAR was selected to further assess the in vivo antioxidant activity against CCl₄ induced oxidative stress.

Safety evaluation of ethanol extract of A. radiata was performed for further proceeding into in vivo studies. Acute toxicity study revealed no behavioral changes and mortality and is nontoxic up to the dose level of 2000 mg/kg b. wt. In sub-acute toxicity studies EEAR did not produce any significant dose-related changes of hematological parameters, serum biochemistry and histopathological observations and so it was considered to be safe as per OECD-2000 guidelines.

The protective antioxidant efficacy of EEAR in vivo was evaluated in albino rats against carbon tetrachloride induced oxidative stress and the treatment was conducted in two phases - acute (10 days) and chronic (8 weeks). Hepatocellular damage caused by CCl₄ resulted in the increased levels of serum SGOT, SGPT, ALP, γGT, LDH and bilirubin. Administration of EEAR brought back the levels to the near normal in a dose and time dependent manner. The condition of renal tissue during CCl₄ intoxication was assessed by measuring serum urea, creatinine and uric acid levels. The changes in the lipid profile such as increase in the levels of triglycerides and cholesterol and decrease in the levels of phospholipids in liver and kidney were observed in CCl₄ induced oxidative stress condition. On the other hand CCl₄ toxicity caused an increase in the levels of all the lipids in brain and heart tissues. All changes were reverted back to near normal levels by the treatment of EEAR.

Oxidative stress markers - lipid peroxidation, protein carbonyls, total sulfhydryls, and xanthine oxidase were measured in control and experimental rats during acute and chronic treatments. The increased levels of lipid peroxidation, protein carbonyls, xanthine oxidase and depletion of total sulfhydryls were observed during CCl₄ induced oxidative
damage to the tissues and these altered levels were reverted to near normal by the administration of EEAR suggesting the antioxidant potential of the extract.

The enzymic (SOD, CAT, GPx, GR, GST and G-6-PDH) and non-enzymic (GSH, vitamin C and vitamin E) antioxidants were remarkably decreased during CCl₄ toxicity. These levels were reverted back to near normal range by EEAR in tissues during both acute and chronic treatments via enhancing the activities of both enzymic and non-enzymic antioxidants and reducing the levels of lipid peroxidation.

To understand the mechanism underlying the protective efficacy of EEAR, isoenzyme pattern of antioxidant enzymes was performed and on the basis of this, the mRNA expression of antioxidant enzymes (SOD, CAT and GPx) were done in the liver of control and experimental rats during chronic treatment. Down regulation of SOD, catalase and GPx gene expression was observed during CCl₄ induced oxidative stress and the administration of EEAR caused a remarkable up-regulation of these antioxidant enzymes. These results suggest that the beneficial effect of ethanol extract of A. radiata is related to its antioxidative property through the enhancement of mRNA expression of these antioxidant enzymes and thus could prevent the progression of oxidative damage caused by CCl₄.

DNA damage due to CCl₄ intoxication resulted in small non-existent heads with large diffused tails of comets. Treatment with EEAR protected against CCl₄ induced DNA damage which was evident from the decreased comet tail lengths.

Histopathological studies were performed to provide direct evidence of the CCl₄ toxicity and protective effect of EEAR on the tissues. Marked disruption of the cell structure was observed in CCl₄ treated rats whereas EEAR treated rats showed only minimal disruption of the cell structure in liver, kidney, brain and heart tissues during acute and chronic treatments. This minimal disruption of cells in various tissues provides additional support to the study that EEAR has protective effect against oxidative stress.
The present findings provide the scientific evidence to the ethnobotanical usage of *A. radiata* which was evident from its potent anti-*Staphylococcus aureus* (*in vitro*) and antioxidant (*in vitro and in vivo*) activities. The potent anti-*Staphylococcus aureus* activity of *A. radiata* is due to the membrane damage evident from ions and protein leakage from the membrane, which may be the possible mechanism of action. *A. radiata* protects against the CCl₄ induced toxicity via its antioxidant properties which is evident from the protection against lipid peroxidation, increase in antioxidant enzymes and their expression at gene level, non enzymic antioxidants, alterations in biochemical parameters and DNA damage. Studies on the histopathology are also in line with the biochemical parameters studied.

**FUTURE DIRECTIONS:**
The spectrum of the biological properties of *A. radiata* is attributed to the synergistic action of phytochemicals like saponins, tannins, glycosides flavonoids and polyphenols. Further investigations on the *in vivo* anti-*Staphylococcus aureus* activity are warranted and also there is need to investigate on the isolation and characterization of the active principle(s) from ethanol extract of *A. radiata* which are responsible for its biological activities. The systematic study of these isolated compounds could be one of the prime sources for the development and therapeutical application of new and potent drugs against *S. aureus* infections and degenerative diseases due to oxidative stress.