SUMMARY AND CONCLUSION

The present study entitled “BIO ACTIVITY GUIDED ISOLATION OF PHYTOCHEMICALS FROM SOME INDIAN MEDICINAL PLANTS” includes the study of 19 Indian medicinal plants namely Crotalaria retusa, Stylosanthes fruticosa, Aegle marmelos, Toddalia asiatica, Toona ciliata, Swietenia macrophylla, Sida acuta, Bougainvilla spectabilis, Polyalthia longifolia, Euphorbia thymifolia, Ficus glomerata, Elittaria cardamomum, Zingiber officinale, Sphaeranthus indicus, Melothria maderaspatna, Madhuca longifolia, Piper nigrum, Clerodendrum phlomidis and Datura metel belonging to 15 botanical families namely Papilionaceae, Rutaceae, Meliaceae, Malvaceae, Nyctaginaceae, Annonaceae, Euphorbiaceae, Moraceae, Zingiberaceae, Compositae, Cucurbitaceae, Sapotaceae, Piperaceae, Verbanaceae and Solanaceae.

Studies on selected Indian medicinal plants and phytochemistry.

Air-dried plant material was successively extracted with hexane followed by ethanol and their percentage yield was calculated. Antimicrobial activity of various extracts (40 in all) against Gram +ve bacteria S.aureus, S.epidermitis, B.subtilis, B.cereus, M.luteus, and Gram –ve E.coli, P.aeruginosa and fungi C.albicans, A.niger was studied by disc diffusion method. The extracts of S.acuta, S.fruticosa, T.ciliata, F.glomerata (bark and leaves), P.longifolia and B.spectabilis showed marked antimicrobial activity. The direct ethanolic extracts of these plants have been used for further studies, and their percentage yield was calculated. HPTLC profile of ethanolic extract of potent plant extracts and its fraction were done. The compounds such as β-sitosterol, linoleic acid and cedrelone were isolated and characterized by using $^1$H NMR, $^{13}$C NMR, IR and MS.
Nine fractions have been collected from each active plant extracts starting with hexane followed by hexane: ethylacetate (9:1,8:2,7:3,1:1,3:7,2:8), ethyl acetate and methanol. All these fractions have been subjected to antimicrobial screening. The finding suggest that *T.ciliata, F.glomerata* (bark) and *P.longifolia* posses potent antimicrobial properties. This study yielded the first report of scientific documentation of antimicrobial activity of *S.fruticosa* and *T.ciliata*.

**Acute toxicity studies**

Acute toxicity study was performed as per OECD guidelines 425, to fix the dose level for pharmacological studies. The findings indicated that *S.acuta, S.fruticosa, T.ciliata, B.spectabilis, F.glomerata* (bark, leaves) and *P.longifolia* were safe when tested in a group of three Swiss albino mice at 2000mg/kg, p.o. The results showed no clinical signs and mortality of the animal therefore an LD$_{50}$ >5000mg / kg body weight may be assumed. Hence the dose (100mg, 300mg and 500mg/ kg body weight) selected for the following pharmacological studies. Wistar albino rats and Swiss albino mice were used for experimental purpose.

**Pharmacological activity- CNS activity**

The ethanolic extract of the plant *S.acuta, S.fruticosa, T.ciliata, B.spectabilis, F.glomerata* (bark, leaves) and *P.longifolia* were used for the pharmacolical studies.

**Antidepressant activity**

Forced swimming test: The ethanolic extracts of 6 plants viz *S.fruticosa, T.ciliata, B.spectabilis, F.glomerata* (bark, leaves) and *P.longifolia* showed significant increase in mean immobility time at 100, 300 and 500mg/kg level tested, where as *S.acuta* extract treated animals showed significant reduction in mean immobility time at 100mg/kg. Increase of dose of *S.acuta* extract causes increase in immobility time.
Locomotion activity

Actophotometer: The ethanolic extract of *S.acuta* at 100, 300 and 300mg/kg, showed significant psychostimulant activity but in case of *F.glomerata* (bark), psychostimulant activity was observed at 300mg/kg and above. The other plant extracts showed significant decrease in locomotor activity. The optimum dose was found to be 300mg/kg, p.o

Analgesic activity

Hot plate method: The analgesic activity was dose dependant. The analgesic activity of *T.ciliata* extract was significant at 100mg/kg, i.p. When compared to vehicle control and other plant extract. At 300mg/kg, i.p the plant extracts *S.fruticosa*, *T.ciliata*, *P.longifolia* and *F.glomerata* (leaves) were showed significant analgesic activity. At 500mg/kg, i.p all the extracts showed significant analgesic activity.

Tail immersion method: The tail withdrawal reflex time in *T.ciliata* was found to be significant in all the three dose level tested. Moreover the effects of *T.ciliata* are comparable at 300mg/kg with that of standard pentazocine at 10mg/kg dose level.

Anti ulcerogenic activity

Aspirin plus pylorous ligation induced ulcer model: The extract of *T.ciliata*, *B.spectabilis* and *F.glomerata* (bark, leaves) were found to be effective in inhibition of ulcer formation at 300mg/ kg, p.o

HCl / ethanol-induced ulcer: The plant extract *F.glomerata* (leaves, bark), *B.spectabilis* and *P.longifolia* were found to be effective choice in protecting the gastric mucosa.

Water immersion stress induced ulcer: It is a significant finding that out of all the extract *P.longifolia* and *F.glomerata* exhibited to afford a distinctively high protection against water immersion stress induced ulcer.
Semi-synthetic modification of cedrelone

The compound cedrelone was isolated from *T. ciliata* hexane extract. The different derivatives prepared are (i) cedrelone acetate, (ii) cedrelone chloroacetate, (iii) 1, 2-epoxycedrelone, (iv) 1, 2-dihydro-1-hydroxyamino cedrelone, (v) isocedrelone, (vi) 1, 2-dibromocedrelone, (vii) 1, 2-dihydrocedrelone, (viii) cedrelone ethyl ether, (ix) bromohydroxy cedrelone, (x) cedrelone Michael adduct, (xi) 5, 6-dihydro cedrelone and (xii) cedrelone 1, 2-epoxy acetate. The semi-synthetically modified products were characterized by spectroscopic techniques like \(^1\)H NMR, \(^{13}\)C NMR, IR and MS.

Out of 12 derivatives cedrelone ethyl ether, bromohydroxy cedrelone, cedrelone Michael adduct, 5, 6-dihydro cedrelone and cedrelone 1, 2-epoxy acetate were reported first time from this investigation. Structural activity relationship was studied for synthetically modified compounds.

Antimicrobial activity of synthetically modified compounds exerted inhibitory response against *S. aureus*, *S. epidermitis*, *B. subtilis*, *B. cereus*, *M. luteus*, *E. coli*, *P. aeruginosa*, *C. albicans* and *A. niger* except *E. coli*. *E. coli* is the least susceptible organism as compared to other organism.

Interestingly isocedrelone and halogen substituted compounds showed higher activity against the aforementioned microorganism except *E. coli*, when compared to other of the compounds. The antifungal activity of cedrelone, cedrelone acetate, 1,2-dihydro-1-hydroxy amino cedrelone, isocedrelone, 1,2-dibromo cedrelone, bromohydroxy cedrelone and Michael adduct was close to that of standard drug ketoconazole and the activity was greater than that observed with other compounds.

The percentage of antimicrobial activity was correlated with C log P value of the cedrelone and semi-synthetically modified compounds. The results were statistically analysed by linear regression analysis. It was observed that C log P value
is non selective in this case. Change of $C \log P$ value has no much influence in the antimicrobial activity of the compounds tested. Cedrelone, cedrelone chloroacetate, 1,2 dibromocedrelone and bromohydroxy cedrelone possess potent antibacterial and antifungal properties.

**Conclusion**

The present study was undertaken with the objective to isolate bioactive molecules from commonly available medicinal plants in India, targeting infectious diseases, and CNS disorder and peptic ulcer. From the results obtained, the following conclusion can be drawn.

From *S.fruticosa* β-sitosterol and linoleic acid were isolated for the first time, using column chromatography.

Antimicrobial activity was remarkable in the following plant extracts *S.acuta*, *S.fruticosa*, *T.ciliata*, *B.spectabilis* *F.glomerata* (bark and leaves), *P.longifolia* and *F.glomerata* (leaves). It is significant to note that though the plant extracts tested showed activity against *P.aeruginosa* they had no activity against the other Gram –ve bacteria *E.coli*. Cedrelone was isolated by extraction with hexane and purified by column chromatography. Cedrelone and its derivatives at 50µg concentration prove to be effective against various pathogenic microorganism *S.aureus*, *S.epidermitis*, *B.subtilis*, *B cereus*, *M.luteus*, *E.coli*, *P.aeruginosa*, *C.albicans* and *A.niger* except *E.coli* and may serve as a choice antifungal agents.

CNS disorder: *S.acuta* at 100mg/ kg was found to be a very good CNS stimulant. The results indicate its prospective inclusion in the formulation of herbal tea. *T.ciliata* ethanolic extract at 100, 300 and 500mg/ kg was found to be excellent analgesic activity and may be used as a substitute for pentazocine like drugs.

Anti ulcerogenic activity: Antiulcer activity of plant extracts indicated that at 300mg/kg ethanolic extract of *T.ciliata*, *B.spectabilis*, *F.glomerata* (bark, leaves) can be
given in combination aspirin to mitigate the side effects during the prolonged therapy with the latter. Studies on cytoprotective activity revealed that *F. glomerata* (bark, leaves) could be effectively used to protect gastric mucosa. The ethanolic extract of *P. longifolia* and *F. glomerata* particularly can be given to prevent stress-induced ulcer.

Out of the 12 semi-synthetically modified derivatives, cedrelone ethyl ether, bromohydroxy cedrelone, cedrelone Michael adduct, 5, 6-dihydrocedrelone and cedrelone 1,2-epoxy acetate were reported first time from this investigation.

Since the pharmacological activity was carried out with crude ethanolic extract of the plants, further studies are needed to isolate the bioactive molecule. Moreover the activity has been conducted only in laboratory animals, the findings can be extrapolated in human cases by further studies, receptor level further studies to understand the mechanism, toxicity studies, safety profile to ascertain the dose, bioavailability, absorption pattern in the body etc.