Chapter 6

DISCUSSION AND CONCLUSION

In this chapter, discussion and conclusion are presented.

6.1 Discussion

*Cyathea gigantea* and *Cyathea brunoniana* belonging to the family Cyatheaceae are on the verge of extinction as it fails to reproduce. Khan et al., (2002) expressed great concern regarding the gradual extinction of *Cyathea gigantea* species in Arunachal Pradesh. The same scenario prevails in other parts of North Eastern part of India also. So, it was the demand of the hour to take care of *Cyathea sp.* in general and it was high time to explore these plants in search of their phytochemicals which can be utilized in development of new drugs and many other products of industrial and pharmaceutical importance before this fern become totally extinct from the Indian scenario. *Cyathea gigantea* and *Cyathea brunoniana* are the two tree fern found in southern Assam. The present state of affairs thus justifies the selection of screening these plants for phytochemical analysis and antibacterial screening.

For the pharmacological as well as pathological discovery of novel drugs, the essential information's regarding the chemical constituents are generally provided by the qualitative phytochemical screening of plant extracts. In the present study, qualitative tests for all sixteen extracts showed significant indication about the presence of metabolites. Steroid and flavonoid were found to be present in the extracts of the
leaves and caudex of both the tree ferns. While alkaloids, tannins and reducing sugar could not be detected in the extracts. Saponin were not uniformly found in both the cases. Singh (2003) also reported the presence of protein, steroid, phenolic compounds, saponin, tannins and starch in *Cyathea gigantea* and only starch in *Cyathea brunoniana*. These findings of phytochemicals were good enough reasons for initiating systematic phytochemical investigation on these two tree ferns.

Before stepping forward the separation of phytochemicals from any of the plant extracts, it is very important to check whether the extracts of plant parts have any biological activity or not. All the 16 extracts prepared from the various plant parts of the two ferns, using a number of solvents, were therefore, tested for antibacterial activity. Acetone and methanol extracts of caudex and leaves of *Cyathea gigantea* and acetone extracts of *Cyathea brunoniana* showed considerable zones of inhibition against *E. coli*, *Streptosoccus sp.* and *Staphylococcus aureus*.

It is not surprising that there are variations in the antibacterial activities of the different extracts of the two tree ferns. This is due to the variation in the nature of the phytochemicals present in the two plants. As usual, the lack of antibacterial activity of some of the extracts could be due to (i) the presence of active constituents in extremely low concentrations, (ii) the simultaneous presence of other metabolite(s) which may act in exactly the opposite manner or (iii) the insolubility of the active constituent(s), if there be any, in the solvents which were used for preparing the extracts. As per Parekh et al. (2007), conformational changes may also occur in some of the chemical constituents of plant parts at the time of drying the sample. This information acted as a guide for the selection of these extracts for the separation of the constituent phytochemicals.

Based on the behavior of extracts on TLC plates as well as their antibacterial activity, only acetone soluble fraction of caudex of both the species were selected for routine chromatography separation. After performing column chromatography, using TLC over silica gel for monitoring the eluates, only the residues from PE-EA (9:1) eluates from both the extracts were found to be pure enough and taken up for spectroscopic identification. A total of four compounds were isolated, all of which appeared to be homogenous on TLC.
Two of the four metabolites were identified as the glyceryl esters of monoenoic fatty acids. It is well known that the esters of fatty acids occur as an intimate mixtures of the stereoisomeric ("Z"- and "E"- and homologous) fatty acid esters. Similar was the situation in the present case too. The major metabolite from the acetone-soluble fraction of the caudex [C(S)] of *Cyathea gigantea* was identified as Glyceryl elaidate, i.e., 2, 3 - dihydroxypropyl E-octadec-9-enoate from detailed spectroscopic analyses. This metabolite has not been reported till date from any tree fern. To our knowledge, glyceryl elaidate had earlier been identified only once and that too by GC-MS by Jaafar et al. (2007) in the stems, flowers and rhizomes of *Etilingera elatior* (Jack) R.M. Smith. The spectroscopic data of glyceryl elaidate are being reported for the first time by us. The second metabolite from the same extract was identified as glyceryl monooleate, i.e. glyceryl Z-octadec-9-enoate or 2,3-dihydroxypropyl Z-octadec-9-enoate. The possible presence of a third metabolite, glycerol-1-propionate, in extremely meager concentration in the same extract was also found out. As far as the two other metabolites are concerned, the spectroscopic data that we could determine were of no use in ascertaining their structures. These two metabolites, probably mixtures, thus remained unidentified.

*In silico* method makes it possible to screen the druglikeness and other parameters of numbers of compounds. ACTELION PROPERTY EXPLORER, an open source ADMET screening server was used to screen the isolated compound. The compound passed the ADMET filters successfully and showed no toxicity. The drug score of compound as depicted by the server was 0.227. As both the isolated compounds are found in cis and trans form, it results the same drug score. Further analysis of this compound for its drug activity screening is required.

Very little work on the phytochemical analysis of both these tree ferns has been reported so far. As referred to earlier, Juneja et al. (1990) reported the presence of hentriacontane, β-sitostenone, β-sitostanone, diploterol, sitosterol, hopan-29-ol and oleanolic acid from the hexane extract of *Cyathea gigantea*. The outcome of our work thus enriched the existing knowledge on the phytochemical constituents of these two tree ferns, which is likely to trigger future research in this direction.
6.2 Conclusion:

Preliminary phytochemical analysis revealed the presence of steroids, flavonoids and saponins in all the 16 extracts of the caudex and leaves of *Cyathea gigantea* and *Cyathea brunoniana*. Acetone extracts of both the plants showed effective antibacterial activity against gram +ve and gram -ve bacteria, whereas only the methanol extract of *Cyathea gigantea* showed antibacterial activity. Based on this information, the acetone extracts of caudex of both the plants were taken up for the separation of the metabolites. As a result, four metabolites could be isolated. Of these, two metabolites of *Cyathea gigantea* were identified as the monoenoates, Glyceryl elaidate and Glyceryl oleate. This is the first report of the isolation of glyceryl elaidate from any tree fern and of its detailed spectroscopic data.

The ACTELION PROPERTY EXPLORER, showed the potentiality of the isolated compound, to become a drug lead as it did not show any toxicity risks and drug score was also impressive.

The present piece of predoctoral research clearly points to the necessity and justification for a future undertaking of a valid two-pronged approach, comprising systematic analysis and bio-monitoring, towards finding out the active constituent(s) of these two tree ferns. It thus, therefore, inclined to think that the work embodied in this Thesis is a significant contribution in the fields of both Phytochemistry and Life Science.

These plants are on the verge of extinction and some measures should be taken to conserve these plants by *in vitro* multiplication for future phytochemical exploration.