

**Chapter 6**



***Conclusion***

## 6. Conclusion

- ❖ A review of literature pertaining to the different aspects of the present line of study has been presented.
- ❖ Detailed description of all the procedure and protocols used in this investigation has been presented.
- ❖ Ferns collected from different areas of Darjeeling town, subdivision of Mungpoo and Siliguri shibmandir area were identified and processed for further experimentation. Identified samples were *Nephrolepis cordifolia* (Nc), *Cyclosorus dentatus* (Cd), *Phymatosorus cuspidatus* (Pc), *Drynaria quercifolia* (Dq), *Dicranopteris linearis* (Dl), *Pteris biaurita* (Pb), *Pteris vittata* (Pv) and *Microsorium punctatum* (Mp).
- ❖ Plant samples were dried, powdered and extracted using different solvent (alcohol, methanol and hot water) and subjected to phytochemical analysis and determination of various biological activities
- ❖ Phytochemical analysis of the samples revealed the presence of phenol, flavonoid, tannin, carbohydrates, reducing sugar, protein and terpenoid in all ferns under study. None of the ferns showed positive result for anthraquinone. Similarly, alkaloid was not detected in any of the samples studied except in *P. biaurita*. Cardiac glycosides were detected in all the ferns except *M. punctatum* and *N. cordifolia*. Further, except *P. biaurita* and *M. punctatum* all the other ferns showed positive result for saponin. Steriod was detected only in *N. cordifolia*, *C. dentatus* and *P. biaurita*.
- ❖ Quantitatively, highest amount of total phenol, protein, total flavonoid, tannin, total sugar and vitamin C was observed in *N. cordifolia*. Conversely, *M. punctatum* had the lowest amount of total phenol, total flavonoid, tannin, reducing sugar, total chlorophyll, chlorophyll a and carotenoid. Highest amount of reducing sugar and total chlorophyll was found in *P. biaurita*. Vitamin E content was highest in *P. cuspidatus* while *D. linearis* had the lowest content. On the contrary, vitamin C content was lowest in *P. cuspidatus*.
- ❖ Extraction of the samples with solvents yielded difference in percentage of yields with highest yield being in methanol than the other two solvents (ethanol and water).
- ❖ Antioxidant activities such as DPPH radical scavenging, hydrogen peroxide scavenging, nitric oxide peroxide scavenging, superoxide scavenging activity and ferric reducing antioxidant power assay (FRAP) performed using the different extracts of the samples revealed *N. cordifolia* and *C. dentatus* to exhibit significantly higher activity than the

other plants studied irrespective of the solvents used. Lowest activity in all the cases was showed by the extracts of *M. punctatum*. Other than DPPH activity (where hot water extracts showed highest activity), methanolic extracts of all the samples revealed highest activity followed by water and ethanolic extracts.

- ❖ PCA analysis using all the factors (phytochemicals) and respective antioxidant activity of each plant was performed. Besides other factors studied, the most positively influencing factors were total flavonoid, total phenols, tannin and lipids as they showed high positive correlation with all the antioxidant activities in almost all the plants.
- ❖ Phenolic compounds identified in the methanolic extracts by HPLC were phloroglucinol, gallic acid, pyrogallol, 3,4-dihydroxybenzoic acid, resorcinol, catechol, catechin, chlorogenic acid, caffeine, caffeic acid, vanillic acid, ferulic acid, salicylic acid and cinnamic acid of which caffeic, ferulic and salicylic acid were the most prominent ones. Huge variation in the concentration of phenolics in each plant was observed.
- ❖ Depending on the antioxidant activities exhibited by the plant samples, two ferns showing higher activity and one showing lowest activity were tested for their antimicrobial properties and *in vitro* antidiabetic activity. Likewise, since among the three solvent extracts, methanolic and hot water extracts revealed comparatively better antioxidant activity, extracts with these solvents were used for antimicrobial and *in vitro* antidiabetic activity. The ferns selected were *N. cordifolia* and *C. dentatus* (higher activity) and *M. punctatum* (lowest activity).
- ❖ Antibacterial studies performed against two gram positive (*Bacillus cereus* and *B. megaterium*) and two gram negative bacteria (*Burkholderia symbiont* and *Serratia marcescens*) using three different concentrations of methanolic and hot water extracts revealed their ability to inhibit the bacterial growth as tested in disc diffusion bioassay with *N. cordifolia* and *C. dentatus* showing better activity than *M. punctatum*.
- ❖ Antifungal studies performed against *Alternaria alternata*, *Curvularia lunata* and *Fusarium oxysporum* by spore germination test and radial growth bioassay revealed that the extracts of *N. cordifolia* and *C. dentatus* was efficient to inhibit the growth of the fungi tested while *M. punctatum* did not exhibit inhibitory activity against any of the fungi studied. Among, the three concentration used for the study, 500mg/mL was found to be effective against these fungi while no inhibition was observed by 250 and 100mg/mL concentration.
- ❖ *In vitro* antidiabetic activity determined using  $\alpha$ -amylase inhibitory activity test revealed that methanolic extracts of the three selected plants showed higher activity than the

aqueous (hot water) extracts. *N. cordifolia* extracts revealed higher activity than *C. dentatus* and *M. punctatum*. However, the activity of the extracts was lesser than the positive control (acarbose) used.

- ❖ Furthermore, an *in vivo* antidiabetic test in streptozotocin-induced diabetic rats was performed using *N. cordifolia* and *C. dentatus* as they were found to be more efficient in inhibiting  $\alpha$ -amylase activity than *Mp* extracts. Though, methanolic extracts showed better activity than aqueous extract, however, for this part of the study aqueous extracts was used considering the hazards that may be caused with methanolic consumption.
- ❖ Before performing an *in vivo* test, acute toxicity test done to analyze the lethal doses of extracts revealed 500 and 250mg/mL doses to be safer for further studies.
- ❖ Streptozotocin-induced diabetic rats treated orally with both the samples and metformin was able to reverse the diabetic induced changes to near normal. Various parameters such as fasting blood sugar level, cholesterol, triglycerides liver enzymes (SGPT and SGOT), serum urea and creatinine was reduced while significant increase in the body weight and HDL-cholesterol level was observed as compared to the diabetic controls.
- ❖ Interestingly, *C. dentatus* extracts showed comparatively better *in vivo* antidiabetic activity than *N. cordifolia* extracts which may be because of the variation in the body responses exerted by several interacting internal factors. On the other hand, higher concentration of *N. cordifolia* revealed lesser reduction in the tested blood parameters than the lower concentration used in the study which may be because of the auto-inhibitory activity of the extracts at higher concentrations or the presence of hyperglycemic compounds, besides hypoglycemic components in the plant extracts.
- ❖ Further, characterization of bioactive compounds was done by partial purification of *N. cordifolia* and *C. dentatus* extract in column using different non-polar and polar solvents at varying gradient.
- ❖ The fractions were tested for their *in vitro* antioxidative and antidiabetic potentials (*in vitro*  $\alpha$ -amylase inhibition assay). In *C. dentatus* fractions, highest and lowest DPPH radical scavenging activity was revealed by *CdB* and *CdD* fraction respectively. On the other hand, *NcE* revealed the highest and *NcB* the lowest activity in *N. cordifolia* fractions. Additionally,  $\alpha$ -Amylase inhibition activity in *C. dentatus* fractions was exhibited highest by *CdC* and lowest by *CdD*. Likewise, *NcC* and *NcB* exhibited highest and lowest activity respectively.
- ❖ The fractions activity was observed to be higher than the crude extracts. This can be expected, since fractionation and purification leads to concentration of the active

compounds in these specific fractions. Moreover, crude extracts would be containing a large number of other compounds and hence the active compounds would be in lesser amounts or its activity may be inhibited by other compounds.

- ❖ The fractions were further characterized using GC-MS which revealed the presence of myriad of compounds containing terpenoids, fatty acids, phytosterols, phenylpropanoids and phenolics,
- ❖ To further understand the mechanism of action that may have been involved in antidiabetic activity revealed by *N. cordifolia* and *C. dentatus* an *in silico* molecular docking studies was performed. The fraction CdC and NcC revealed better  $\alpha$ -amylase inhibitory activity, thus compounds identified in these fraction was docked with the target proteins/enzymes involved in glucose metabolism such as glucokinase (IV4S), fructose 1,6- biphosphatase (2JJK), 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (2BEL) and peroxisome proliferators activated receptor gamma (3DZY). Results revealed that amongst many other compounds, corynan-17-ol, 18,19- didehydro-10-methoxy-acetate (ester) and propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandro-8-en-17-yl)- possess better interaction with almost all the proteins/enzymes involved in glucose metabolism. Thus, the plausible mechanism of antidiabetic activities exhibited by *N. cordifolia* and *C. dentatus* may be due to the alteration in the activity of these proteins/enzymes. Further, these compounds may be considered as potential antidiabetic agents as hit structures for designing potent and specific drugs.
- ❖ Presence of various bioactive constituents in these ferns makes them the potential source for exploiting their applications in pharmaceuticals. However, further isolation and characterization of each compound responsible for the activity in addition to elucidation of the mechanism involved remains to be explored.