ABSTRACT

Plants and plant-derived natural products have been the integral part of traditional healthcare system since early human civilization. The use of synthetic drugs over natural products prevailed for many years with the advancement in the synthetic drug discovery. However, with numerous illustrations of adverse effects of synthetic drugs during past decades, there has been a tremendous rise in the search of natural and safer drugs. Plants with the presence of various phytochemicals or bioactive compounds provide a safer and cost effective drug development resource.

The change in the life style of human population has increased the rate of development of various life threatening oxidative stress related diseases like cancer, diabetes, atherosclerosis, arthritis, Alzheimer's disease, other neurodegenerative disorder etc. Plants are known as rich source of natural antioxidants as the phytochemicals present in them have been reported as potent free radical scavengers or quenchers. Besides antioxidants, herbal medicines have been used as antimicrobial, antiviral, anti-inflammatory, anti rheumatid, antiallergic etc.

Likewise, use of ferns and fern allies in traditional medicines has a long history tracing back to 2000 years. Ferns have been expected to have many more useful metabolites than other plants as they have survived through various environmental changes from Paleozoic times. Despite being luxuriantly available and possessing economical, ecological and medicinal values very less attention has been given towards the utility of pteridophytes. Considering the importance of natural products and its application in pharmaceuticals the present study has been undertaken to screen and quantify various phytochemicals present in some locally available ferns of Darjeeling district, North Bengal region. Furthermore, biological activities like antioxidant, antimicrobial and antidiabetic have also been studied. Subsequently, partial purification, isolation and elucidation of the chemical structures of some active compounds have been performed. Further, an *in silico* molecular docking approach has been employed to understand the mechanism and identify some potential lead compounds against some proteins involved in glucose metabolism.

A review of literature has been compiled and presented on the investigation of various bioactive constituents present in the plants, their antioxidant, antimicrobial and antidiabetic activity. Subsequently, brief discussion on phytochemicals screened in ferns and their activities mainly focusing on antioxidant, antimicrobial and antidiabetic properties has been presented. Ferns were collected from different areas of Darjeeling town, subdivision of

Mungpoo and Siliguri Shibmandir area and were identified as *Nephrolepis cordifolia* (*Nc*), *Cyclosorus dentatus* (*Cd*), *Phymatosorus cuspidatus* (*Pc*), *Drynaria quercifolia* (*Dq*), *Dicranapteris linearis* (*Dl*), *Pteris biaurita* (*Pb*), *Pteris vittata* (*Pv*) and *Microsorum punctatum* (*Mp*). The plant samples were dried, powdered and extracted using different solvent (alcohol, methanol and hot water). The powdered samples were qualitatively screened for the detection of phytochemicals present in the plants. Phytochemical analysis of the samples revealed the presence of phenol, flavonoid, tannin, carbohydrates, reducing sugar, protein and terpenoid in all the ferns studied. 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*.

Quantification of the bioactive compounds revealed *N. cordifolia* to contain highest amount of total phenol, protein, total flavonoid, tannin, total sugar and vitamin C. On the other hand, *M. punctatum* had the lowest amount of total phenol, total flavonoid, tannin, reducing sugar, total chlorophyll, chlorophyll a and carotenoid. *P. biaurita* had the highest amount of reducing sugar and total chlorophyll. 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.*

Difference in the extraction yield was observed with the difference in the solvents used. The percentage of yield was highest in the methanolic extracts followed by hot water and ethanolic. These extracts exhibited varying degrees of antioxidant activities as determined by DPPH radical scavenging activity, hydrogen peroxide scavenging activity, nitric oxide scavenging activity, superoxide radical scavenging activity and ferric reducing antioxidant power. *N. cordifolia* and *C. dentatus* exhibited the highest antioxidant activities irrespective of the solvents used and *M. punctatum* the lowest antioxidant activities. Unlike hot water extracts in DPPH activity, methanolic extracts of all the plants revealed significantly higher activity than the other extracts.

Among the various influential factors responsible for the antioxidant activities, principle component analysis (PCA) revealed total flavonoid, total phenols, tannin and lipids to be the most positively influencing factors as high positive correlation with the activities was observed in almost all the plants studied.

Various phenolics present in the methanolic extracts of all the plant samples were identified and quantified using High Performance Liquid Chromatography analysis. The phenolic compounds identified 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. The most prominent groups of phenolics present in all the samples were caffeic, ferulic and salicyclic acid. Huge variation in the concentration of phenolics in each plant was observed.

Furthermore, antibacterial studies revealed that the selected fern extracts (methanolic and hot water extracts) were efficient in inhibiting the growth of the bacteria studied. Studies was performed against two gram positive (*Bacillus cereus* and *Bacillus megaterium*) and two gram negative bacteria (*Burkholderia symbiont* and *Serratia marcescens*) using three different concentrations of the selected plant extracts. The ferns selected were *N. cordifolia* and *C. dentatus* (higher antioxidant activities) and *M. punctatum* (lowest antioxidant activities). 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 activity. Methanolic extracts of all the three plant samples revealed comparatively higher inhibitory activity than the water extracts against all the bacteria studied.

The plant extracts was also found to be efficient in inhibiting the growth of all the three fungi studied i.e, *Alternaria alternata*, *Curvularia lunata* and *Fusarium oxysporum* as evaluated through spore germination test and radial growth bioassay. Spore germination of all the fungi studied was efficiently inhibited by the extracts of *N. cordifolia* and *C. dentatus* while *M. punctatum* extracts was not able to inhibit the germination of any of the fungi studied. Among, the three concentrations used for the study, 500mg/mL was found to be effective against these fungi while no inhibition was observed by lower concentrations. Since, *M. punctatum* extracts was unable to inhibit the spore germination of any of the fungi used, radial growth bioassay was performed using only *N. cordifolia* and *C. dentatus* extracts. Varying degree of fungal growth inhibition was exhibited by both the extracts. Methanol and hot water extracts of *N. cordifolia* was found to be marginally better in inhibiting the fungal growth than *C. dentatus*. Methanolic extracts revealed higher activity than hot water extracts against all the fungi studied.

In vitro α-amylase inhibitory activity revealed that methanolic extracts of the three selected plants showed higher activity than the aqueous extracts. Among the sample, *N. cordifolia*

extracts revealed higher activity than *C. dentatus* and *M. punctatum*. The activity of the extracts was lesser than the positive control acarbose used. Since, *N. cordifolia* and *C. dentatus* extracts were found to be more efficient in inhibiting α-amylase activity than *M. punctatum* extracts, *in vivo* studies was conducted using only *N. cordifolia* and *C. dentatus*. 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.

An *in vivo* antidiabetic assay performed on streptozotocin-induced diabetic rats revealed extracts to be efficient in normalzing the various blood parameters altered in the rats with the induction of diabetes. Various parameters monitored was change in body weights, the fasting blood sugar level, cholesterol, triglycerides and HDL-cholesterol level, SGPT and SGOT level and serum urea and creatinine level. 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 may be because of the presence of hyperglycemic compounds, besides hypoglycemic components in the plant extracts. Interestingly, *C. dentatus* extracts showed 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.

Further, characterization of bioactive compounds present in *N. cordifolia* and *C. dentatus* extract was done using column chromatographic technique. The column was eluted with different non-polar and polar solvents at varying gradient. The eluted fractions were tested for their *in vitro* antioxidative and antidiabetic potentials. Results revealed that in *C. dentatus* fractions, highest and lowest DPPH radical scavenging activity was revealed by *Cd*B and *Cd*D fraction respectively. In *N. cordifolia* fractions, *Nc*E revealed the highest and *Nc*B the lowest activity. Additionally, *in vitro* α-amylase inhibition activity in *C. dentatus* fractions was exhibited highest by *Cd*C and lowest by *Cd*D. Likewise, *Nc*C and *Nc*B exhibited highest and lowest activity respectively. In both the cases fraction (eluate) activity was observed to be higher than the crude extracts.

Each of the fractions (eluates) were further characterized using GC-MS which revealed the presence of myriad of compounds containing terpenoids, fatty acids, phytosterols, phenylpropanoids and phenolics,

The compounds identified in the fraction *Nc*C and *Cd*C revealing better α-amylase inhibitory activity was docked with some of the proteins involved in glucose metabolism such as

Glucokinase (IV4S), Fructose 1,6- bisphosphatase (2JJK), 11β-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-trimethylandrost-8-en-17-yl)- possess better interaction with almost all the targeted proteins 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. Further, these compounds may be considered as potential antidiabetic agents as hit structures for designing potent and specific drugs.

Presence of diversified type of bioactive compounds in these ferns makes them the potential source for exploiting their application in pharmaceuticals.