ABSTRACT

The change in the life style of human population during the last few decades has given rise of several harmful diseases which are mainly the result of free radicals. Free radicals are generated due to oxidative biochemical processes that are essential for existence of living cell. These free radicals are responsible for diseases like cancer, diabetes, atherosclerosis, arthritis, Alzheimer's disease, other neurodegenerative disorder etc.

The plants are known for their therapeutic nature from ancient past to the wise peoples of our country. During the development of our culture and civilization plants always were given supreme priority as well as respect for their utility in our ancient medical science Ayurveda. For the last few years lots of work has been done on the antioxidative and antidiabetic activity of different plants from India and abroad. Due to its wide and extremely diverse geographical distribution India is rich in plant diversity that can be explored for development of natural therapeutics to treat life style diseases.

Northern part, demarcated by the river Ganges, of the state West Bengal is known as North Bengal and is recognized for its richness in biodiversity. This part of land is also dominated by the presence of several wetlands which are rich for their biodiversity. Considering the importance of natural products and its application in pharmaceuticals the present study has been undertaken to document the available wetland plants with ethnobotanical uses from six districts of West Bengal. Furthermore, biological activities like antioxidant, antimicrobial and antidiabetic and anticancer have also been studied in selected plant species. Subsequently, characterization of active principles by HPLC, GC/MS and FTIR was done. Further, *in silico* molecular docking approach has been employed to understand the mechanism active constituents against the protein peroxisome proliferators activated receptor gamma (3DZY).

A review of literature has been compiled and presented on the ethnobotanical study of plants and their uses, compilation of various bioactive constituents present in the plants, their antioxidant, antimicrobial and antidiabetic activity. Subsequently an extensive survey was conducted between the common peoples of the districts of north Bengal regarding the uses of different plant species. Survey of the wetland plants reveals that the local community of North Bengal consume majority of the plants in

their daily diet. A total of 58 wetland plant species belonging to 42 families were recorded as ethnomedicinally important during the study. It has been also observed that some are sold in daily market regularly e.g *Amorphophalus paeoniifolius* (Dennst.), Nicolson, *Bacopa monnieri* (L.)Pennell., *Colocasia esculenta* (L.)Schott., *Diplazium esculentum* (Retz.)Sw., *Enydra fluctuans* Lour., *Euryale ferox* Salisb., *Glinus oppositifolius* (L.) A. DC., *Hygrophila auriculata* (Schumacher) Heine., *Ipomoea aquatica* Fossk., *Marsilea minuta* L., *Portulaca oleracea* L., *Trapa natans* L., *Nasturtium officinale* R.Br.

Finally, a total of fifteen plants were selected for study. All the plants were dried and mounted on herbarium sheet following the proper protocol and submitted to the NBU Herbarium for their authentication and subsequently the accession Nos. were also obtained against each of the samples.

After the proper identification plants were dried and crushed in to powder and extracted by using methanol as solvent. Flask extraction method was followed in this study and it was observed that the extractive yield percentage ranged from 8.32% to 12.52%. The highest extractive value was recorded in the sample *Marsilea minuta* and lowest was recorded in *Amaranthus spinosus.*

Different phytochemicals including primary and secondary metabolites were screened in the powdered plant samples. It was observed that saponins (12), alkaloids (09), phenolics and flavonoids (11) and tannin (12) were the predominant phytochemicals among the tested plants.

Plants used in this study were also tested for the presence of different biochemical components such as carbohydrate, protein and amino acid, phenol etc. The protein content was found to be significantly highest in the powdered sample of *A. spinosus* and lowest in *Pilea microphylla*. Similarly, significant differences were present in the free amino acid content of the 15 samples under study. Among the plant samples, *Glinus oppositifolius* contained the highest amount of amino acids whereas *Cryptocoryne retrospiralis* had the lowest. In case of phenol, the powdered sample of *Hydrocotyle sibthorpioides* had significantly ($p<0.05$) highest phenol content compared to all the other plant samples. And the lowest phenol content was recorded in *Portulaca oleracea*. Similarly, the total flavonoid content of *Amaranthus viridis* was highest and in *Portulaca oleracea* it is lowest. Significantly highest soluble sugar content was recorded in *Eclipta prostrata* and lowest concentration was detected in *Ipomoea aquatica*. Ascorbic acid content was found to be highest in *H. sibthorpioides*

and lowest in *Glinus oppositifolius*. On the other hand, the carotenoid content was found to be highest in *Amaranthus viridis* and lowest in *Ipomoea aquatica*. Total chlorophyll content was found to be highest in *Eclipta prostrata* and the least amount was observed in *Diplazium esculentum*.

After the phytochemical screening and estimation of biochemical component in plant powder the antioxidant activity of the plant methanolic extracts were evaluated. All the plant extracts showed varied degree of DPPH scavenging activity mostly ranged from 2.96-73.19 %. Among the plant extracts, the lowest IC50 value for DPPH was recorded for *H. sibthorpioides* (1.539 ± 0.065) and the highest IC50 value was recorded for *Enhydra fluctuans* (9.672 ± 0.082).

ABTS free radical scavenging activity of all the plants extracts was found to increase in a dose dependent manner for the range of concentrations tested (0.1, 0.5, 1, 2, 4, 6, 8, 10 mg/ml). All the extracts showed variable ABTS scavenging activity that mostly ranged from 04.96-76.09%.

The ferric reducing power of the positive control (ascorbic acid) was significantly higher than all extracts. The FRAP ability of extracts are given in ascending order *Pilea microphylla* > *Diplazium esculentum* > *Portulaca oleracea* > *Glinus oppositifolius* > *Marsilea minuta* > *Amaranthus spinosus* > *Hygrophila auriculata* > *Hydrocotyle sibthorpioides* > *Ipomoea aquatica* > *Amaranthus viridis* > *Phyla nodiflora* > *Enhydra fluctuans* > *Eclipta prostrata* > *Cryptocoryne retrospiralis* > *Barringtonia acutangula*.

All the plant extracts was tested for hydroxyl radical scavenging activity which was found to increase in a dose dependent manner for the range of concentrations tested (0.1, 0.5, 1, 2, 4, 6, 8, 10 mg/ml). All the extracts showed variable OH scavenging activity that mostly ranged from 8.34-81.23%.

All the plant extracts was tested for NO scavenging activity which was found to increase in a dose dependent manner for the range of concentrations tested. All the extracts showed variable NO scavenging activity that mostly ranged from 04.65-60.29%.

All the fifteen plant extracts was tested for superoxide radical scavenging activity which was found to increase in a dose dependent manner for the range of concentrations. All the extracts showed variable superoxide scavenging activity that mostly ranged from 20.53-65.14%. Among the plant extracts, the lowest IC50 value

was recorded for *H. sibthorpioides* (1.492 ± 0.085) and the highest IC50 value was recorded for *G. oppositifolius* (8.590 ± 0.062).

Metal chelating activity was also found to increase in a dose dependent manner for the range of concentrations tested (0.1, 0.5, 1, 2, 4, 6, 8, 10 mg/ml). All the extracts showed variable Metal chelating activity that mostly ranged from 12.23-84.67%.

In vitro α-amylase activity assay was also performed to determine whether any antidiabetic property is present in the plant samples under study. Dose dependent inhibition activity was also observed in this case. All the extracts showed variable α-amylase inhibitory activity that mostly ranged from 8.14-86.77%. Among the plant extracts, the lowest IC50 value was recorded for *C. retrospiralis* (0.69 ± 0.062) and the highest IC50 value was recorded for *H. auriculata* (6.293±0.144).

Based on the results of all previous experiments, finally two plant samples showing overall best performance were selected for all further tests. These plants samples were *C. retrospiralis and H. sibthorpioides.*

After the selection of two plants antibacterial and antifungal activity of the two plant extracts were evaluated against *Bacillus subtilis, Escherichia coli* and *Mycobacterium avium* and three fungal pathogens. It was observed that both the isolates exhibited antimicrobial activity against *B. subtilis* and *E. coli*. No inhibition was observed against *Mycobacterium avium C. retrospiralis* showed better antibacterial activity in comparison to control against the test organisms. The spore germination of the fungal pathogens was inhibited by the methanolic plant extracts. Inhibition was recorded higher in *C. retrospiralis* in comparison to *H. sibthorpioides* in all three plant pathogenic fungus.

Finally *in vivo* study was conducted to determine the antidiabetic activity of two plant extracts. Rats were induced to be hyperglycaemic by STZ injection. Results were recorded after 25 days of induction of diabetes. The body weight of the diabetic control was found to have decreased significantly in diabetic control (set II) when compared to the normal rats (set I). However, an oral administration of *C. retrospiralis* and *H. sibthorpioides* (200 & 400 mg/kg b.w.) and metformin to diabetic rats reversed the body weights changes to near normal. The hypoglycaemic effect of the extracts *C. retrospiralis* and *H. sibthorpioides* was recorded by measuring the fasting blood glucose levels in day 0, day 1, day 5, day 10, day 15, day 20 and day 25. It was observed that both the extracts could lower blood glucose level almost as effectively as metformin. Triglycerides, LDL- cholesterol, cholesterol levels were reduced and HDL

cholesterol level was increased significantly in standard drug (10 mg/kg) treated as well as extract (*C. retrospiralis, H. sibthorpioides*) treated groups. In case of liver enzyme such as SGPT and SGOT a significant increase was observed in diabetes induced rat. Although, increased level of SGPT and SGOT in the diabetic induced rats was decreased significantly with subsequent administration of the standard drug and the plant extracts in a dose dependent manner. Similar type of result was also observed in case of urea and creatinine.

Plants extracts were then characterized for their bioactive compounds. FTIR study revealed the presence of -O-H, –C=O and –C-C stretching in *C. retrospiralis.* Similar type of FTIR spectra was also recorded in *H. sibthorpioides*.

HPLC profiles of *C. retrospiralis and H. sibthorpioides* along with the other plant extracts were done and the analysis revealed the presence of a wide array of phenolic compounds. A total of 70 major peaks were detected across the plant extracts which were represented by individual peaks with different retention times in the chromatogram. Among the plant extracts, *Marsilea minuta* recorded the maximum number of phenolic compounds with the presence of 61 major peaks pointing out towards the presence of a wide range of phenolic compounds. In *H. sibthorpioides*, however only 15 peaks could be obtained and in *C. retrospiralis*, only 12 peaks could be recorded.

The methanolic extract of *C. retrospiralis* and *H. sibthorpioides* were further subjected to GC-MS for partial characterization of the compounds present in the fractions. *C. retrospiralis* methanolic extract were identified to contain nine different compounds, and *H. sibthorpioides* methanolic extracts were identified to contain ten different compounds.

In silico molecular docking with the compounds revealed by GC/MS showed that identified compounds were able to bind the catalytic site of Peroxisome proliferators activated receptor gamma (3DZY). Similarly docked conformation of different compounds *H. sibthorpioides* along with important amino acid residues of 3DZY was tested. The compound Corynan-17-ol,18,19-didehydro-10-methoxy acetate (ester) which was obtained from *H. sibthorpioides* possessed good binding affinity (-9.95 kcal/mol) by binding with amino acid residues ILE268, ALA 271, GLN275, TRP 305, ASN 306, LEU309, PHE 313, ARG 316, LEU 326, ALA 327, VAL 342, ILE 345, LEU 426, HIS 435.