## 6. CONCLUSION

- ✓ A review of literature pertaining to the different aspects of the present line of study has been presented.
- ✓ Ethnobotanical survey and selection of plant materials were done accordingly.
- ✓ Detailed description of all the procedure and protocols used in this investigation has been presented.
- ✓ Wetland plants collected from different areas of North Bengal were identified and processed for further experimentation. Identified samples were *Cryptocoryne retrospiralis* (Roxb.) Kunth., *Barringtonia acutangula* (L). Gaertn., *Hydrocotyle sibthorpioides* Lam., *Eclipta prostrata* (L.) L., *Enhydra fluctuans* DC., *Amaranthus spinosus* L., *Marsilea minuta* L. *Amaranthus viridis* L., *Glinus oppositifolius* (L)Aug.DC, *Portulaca oleracea* L., *Hygrophila auriculata* (Schumach) Heine., *Phyla nodiflora* (L) Greene., *Pilea microphylla* (L) Liebm., *Diplazium esculentum* (Retz) Sw., *Ipomoea aquatica* Frossk.
- ✓ Plant samples were dried , powdered and extracted using methanol and subjected to phytochemical analysis and determination of various biological activities
- ✓ Different phytochemicals including primary and secondary metabolites were screened in the powdered plant samples. In all the plant samples except *C. retrospiralis*, *A. viridis* and *I. aquatica* saponins were detected. Presence of alkaloids was detected in 9 plant samples except *H. sibthorpioides*, *A. spinosus*, *M. minuta*, *A. viridis*, *P. oleracea* and *I. aquatica*. The phenolics and flavonoids were detected in all the plant samples except *P. oleracea*. In *B.acutangula*, *E. prostrata* and *G. oppositifolius*, the phytochemical test for steroids showed positive result. In *C. retrospiralis*, *A. viridis*, *P. oleracea*, *P. nodiflora*, *P. microphylla* and *I. aquatica*, carbohydrates were not detected. The phytochemical screening showed positive results for the presence of anthroquinones only in case of *B.acutangula*, *P. nodiflora*, *D.esculentum*; but in other samples anthroquinones were not detected. Only *C. retrospiralis* and *D.esculentum* showed negative results for amino acid. Reducing sugars were not traced in *C. retrospiralis*, *P. oleracea* and *P. microphylla*. Also,

phytochemical screening of resins revealed their presence only in case of *B.acutangula*, *E. prostrata* and *I. aquatica*. On the other hand, cardiac glycosides were present in *A.spinosus*, *A. viridis*, *G. oppositifolius, P. oleracea* and tannins were detected in all the samples except *A.spinosus*, *D.esculentum* and *I. aquatica*. Likewise, phytochemical screening of glycosides revealed their presence in *C. retrospiralis*, *H. sibthorpioides*, *A.spinosus*, *A. viridis*, *G.oppositifolius*, *P. oleracea* and *P. nodiflora*. However, protein was not detected in *P. nodiflora* and *P. microphylla*; and terpenoids were detected in *C. retrospiralis*, *B.acutangula*, *E. prostrata*, *E. fluctuans*, *P. oleracea*, *H. auriculata.*

- ✓ Quantitativley, amount of total phenol, total flavonoid, protein, free amino acids, total sugar, reducing suger, chlorophyll, carotenoid and ascorbic acid content were estimated in these plants. The protein content observed maximum in *A. spinosus* and the amino acid content was recorded highest in *G. oppositifolius*. In *H.sibthorpioides* highest phenol content was recorded and in *A.viridis* highest flavonoid content observed. The total suger content of *E.prostrata* ranked highest and highest reducing suger content was recorded in *P. oleracea.*Highest ascorbic acid content observed in *H.sibthorpioides* and the carotenoid content estimated maximum in *A.viridis.*Total chlorophyll and Chl-a was recorded highest in *E.prostrata* and highest Chl-b content recorded in *A.spinosus* .
- ✓ Extractive values for these fifteen plants with methanol differ from 12.52% to 8.32%. Highest yield was achieved in *M. minuta* and lowest in *A.spinosus* .
- ✓ In the present study, methods such as DPPH radical scavenging, ABTS scavenging activity FRAP assay, $OH^-$ scavenging activity, nitric oxide scavenging, superoxide scavenging and Metal chelating activity was employed.The DPPH scavenging activity mostly renged from 2.96 – 73.19 %. Among the plant extracts, lowest IC50 value for DPPH was recorded for *H.sibthorpioides* (1.539±0.065) and the highest IC50 value was recorded for *E.fluctuans* (9.672±0.082). ABTS scavenging activity mostly ranged from 04.96-76.09%. In case of FRAP assay highest activity was recorded in *B. acutangula* whereas lowest activity was exhibited by *P. microphylla.* Similarly all the extracts showed variable $OH^-$ scavenging activity ranged from 8.34-81.23%. The NO

scavenging activity ranged from 04.65-60.29%. Almosr all the extracts showed variable superoxide scavenging activity. Among the plant extracts the lowest IC50 value was recorded for *H.sibthorpioides* and highest IC50 value recorded for *G. oppositifolius*. The metal chelating activity 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 metal chelating activity that mostly ranged from 12.23-84.67%.

- ✓ *In vitro* α-amylase activity assay was also performed to determine wheather any antidiabetic property is present in the plant samples under study. All 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* and the highest IC50 value was recorded for *H.auriculata*
- ✓ Based on the results of all previous experiments, finally two plant samples showing overall best performance were selected for further experiments *eg*. antimicrobial properties, *in vitro* antidiabetic activity, Cytotoxic effect etc.
- ✓ Antifungal studies performed against *Alternaria alternata*, *Curvularia lunata* and *Fusarium oxysporum* by spore germination test revealed that the extracts of *C. retrospiralis and H. sibthorpioides* were efficient to inhibit the growth of the fungal spore upto certain level. 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.
- ✓ Furthermore, an *in vivo* antidiabetic test in streptozotocin-induced diabetic rats was performed using *C. retrospiralis* and *H. sibthorpioides* as they were found to be more efficient in inhibiting α-amylase activity than *other* extracts.
- ✓ Before performing an *in vivo* test, acute toxicity test done to analyse the lethal doses of extracts revealed 500 and 250 mg/mL doses to be safer.
- ✓ Streptozotocin-induced diabetic rats treated orally with both the samples and metformin was able to reverse the diabetic induced changes to upto certain level. Various parameters such as fasting blood sugar level, triglycerides, LDL-cholesterol,total cholesterol, liver enzymes (SGPT and SGOT), serum urea and creatinine was reduced while significant increase in the body weight and HDL-cholesterol was also observed.

- ✓ The main stretching vibrations of *C. retrospiralis* appear at 3394cm$^{-1}$ (broad), 2924 cm$^{-1}$2852 cm$^{-1}$, 2265 cm$^{-1}$2065cm$^{-1}$, 1640cm$^{-1}$, 1407cm$^{-1}$ and1237 cm$^{-1}$. Absorption bands in the 3600-3200 cm$^{-1}$ regions are from O-H stretching vibrations. Similar type of FTIR spectra of *H. sibthorpioides*. The main stretching vibrations of *H. sibthorpioides* appear at 3397cm$^{-}$1 (broad), 2926 cm$^{-}$ $^{1}$2855 cm$^{-1}$, 2265 cm$^{-1}$, 2065 cm$^{-1}$, 1648cm$^{-1}$ and 1411cm$^{-1}$. From the FTIR spectra it confirmed that *C. retrospiralis* and *H. sibthorpioides* contain similar types of functional groups.
- ✓ 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 1,3- Diazocane -2 thione, E 11 - Methyl-12-tetradecan-1-ol acetate, Hexadecanoic acid- methyl ester, Phytol, 9, 12- Octadecadienoic acid, Isopropyl Stearate, 6,9,12-Octadecatrienoic acid, Phenylmethyl ester, Testosterone Enanthate, n-Hexadecanoic acid and *H. sibthorpioides* methanolic extracts were identified to contain ten different compounds 3 Eicosyne, Morphinan- 3,14-diol, 4,5-epoxy(5 á), 9,12,15-Octadecatrienoic acid, 2,3-dihydroxy propyl ester, Corynan-17- ol,18,19-didehydro-10-methoxy acetate, Curan-17-oic acid, 2,16-didehydro-20-hydroxy-19-oxo, methyl ester, 10-Octadecanoic acid, methyl ester, Z,E-3,13-Octadecadien -1 ol, Thujopsene, 3-Cyclohexen-1-ol, 4- methyl-1-1(1-methylethyl), Hexadecanoic acid- methyl ester.
- ✓ To further understand the mechanism of action that may have been involved in antidiabetic activity revealed *C. retrospiralis* and *H. sibthorpioides* an *in silico* molecular docking studies was performed. The molecular docking results showed that the compound testosterone enanthate obtainined from methanol extract of *C. retrospiralis*, possess good binding affinity (-11.16 kcal/mol) with 3DZY showing interaction with protein residues ILE 268, ALA 271, ALA 272, GLN 275, TRP 305, ASN 306,LEU 309,ILE 310, PHE 313,ARG 316, LEU326, ALA 327, ILE 345, PHE 346, VAL 349, CYS 432, HIS 435, LEU 436 as compared to commercial drug metformin (LYS431, GLU434, THR737, GLN741, GLN744, TYR777).

- ✓ 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, GLN 275,TRP 305, ASN 306, LEU 309,PHE 313, ARG 316, LEU 326, ALA 327, VAL 342, ILE 345,LEU 426,HIS 435.
- ✓ The methanolic extract of *C. retrospiralis and H. sibthorpioides* showed significant cytotoxicity against the human hepatocarcinoma cell line ($HepG_2$). At maximum concentration of the plant extracts tested i.e 500 µg/ml, the cell death increased to 57.12 and 68.32 respectively. The LD50 value of the *C. retrospiralis* and *H. sibthorpioides* extracts was determined to be 366.70 µg/ml and 375.5 µg/ml subsequently.
- ✓ Presence of various bioactive constituents in these plants 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.