

SUMMARY

Present work deals with the “Studies of phytochemical and antioxidant properties of selected liverworts of Darjeeling Himalaya”. A brief introduction of the work and a review of literature are presented in the beginning of the thesis. The review starts with detailing of the status of liverworts in India and their uses in traditional medicines. Since time immemorial plants are used for the treatment of different ailments and scientific reports indicated the application of liverworts in traditional medicine. It has been proved that phytochemicals present are responsible for the biological activities showed by plant. In this context, work done for the evaluation of phytochemicals present in liverworts worldwide and in India was reviewed. Presence of many biologically active phytochemicals has been recorded and some among them are even reported to be unique to the plant kingdom. The conceptual background of free radicals and their harmful effects in human health was presented. Free radicals generated during different metabolic processes in the body at their optimized concentration have beneficial role in cell signaling and other cellular functions. However excess free radical accumulation in the body leads to severe deterioration of the human health. Antioxidants are the first line of defense to combat the harmful effect of free radicals generated during oxidative stress. When endogenous antioxidant protection system fails, intake of antioxidants from external sources is the only solution. So the source of dietary antioxidants and their mechanism of action against free radicals were schematically presented. However, high doses of dietary uptake of synthetic antioxidants have major side effects like carcinogenic effect. Plant secondary metabolites have a great potential to act as a free radical scavengers. Thus, in this study potential of liverworts to act as free radical scavengers was reviewed. Apart from the anti-oxidative activity liverworts are reported of having many other pharmacological activities. Besides the antioxidative role, the present work also aims to study their anti-diabetic potential and cytotoxic activity of liverworts against kidney cancer cell line. Therefore, literature on the anti diabetic and cytotoxic role of liverworts against specific cancer cell lines is also reviewed.

Liverworts are important component of biodiversity however; they are usually neglected due to their small size and less economic values. As a result of this many species are being threatened and some are even on the verge of extinction. So, the threats as well as the conservation technique undertaken for the protection of liverworts in India were reviewed. Apart

from conservation, liverworts are receiving limited attention in laboratory researches too. Restricted geographical distribution pattern, seasonal availability and problem of sample availability in sufficient amount for research are restricting researches on this group of plants. One of the main solutions to these problems is to propagate and multiply the plants *in vitro*. Thus, literatures on *in vitro* culture method of liverworts were reviewed. Optimum condition for *in vitro* growth, increase or decrease in pharmacological activity and phytochemical constituents of liverworts when grown under artificial growth condition were discussed under this topic. In natural habitat, different physical factors affect the species richness and abundance of liverworts. Due to small body structure and less economic importance, liverworts are usually considered as weeds and their habitats are being destroyed without even being noticed. Thus, facilitating the concepts of *ex-situ* conservation might help in the conservation and familiarization of liverworts to common people. Sustainable conservation requires good knowledge about ecological factors that affects the diversity, distribution and abundance of liverworts. Thus, literature on the study of physical and biochemical factors affecting the richness and abundance of liverworts was critically studied.

In the next chapters, results of the work performed for fulfilling the objectives was presented and the important outcome of the works has been discussed. Eleven liverworts were selected for the study in present work considering their abundance in nature and the use in traditional medicines. Free radical scavenging potential was screened through DPPH[•], ABTS⁺, nitric oxide, superoxide, metal chelation and ferric reducing method. Promising free radical scavenging activities were obtained from liverworts *Plagiochasma cordatum*, *Asterella wallichiana*, *Lunularia cruciata*, *Marchantia paleacea* and *Marchantia nepalensis* among the studied liverworts. Type 2 diabetes mellitus is caused by the reduction in insulin sensitivity which results to a condition called hyperglycemia. Postprandial hyperglycemia can be lowered by inhibiting the activity of enzyme α -amylase and α -glucosidase. *Marchantia subintegra* showed highest α -amylase and α -glucosidase inhibitory activity which was almost similar to that of standard drug Metformin. Oxidative stress also increased under sustained hyperglycemia. Therefore antioxidant therapies that targets diabetes induced oxidative stress can be useful to prevent downstream complications of diabetes. Cytotoxic activity of liverworts was assessed by measuring their toxicity to human kidney cancer cell line (ACHN). Cytotoxicity value ranging between ≤ 20 and 1000 $\mu\text{g/ml}$ (IC_{50}) are considered active and extracts having cytotoxicity value

more than 1000 µg/ml are considered inactive. *Plagiochasma cordatum*, *Asterella wallichiana*, *Lunularia cruciata*, *Marchantia paleacea* and *Marchantia nepalensis* were found to have active in controlling the proliferation of human kidney cancer cell line cells.

As the phytochemicals present in plants are responsible for their pharmacological properties, qualitative and quantitative estimation of phytochemicals was done. Significant amount of polyphenolic components were recorded in studied liverworts. Apart from polyphenolic group, presence of other important group of secondary metabolites like terpenes was recorded. Data analysis by software programs such as Pearson Correlation test and Principal Component Analysis (PCA), showed a positive correlation of phyto-compounds with antioxidant and anti-diabetic activities.

Life strategies of bryophytes are system of co-evolved adaptive qualities. They are successful colonizers and can survive extreme environmental conditions. Survival of extreme environmental condition leads to different physical and chemical adaptations in the bryophytes. One of the important strategies adapted by plants is the generation of strong antioxidant defense system. Thus, the changes in antioxidant defense system under favorable (rainy season) and unfavorable growth conditions (season with scanty water in the environment) was studied. *Marchantia paleacea* was selected as the model plant for this work. Result of the study suggested that free radical scavenging activity increases in unfavorable growth condition. The polyphenol synthesis was also found to increase during unfavorable condition of growth and it was assumed that increased polyphenolic compounds might have contributed to enhanced free radical scavenging activity.

One of the main objectives of the present work was *in-vitro* bioassay guided isolation of bioactive phytochemicals. Considering medicinal value, abundance in nature and promising free radical scavenging, anti-diabetic and cytotoxic activity, *Marchantia paleacea* was selected for *in-vitro* bioassay guided purification of bioactive compounds having promising anti-diabetic activity. Purification of phyto-compounds by column chromatography identified nineteen phyto-compounds having potential antioxidant and anti-diabetic activity. Network pharmacology is an important approach in drug development and discovery process. After the identification of bioactive compound through GC-MS analysis and NIST Library matching, the underlying mechanism of action of these compounds at cellular level was studied by network pharmacology followed by KEGG Metabolic Pathway analysis. This approach finally lead to the

identification of phytochemicals namely, 5-hydroxy-2-(4-methoxyphenyl), 4-H-1 Benzopyran-4-one; Pimaric acid; 5 hydroxy-7-methoxy-2-phenyl, 4H-1-Benzoyran-4-one; Palustric acid and 4',5,7-Trihydroxy isoflavone which directly targets the main human proteins that are involved in the insulin signaling pathway. The protein target of these phytochemicals was PPARG, GSK3B, PTPN1 and ADORA1. By acting either as the agonist or the antagonist of these protein targets identified phytochemicals was found to increase the insulin sensitivity in insulin resistant pathway. Flavononolic compounds, 5-hydroxy-2-(4-methoxyphenyl), 4-H-1 Benzopyran-4-one and 5 hydroxy-7-methoxy-2-phenyl, 4H-1-Benzoyran-4-one present in *Marchantia paleacea* were found by network analysis to increase insulin sensitivity by acting as the agonist of protein PPARG in the insulin signalling pathway. Similarly flavonoid 4',5,7-Trihydroxy isoflavone along with 5 hydroxy-7-methoxy-2-phenyl, 4H-1-Benzoyran-4-one were predicted to prevent insulin resistant by acting as an antagonist to the GSK3B protein. Network analysis showed terpenes like Palustric acid and Pimaric acid present in the bioactive fraction of *Marchantia paleacea* by acting as agonist as to PTPN1 protein can reduce the insulin sensitivity. Phytochemical-protein-disease network showed that 5-hydroxy-2-(4-methoxyphenyl), 4-H-1 Benzopyran-4-one and 5 hydroxy-7-methoxy-2-phenyl, 4H-1-Benzoyran-4-one also targets protein ADORA1 which is responsible for causing metabolic disorder.

Number of research works going on to study about pharmacological activity phytochemical constituents of bryophytes are considerably low. Main reason that constrains the research work on this group of plants is the problem of sample availability in sufficient amount to carry on the sophisticated analytical work. *In-vitro* culture technique was tried to establish for liverwort *Lunularia cruciata* in this work as a contribution to resolve the problem of sample unavailability. On the basis of bio-prospective value and low availability in nature *in vitro* culture was established with *Lunularia cruciata*. *In vitro* propagation was initiated from gemmae. Continuous illumination of 4000-5000 lux at $21^{\circ}\pm 2^{\circ}\text{C}$ initiated germination of gemmae after 8-12 days in a half strength Murashige and Skoog media. Profound effect of growth regulators benylaminopurine (BAP) and naphthelen acetic acid (NAA) was noticed on the growth and multiplication of thalli. Young thalli then grew continuously to develop dichotomously branched thalli after 12 weeks. Hormone combination (BAP 2 mg/L and IAA 0.5 mg/L) initiated differentiation of thallus and rhizoids from the gemmae. Further the phytochemicals composition and biological activities of naturally grown and *in vitro* grown

liverwort was compared to determine if there occurs any change in biological activity or phytochemical composition when *in vitro* condition was used for the propagation. Considering the bio prospective value and lower availability in natural condition, in the present work *in vitro* culture was initiated from liverwort *Lunularia cruciata*. No significant changes in the bioactivity were seen. Phytochemicals constituents of two differently grown plants also did not varied much.

Changing atmospheric scenario is posing serious threat to world's biodiversity. Area of study of the present work is one of the famous tourist places. Thus increasing tourism as well as urbanization in this area are causing serious anthropogenic threats to bryophytes as they are uncommon to people and are usually considered as weeds. So an attempt was made to demonstrate the effects of different physical and chemical factors on species richness and abundance of epiphytic liverworts and ground liverworts. Moisture content of the substrate was found to be best correlated with liverwort abundance in positive manner. Light intensity also influenced the abundance immensely. Liverwort abundance was found to reduce dramatically under abrupt exposure to light. Age of the tree on which the epiphytic liverworts grow also influences the density of the liverworts. With increase in the age of the tree increase in the density of liverworts was recorded. Biochemical nature of the tree bark such as phenol, flavonoid, tannin, steroid content, pH did not exert much effect on the liverwort abundance in the present work. However terpenoid content of the trees in case of epiphytic liverworts found to exert a negative effect of their abundance. In case of liverworts growing on the ground, moisture content of the substrate and light intensity were the most important factors influencing the abundance. Other factors like pH, carbon, nitrogen, phosphorus, potassium, sulphur content and carbon nitrogen ratio of the soil didn't influence the abundance of liverworts. By discussing the important outcomes of objectives of the present work, the result and discussion chapters were concluded. At the end thesis writing was ended with short conclusion of the framed work.