

ABSTRACT

For long time bryophytes have been neglected as a source of biologically active substances but the situation has changed now as many new biologically active compounds have been found in this group. Bryophytes can serve as the source of new therapeutically active phytochemicals. Till date, only a negligible number of species has been studied in detail. Therefore, present work aims to explore the phytochemical and pharmacological properties of some selected liverworts of Darjeeling. Accumulation of free radicals generates oxidative stress that plays major part in development of chronic and degenerative illness. Several reports document the use of antioxidants in reducing the level of oxidative stress. The antioxidant activity in present work was assessed by DPPH; ABTS⁺, superoxide, nitric oxide scavenging, metal chelating and reducing power assay. All the studied liverworts showed a promising antioxidant activity.

Diabetes mellitus is a chronic syndrome characterized by increase in the hepatic glucose production (hyperglycemia). α -amylase and α -glucosidase are the main enzymes involved in the digestion of carbohydrates. Thus by inhibiting the activity of these enzymes postprandial hyperglycemia can be controlled. In present study, highest α -amylase and α -glucosidase inhibitory activity was shown by *Marchantia subintegra*.

Anti-cancer activity was screened by studying the anti-proliferative activity against Human renal cancer cell line (ACHN). Anti-proliferative activity of studied liverworts against ACHN ranged between 69.15 and 308.98 $\mu\text{g/ml}$ (IC₅₀). Among studied liverworts *Plagiochasma cordatum* showed highest cytotoxic effect against ACHN.

The phytochemicals present in the liverworts were screened by quantitative, qualitative phytochemical analysis and Thin Layer Chromatography (TLC). In *Marchantia emarginata* highest amount of phenol was recorded and the highest content of flavonoids was recorded in *Plagiochasma cordatum*. Estimation of ortho-dihydric phenol content using Arnov's reagent showed highest ortho-dihydric phenol content in *Dumortiera hirsuta* while, tannin content was highest in *Asterella wallichiana*. Qualitative phytochemical analysis confirmed the presence of steroid, tannin, triterpenoid, amino acid, resin, cardiac glycoside, alkaloid, flavonoid, reducing sugar, anthraglycoside and glycoside in the studied liverworts.

The correlation between the phytochemicals and pharmacological activities was studied by Pearson Correlation Coefficient (PCC) and Principal Component Analysis (PCA).

Result illustrated positive correlation between the pharmacological activity and phytochemical content. Phenolic content of liverworts was directly correlated with their DPPH[·], superoxide scavenging activity and reducing power. Similarly orthodihydric phenol content of liverworts was found to be positively correlated with their α -amylase inhibitory activity.

Bryophytes are successful colonizers whose life strategies are system of co-evolved adaptive qualities. An unfavourable growth condition leads to a stage when there occurs a little intracellular water content and reduced metabolic activity resulting in irrevocable damage to lipids, protein and nucleic acids by the production of Reactive species. To cope up with this condition an important strategies adapted by bryophytes is the production of strong antioxidant defence system. Study was done to evaluate the changes in antioxidant potential and phytochemical content of liverworts during unfavourable growth condition. The result showed significant increase in antioxidant activity during an unfavourable period of growth *i.e.* dry season (October –April). The phenol and flavonoid content also increased significantly during dry season. However, orthodihydric phenol content of studied liverwort was higher during rainy season. This result can be helpful in finding appropriate growth conditions for harvesting compounds with pharmacological value.

Purification and characterization of the phytochemicals with anti-diabetic potential from liverworts was done. *Marchantia paleacea* was selected for the purpose for its high bio prospective value and abundance in Darjeeling. Bioassay guided purification of *M. paleacea* yielded two bioactive fractions, FF1 and FF3. GC-MS analysis confirmed the presence of 19 phyto-compounds in these fractions. Network pharmacology is an important approach for drug discovery and development process. Through Network analysis, five phytochemicals was selected from 19 isolated compounds that specifically targeted important proteins associated with type 2 diabetes mellitus. This result highlighted the potential of *M. paleacea* to act as a source of phytochemicals having therapeutic potential against diabetes.

Chemical analysis on liverworts is restricted mainly due to their inadequate availability in nature, seasonal dependency and habitat specificity. These impediments, however, can be successfully addressed by *in-vitro* propagation of desired species. An attempt was made to initiate *in-vitro* culture of *Lunularia cruciata*. Culture was initiated from gemmae by giving continuous illumination of 4000-5000 lux at $21^{\circ} \pm 2^{\circ}\text{C}$. Half strength Murashige and Skoog media was most suitable medium for the propagation. Benzyl

aminopurine and naphthalene acetic acid (2: 0.5 mg/L) was the successful hormonal combinations. After proper *in vitro* growth of *L. cruciata*, a study was done to find out the difference in the pharmacological activities and phytochemical content of *in-vitro* and naturally grown *L. cruciata*. Antioxidant potential was similar in *in-vitro* and naturally grown plants. Alpha-glycosidase inhibitory activity was high in *in-vitro* grown plants while alpha-amylase inhibitory activity was better in naturally grown plants. GC-MS analysis showed the presence of similar phytochemicals in both *in-vitro* and naturally grown plants which validated the use tissue cultured plants as a substitute for naturally grown plants to overcome the shortcomings restricting the use of liverworts for analytical purposes.

Nowadays increasing anthropogenic activities is posing serious threats to natural habitat of liverworts. Thus, study of factors that limits the growth of liverworts is highly essential from the conservational point of view. Survey related with ecology of epiphytic liverworts was done on fifty *Cryptomeria japonica* trees in Senchal Forest, Darjeeling. Factors like moisture, light intensity, age and the diameter of tree at breast height showed profuse influence on the abundance of epiphytic liverworts while biochemical properties of bark like phenol, orthodihydric phenol, flavonoid, tannin, sugar content and pH didn't show any remarkable effects; however the enhanced terpenoid content of the tree bark restricted the abundance of epiphytic liverworts. Similarly, study of the sites of growth of soil liverwort illustrated moisture content of the soil and light intensity to be the most important factors influencing the growth of liverworts. This work highlights the importance of old trees, canopy cover, moisture etc for maintenance of microclimate suitable for liverwort growth and suggests the responsible government authority to look at the activities resulting in habitat destruction of liverworts.