ABSTRACT

Background: Wild edible herbs are important natural commodity for rural areas either to be used for household consumption, for income generation through trade or in medicine formulations. Edible herbs have been used as a source of medicine and a nutritious food by various tribes of Northeast India for millennia. In the face of concern over crisis for more effective treatment of ailments scientists are urging for more investigation into bioactive components of traditionally used plants. In the well-developed realms of medicinal plant research, information gathered from indigenous knowledge holders have immensely contributed to identifying valuable plants and their associated bioactive compounds. The specific expertise of ethnomedicine knowledge holders has at all times been the initial spur towards further research on medicinal plants and will remain as the foundation for the progress in ethno medicine research.

The general aim of the research was to study and document the indigenous knowledge of traditional healers on using wild edible medicinal plants that occur in Goalpara district of Assam, India and to scientifically investigate the bioactive compounds and their role in three selected plant species from the area. This new knowledge achieved through scientific investigation will develop an understanding of the use of these natural resources and their sustainable management.

Methodology: The study began with the survey on ethnomedicinal plants used by traditional healers of Goalpara district of Assam, India. Three most cited and popularly used ethnomedicinal plant species of this region were selected for further scientific investigations. The three plant species were taxonomically identified by experts from Gauhati University and Bodoland University. For more precise authentication, molecular identification of the three plants was achieved through DNA Barcoding technique. The proximate composition of crude extracts of leaf samples were examined by standard food analysis methods of the Association of Official Analytical Chemists' (AOAC). Individual elements were screened and quantified using Inductively Coupled Plasma - Optical Emission Spectrometry

(ICP-OES) mineral analyzer. For gaining more perspective into its nutritional standpoint, amino acids in the samples were identified and quantified using Ultra Peformance Liquid Chromatography (UPLC). Fatty acid composition was determined by Gas Chromatography Mass Spectrometry (GCMS). Antinutritional components as alkaloid, oxalate and tannin were also investigated by standard techniques as they exert a negative impact on nutrient utilization. Phyto-chemical constituents and bioactive compounds in the leaf extracts were detected by qualitative phytochemical screening and GCMS. For the above analysis four crude extracts of leaves for each sample were prepared by extraction with water, methanol, hexane and chloroform. For the identification of functional groups in compounds of sample, Fourier transform infrared spectroscopy (FT-IR) analysis was performed. Methanol extracts were chosen for additional anticancer research in two human cancer cell lines, HeLa cells and MCF-7 cell lines using the MTT assay based on the outcomes of the phytochemical investigations. Acridine orange (AO)/Ethidium bromide (Et-Br) staining and flow cytometry was used to investigate the induction of apoptotic morphological abnormalities.

Results and Discussion: Through a survey study on ethnomedicinal plants of Goalpara district 121 numbers of plants with ethnomedicinal importance were identified. This thesis reports on three chosen ethnomedicinal plants from the areas along with their molecular taxonomy, pharmacological and nutritional studies. In DNA Barcoding studies, *Zanthoxylum oxyphyllum* could be identified upto genus level using rbcL gene. *Rotheca serrata* could be identified upto genus level using ITS1 gene and using ITS2 gene *Blumea lanceolaria* was identified upto species level. Good universality was demonstrated by the ITS and rbcL sections indicating the effectiveness of these loci as DNA barcodes.

Based on a comparison of the nutritional contents detected in our samples and the recommended daily allowances (RDA), it was found that the plants under investigation may serve as an excellent source of micronutrients, proteins and carbohydrates as supplements. The result of mineral analysis testified that individual mineral concentrations in the three samples were within the permissible levels for daily intake of minerals set by the Food and Nutrition Board, Institute of

Medicine, US, thereby signifying that these ethno medicinal plants positively can substantially contribute the required minerals in the diets of consumers. The three plant samples tested negative for heavy metals through heavy metal test. Antinutrients were detected in the samples and quantitatively they were found to range within the permissible limit for intake by humans and animals. Findings of this study have also clearly made known the presence of most of the essential and non essential amino acids which is indicative of the fact that the studied plants can possibly contribute towards mitigating protein deficiency. Study on phytochemicals showed that they were higher in methanolic and aqueous extract in comparison to chloroform and n-hexane-extracts. Comparing among the three samples it was revealed that crude extracts of *B. lanceolaria* is a potential source of naturally occurring bioactive compounds for use in traditional medicines. The various functional groups were identified using the mid-infrared (4000-400 $cm^{-}1$) portion of the FTIR spectrum. The obtained results showed a wide variety of bioactive compounds from various functional groups, including alkenes, alkanes and aromatics. The choice of solvent used for extraction process has a significant impact on the identification and successful extraction of physiologically active bioactive compounds from plant material. The current research showed that polar solvent viz., methanol and aqueous solvent extracts produced higher yields of flavonoids and phenols and therefore has better antioxidant potential. Methanolic plant extracts cytotoxically affected both cancer cells in a concentration and time dependent manner. Cells treated with effective concentrations showed distinct morphological alterations associated with apoptosis. The results showed that plant extracts might induce apoptotic cells as evidenced by the orange-red fluorescence that increased in extract-treated cells in a dose-dependent manner. The ability of all plant extracts to induce cell death in MCF-7 cells at an early stage of apoptosis and in HeLa cells at a late stage of apoptosis in all plant species was demonstrated by the overall alterations in the cell population.

Conclusion: This study significantly contributes to global initiative of investigating new compounds with pharmaceutical properties from ethnomedicinal plants of Goalpara district, Assam. The research develops a database of Goalpara's,

ethnomedicinal plants and their use for treatment of various ailments. According to this research, the ethnomedicinal plants are a free food resource which also contains a range of bioactive compounds that have a wide range of scope for applications in pharmaceutical, cosmetic and nutraceutical products as well as for health benefits. This research gives scientific evidence for the traditional knowledge applications of using *Z. oxyphyllum*, *R. serrata* and *B. lanceolaria* leaves in treating a range of ailments.

Keywords: Anticancer, Bioactive, Medicinal, Northeast India, Nutritional, Pharmacological, Taxonomy, Traditional healers