ABSTRACT

Plants have always played an integral part of life due to the fact that it contains various secondary metabolites as a defense mechanism against various stress and diseases. These secondary metabolites can also be utilized by humans against numerous therapeutic purposes. Researchers have been showing keen interest in plant originated novel drugs due to its safety, efficacy, and cost-effectiveness. The search for naturally occurring medicines could be explored primarily by documenting the traditional knowledge of herbal practitioners who have been learning and practicing this therapy over the centuries. The medium of passing this knowledge is still merely confined to verbal interaction from one generation to the next. Due to the lack of interest in younger generation in this field and the presence of modern healthcare system in the society, this knowledge is depleting gradually. A comprehensive documentation of this knowledge has become an utmost priority for the conservation of ethnic knowledge from a socio-cultural aspect as well as for its utilization in the development of modern medicine.

Sikkim is one of the smallest and less populated states of India with more than 80% of its geographical area covered by forest with rich flora and fauna with a large storehouse of medicinal plants. The indigenous people of Sikkim have developed their traditional knowledge of ethnomedicine over the years due to necessity in the early days when there was a lack of modern medicine. This study has focused on documentation of traditional knowledge of herbal medicine from the herbal practitioners who have the experience of several years. The area selected for this study was West district of Sikkim because of the fact that the area was less explored in terms of ethnomedicinal survey as compared to the rest of the state. After the survey conducted in 9 different villages of the west district of Sikkim, the few important observations were the confinement of traditional knowledge amongst elderly people (above the age of 60 years old) and no younger generations were found to be carrying forward the legacy. The literacy rate was low with only 64.29% while the rest of the healers being completely illiterate. The educational qualification is important to understand the importance of merits and demerits of using the plants for treatment of diseases such as conservation of the endangered species along with adopting appropriate safety measures during therapeutic procedures. Data was collected from 14 herbal practitioners in total and 36 medicinal plants were collected

which were used for the preparation of 46 herbal formulations. On the basis of effectiveness and availability, 11 formulations for selected for further study.

The demand and supply of large amount of medicinal plants in the market as well as in traditional therapeutic system could lead to adulteration specially when they are in dry or powder form. A standard has to be established for any powder drugs or herbal formulations to check the quality control and authentication of the same. We have carried out some simple and basic yet effective standardization techniques for these 11selected formulations through pharmacognostic study. Organoleptic tests, powder microscopy, physicochemical analysis (ash values, extractive values, pH etc.), fluorescence analysis, thin layer chromatography (TLC) were performed and a standard for each of the formulations were established.

The therapeutic effect of plants is due to the presence of various active phytoconstituents present in them. Hence, preliminary study for phytochemical estimation was carried out through qualitative methods to detect the phytoconstituents such as amino acids, steroids, flavonoids, and resins. It was done on the 10 different solvent extracts of each of the 11 different herbal formulations. The extracts were prepared through successive extraction method in 10 different solvents from low to high polarity. It was basically done to find out the solvent which is most suitable for the extraction of a particular formulation.Results showed the presence of these active phytoconstituentsin almost all the formulations varying in the amount depending on the various solvent extracts. On the basis of this result, 9 formulations were selected for further study.

The fact that overwhelming generation of free radicals or reactive oxygen species (ROS) in the body from various cellular processes lead to oxidative stress in cells and may promote several diseases such as cardiovascular diseases, diabetes, arteriosclerosis, cancer etc. Antioxidants act as defense mechanism against oxidative stress. Several synthetic antioxidants are there but they do come with harmful side effects. Plants are source of safe and natural antioxidants thus in this study, we have evaluated the antioxidant activity of the herbal formulations we have selected. The results were outstanding in some solvent extracts of all the formulations. However the extract that stood out was, aqueous extract of *Fraxinus floribunda* with highest phenol (712.130±0.26mg GAE/g EW), flavonoids (56.330±0.22 mg QE/g EW),

tannin (823.450±0.65 mg TAE/g EW) and steroid content(1.29±0.05 mg SE/g EW). The same extract also showed lowest IC_{50} in radical scavenging assays such as DPPH, superoxide, nitric oxide and in metal chelation as well as ferric reducing antioxidant potential.

Antimicrobial activity of these formulations (aqueous and alcoholic extracts) against two Gram negative and three Gram positive bacteria were evaluated comparing it with standard streptomycin. Some of the selective extracts showed potential antimicrobial activity with the best activity shown by an herbal formulation used for the treatment of food poisoning prepared from the roots of *Plumbago zeylanica*. Hence, it supports the use of this formulation in traditional medicine since food poisoning could also be caused by a bacterial contamination.

Cancer is another alarming threat globally in the recent time and there is a huge demand of anticancer lead molecules especially from a natural source. We attempted to access a preliminary anticancer activity of these formulations in some selective solvent extracts which had already showed better antioxidant activity. On the basis of human liver cell line (WRL-68) and MTT assay, the cytotoxic activity of the formulation was not very impressive but amongst all, the formulation used for the treatment of arthritis prepared from three different plants (*Viscum articulatum*, *Rheum acuminatum* and *Astilbe rivularis*) showed better activity than the rest with lowest IC_{50} value (173.44±9.82 μg/ml).

Diabetes is one more common metabolic disorder causing significant morbidity and mortality due to microvascular and macrovascular complications. The discovery of natural antidiabetic agent would be a boon to the world considering the present scenario of its growth. From the result of the *in vitro* antidiabetic assay based on inhibition of α-glucosidase enzyme, it could be concluded that the formulation was having remarkable antidiabetic activity. However, the aqueous extract of *F. floribunda* bark showed the highest activity with lowest IC_{50} value (0.011±0.002 mg/ml).

Hypertension is another concerning medical condition in the recent times leading to increasing risk of cardiovascular diseases with high mortality and morbidity worldwide. In this study we have evaluated the anti-hypertensive activity of the formulations on the basis on the inhibition of angiotensin converting enzyme.

Out of all, only two formulations *i.e. F. floribunda* bark and a formulation used for the treatment of hypertension prepared from *Berberis asiatica* (abbreviated as BP) in traditional system could inhibit the enzyme with highest activity in the latter with lowest IC_{50} value (55.25±5.59 mg/ml) thereby supporting the use of the formulation in traditional therapeutic system.

Based on the result of *in vitro* assays, *F. floribunda* was selected for the *in vivo* assays. In addition, *F. floribunda* bark was extracted through four different methods such as normal boiling, pressure boiling, Soxhlet extraction and through cold percolation to obtain the extract with highest bioactivity. Based on the above assays to measure antioxidant activity, the extract obtained through pressure boiling was the best extract with highest bioactivity. It was selected to evaluate *in vivo* anti-inflammatory, hepatoprotective and antidiabetic activity. Anti-inflammatory activity was observed in the extract with significant ($p < 0.05$) reduction of paw oedema formation (0.833 ± 0.01 ml) compared with standard, aspirin at 100mg/kg body weight of the extract. For hepatoprotective activity, the liver injury indicators (SGOT, SGPT, ALP, bilirubin levels) were significantly reduced after treatment with the extract showing a potential hepatoprotective activity. For antidiabetic activity, streptozotocin induced diabetic rats were taken and of bodyweight, glucose levels, lipid profile (TGL, TCL, LDL and HDL) and liver histopathology were studied. Based on these parameters, the *F. floribunda* extract was considered as a potential antidiabetic agent in favour of supporting its use in traditional system.

The compound responsible for the above bioactivity was isolated with partial purification through column chromatography and TLC followed by GC-MS and NMR analysis. The GC-MS analysis revealed the presence of 10 bioactive compounds some of which were flavones (antibacterial, antidiabetic, antioxidant), Coumarin,6-amino-3-phenyl (anti-inflammatory, antibacterial), Acacatechin (anticancer, antibacterial), some fatty acids and 2(1H)-Quinolinone hydrazone (antidiabetic, antimicrobial, anti-inflammatory). The compound with high abundance was 2(1H)-Quinolinone hydrazone also known as 2-hydrazinoquinoline with 25.7% occupancy. Hence the extract was again analysed through H_1 NMR analysis to obtain a specific compound responsible for bioactivity. The extract was separated through TLC and the bands observed in the TLC plate were subjected to antidiabetic assays. The bands with highest activity extracted and used for NMR

analysis along with the NMR study of the standard, 2-hydrazinoquinoline to compare their structures. From the spectra generated through H_1 NMR analysis, the peaks from both the extract and the standard were compared and studied which lead the conclusion that the extract possessing high antidiabetic activity contained 2-hydrazinoquinoline in it. Thus, it is possible that antidiabetic activity of *F.floribunda* extract could possibly be due to the presence of 2-hydrazinoquinoline in it.

From the overall study it was observed that traditional knowledge of herbal medicine requires a high priority for documentation and conservation. Some of the formulations were very impressive in terms of the pharmacological activities and these should be studied further in details with more attributes and sophisticated purification techniques for better understanding and to find out the lead compound responsible for their bioactivity.