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## Summary

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Darjeeling Himalayan region possesses close similarities among multilingual and multiethnic populations from Nepal to Sikkim and Bhutan. Different ethnic communities, *viz.* Nepalese (Gorkha, in general), Bhutia, Lepcha, Limboo, Manger etc. form the main inhabitants of this belt. The knowledge of traditional medicinal plants, used by the natives is entirely surviving on folklores, and recording of such knowledge is the only means of restoration of this valuable ethnic information (Das and Mandal, 2003). This can be achieved by surveys and interviews with the local tribals along with specimen collection and their preservation followed by proper identification. The plants, used as ethnomedicine in this region, have a great potential, and can be exploited to scientific propagation for serving a greater spectrum of human lives. The medicinal plants, used for long by various tribal folks, are known to produce several important therapeutic compounds. Hence, the study was taken up with an attempt of a moderate survey of the plants of Darjeeling Himalayan region imparting a special focus on their folklore use against different diseases by the local people, belonging to the Nepalese community mainly. Another objective was to rationalize the medicinal attributes in terms of the antioxidant property of the plants, since antioxidants are considered beneficial in combating various reactive oxygen species (ROS), which induce oxidative damage to biomolecules that lead to several degenerative diseases, *viz.* artherosclerosis, heart diseases, cancer, malaria etc. In addition, the plants were also tested for their efficacy in preventing potential infection which is becoming an important cause

of health hazard in the developing countries. The antimicrobial activities of various plant extracts and essential oils have vast potential for raw and processed food preservation, as pharmaceuticals and as alternative medicines for natural therapies. These are regarded as GRAS (Generally Recognized As Safe) substances. For a comprehensive view of the outcome of the results are summarized below.

A total of 106 plants were surveyed to be commonly used by the local people inhabiting three hilly Subdivisions of the district of Darjeeling for treating different ailments. These were taxonomically identified and sample specimens were kept in a reference repository (National Gene Bank for Medicinal and Aromatic Plants (NGBMAP), Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India), and their traditional uses have been documented. This would be helpful in identifying the problems of erosion of the knowledge about the ethnic system of medicine and taking remedial measures.

Methanolic extracts of 38 different plant parts were screened for their *in vitro* antioxidant activity (DPPH<sup>•</sup>-scavenging, reducing power and metal-chelating power) which was correlated with their chemical composition (polyphenol and flavonoid contents) using principal component analysis. Flavonoid content, DPPH<sup>•</sup>-scavenging ability and reducing power were shown to be highly loaded on factor 1 (PC1) with loadings 0.555, 0.929 and 0.795, respectively. The statistical analysis of the results suggested the key components responsible for the antioxidant activity.

Using the most promising 14 plants, their antioxidant activity was analysed further using a wide range of well-accepted protocols (ABTS<sup>•+</sup>-scavenging, <sup>•</sup>OH-scavenging, anti-lipid peroxidation and oxygen radical absorbance capacity). *Fragaria nubicola* root (FNR) extract showed a superior capacity, in all the tests.

The best candidate, FNR was then assessed for its antioxidant properties (DPPH<sup>•</sup>-scavenging, ABTS<sup>•+</sup>-scavenging and reducing power) and for relevant biological models, *viz.* lipid, protein and DNA in a concentration dependent manner. Lipid peroxidation was induced by Fenton reagent as well as by free radical, 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH); protein (bovine serum albumin) damage from radiation, while Fenton induced DNA damage was found to be successfully controlled by FNR.

It was also screened for its chemopreventive activity against two human cancerous cell lines (A-549 and MCF-7) and found promising. It prevented proliferation of both lung and breast carcinoma cells (being more toxic to lung cancer cell line), without being toxic to the normal cells (INT-407). The medicinal property of the extract may be due to its constituent hydroxyl acids (gallic acid, dihydrocaffeic acid, caffeic acid etc.), as revealed by the HPLC analysis of its acid-hydrolyzed product.

Most of the extracts of plants (*Leucas indica*, *Tectaria cicutaria*, *Physalis minima*, *Physalis peruviana* etc.) also showed "good" to "excellent" inhibitory activity against Gram-positive bacteria, while a few were also active against Gram-negative ones. The extracts of *Rubia manjith*, *Fragaria nubicola* fruit and root, *Duchesnea indica*, *Astilbe rivularis* and *Amaranthus viridis* showed effectiveness against both Gram-negative and Gram-positive bacteria. Besides FNR, the extracts of *Heracleum nepalense* seeds and root tubers of *Stephania hernandifolia* showed good antioxidant as well as antimicrobial properties. *H. nepalense* was found to contain several furanocoumarins of which three were isolated chromatographically and identified. The furanocoumarins were found to be byak-angelicol, sphondin and furropinnarin. The identification was achieved by comparing their 1H-NMR with the available literature. All these compounds showed a reasonably good antimicrobial activity. On the contrary, *S. hernandifolia* appeared to be a good source of alkaloids, although their complete identification could not be carried out.

The antimicrobial and antioxidant activities of the purified furanocoumarins were screened individually following chemical models (DPPH<sup>•</sup>-scavenging and <sup>•</sup>OH-scavenging) and using biologically relevant models (anti-lipid peroxidation and DNA protection), in a concentration dependent manner. Among the isolated

compounds, sphondin responded poorly in all the antioxidant tests in both the models as well as in the antimicrobial assays, while byak-angelicol was the best candidate. Furopinnarin, possessed the intermediate potential.

The study, thus, provided us with information about the common ethnomedicinal resource of Darjeeling Himalayan region. Among the 38 selected plants, three were analysed for their phenolic constituents and found prospective in alleviating human sufferings.