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Since time immemorial plants have been the source of medicine for various ailments as well as for health protection and health promotion purposes. Knowledge about medicinal property of some plants generated mostly through trial and error and curiosity and the knowledge percolated through generations with refinements out of practical experience. With the advent of modern synthetic medicines, particularly since the beginning of 20\textsuperscript{th} century, there developed a tendency to neglect plant based traditional medicines as outdated and irrelevant. However, soon it was realised to be wrong as it became apparent that modern synthetic medicines also have their limitations like side effects, long term biosafety problems etc.

1.1 Importance and dependance on medicinal plants

Against this backdrop herbal medicines and products are in great demand in the present fast developing world. These herbal products and medicines seem to be more trusted products as it is believed to be safer in contrast to the synthetic medicines present in the market whose biosafety and impact on environment are questionable. It is believed that over three-quarters of the world population relies mainly on plants and plant extracts for healthcare. Of the 2,50,000 higher plant species on earth, more than 80,000 species are reported to have at least some medicinal value and around 5,000 species have specific therapeutic value that are scientifically assessed (Joy \textit{et al.}, 1998). According to the World Health Organization (WHO), as many as 80\% of world’s population depends today on traditional medicine for their primary health care needs (Azaizeh \textit{et al.}, 2003). WHO has recognized the effectiveness of traditional
system of medicines and its safety (Tannan and Tannan, 2006). In China, one of the most developed countries, out of 15,000 recorded medicinal plants, 1,700 species are in common use. In India, at least 2,500 are utilized for medicinal purposes, out of 18,000 species of medicinal plants recorded in the country. There are about 46,000 licensed production units manufacturing traditional remedies of Indian systems of medicine and homeopathy (Alok, 1991; Anyinam, 1995). China leads the export of medicinal plants and related products which is evident from the fact that 20% of land area is used for cultivation of medicinal plants which is greater than the USA who is the second largest supplier (De Silva, 1997). Medicinal plant trade in India is substantial with total turnover of Rs. 2,300 crores of ayurvedic and herbal products. Despite its substantial domestic trade and its long experience with herbal medicines, India has not been able to capitalize on this by promoting its use in developed country markets (Kuipers, 1997). The Export-Import Bank of India, in its report for the year 1997, puts medicinal plants related trade in India at $3.2 billion and the same is growing rapidly. According to WHO, the international market of herbal products is around $6.2 billion, which is poised to grow to $5 trillion by the year 2050. The World Bank in its latest report on the potential of India’s forests to generate income has praised efforts of Madhya Pradesh and Assam in marketing medicinal plants. However, the report states that the country’s natural resources are not being fully exploited. India’s share in the global export of medicinal plants is just 0.52%, not withstanding it’s having 15,000 species of such plants (Kumar and Janagam, 2011). Hence, from the above statistics, it is clear that medicinal plants are valuable resource for a developing country like India as it can boost the economic status of India.
The secret of proper utilisation of these medicinal plants prevails in the rural areas, where people depend on these herbs regularly. Rural societies, various ethnic groups, particularly tribal dominated areas, far away from urban places are considered as storehouse of various plants of medicinal and other values. Different ethnic groups uses these plants in various forms and do not depend on allopathic medication as much as people of urban areas do. In fact, the practice of ethnomedicine is an important vehicle for understanding indigenous societies and their relationships with nature (Anyinam, 1995). However, with globalisation; change in life style, change in social and moral values and change in economic activities many such valuable plants are on the verge of being lost due to neglect, disuse, etc. Habitat loss due to deforestation, urbanisation is aggravating the same. Therefore, search for plants with healthcare values and their evaluation deserve priority.

1.2 Medicinal Plants and anti-stress activity

In general sense, medicinal plants mean plants or plant products which can cure a prevailing ailment. But in broad sense it also includes those plants that have health protective and health promoting effects. In accordance with the dictum “prevention is better than cure” the second category plants are equally important. Plants with adaptogenic property belong to the second category. The concept "Adaptogen" was coined in 1947 by the Russian scientist, Lazarev when he discovered the adaptogenic effect of dibasol (2-benzylbenzimidazol) with aim to stimulate non-specific powers of resistance and protection against stress and stress related diseases (Lazarev, 1958). He defined these medically-effective substances, "adaptogens," which means substances meant to put the organism into a state of non-specific
heightened resistance in order to better resist stresses and adapt to extraordinary challenges to general health.

Adaptogen or adaptogenic agents can be divided into following three groups (Pawar and Hugar, 2012):

(1) Those containing phenolic compounds or having structure similar to phenolic compounds such as phenylpropanoids, phenylethane derivatives, and lignans that have structural resemblance to catecholamines. Such compounds have an effect on the sympathoadrenal system and possibly induce an effect in the early stages of the stress response (Wagner and Norr, 1994; Wagner, 1995).

(2) Those containing tetracyclic triterpenes such as cucurbitacin R diglucoside which structurally resemble the specific corticosteroids that inactivate the stress system to protect against overreaction to stressors (Panossian et al., 1999).

(3) Oxylipins are unsaturated trihydroxy or epoxy fatty acids structurally similar to leukotrienes and lipoxines (Panossian et al., 1999).

The first group of adaptogenic extracts named compounds are reported from the roots and rhizome of *Eleutherococcus senticosus* and *Rhodiola rosea*, as well as extracts of *Schizandra chinensis* fruits. The second group of adaptogenic substances are found in extracts of *Morus alba* and *Withania somnifera*. The third group of adaptogenic compounds have been found in *Morus alba* and *Glycyrrhiza glabra*.

Anti-stress agents apparently increase the ability of the stress system to respond to stress stimuli in a manner that tends to preserve homeostasis, particularly by modulating the biosynthesis of eicosanoids including prostaglandins E₂ and F₂, 5-hydroxyeicosatetraenoic acid (5-HETE), 12-HETE, and leukotriene B₄. Moreover, adaptogens also appear to regulate the basal level of the arachidonic acid which is a
precursor of the eicosanoids responsible for synthesis of leukotriene, 5-HETE which is associated with tolerance to various stressful conditions, such as immobilization, heavy physical exercise, and radiation injury, etc. Although there is a difference in the mode of action and pharmacological activity of different adaptogens, it is difficult to relate these in a satisfactory way to the differences in adaptogens’ various effects. However, the mechanisms of action of adaptogens are mainly related to effects on the neuroendocrine-immunological system (Sprygin et al., 1988, Panossian et al., 1988, Panossian et al., 1989). It was further found that the stress protective activity of adaptogens was associated with regulation of homeostasis via several mechanisms of action, which was linked with the hypothalamic pituitary-adrenal axis and the regulation of key mediators of stress response, such as molecular chaperons (e.g., HSP70), stress-activated c-Jun N-terminal protein kinase 1 (JNK1), Forkhead box O (FOXO) transcription factor DAF-16, cortisol and nitric oxide (Panossian and Wikman, 2010).

1.3 Scenario of medicinal plants in India and North east India

India’s enormous biodiversity and thousand years old rich traditional knowledge base make excellent basic materials for research in this area. Ethnomedicines are of particular relevance in developing countries like India, where modern health service is limited (Jamir et al., 1999; Buragohain, 2008). India is one of the 12 biodiversity centres consisting of 16 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes. There is 15,000-20,000 plant species having good medicinal values. However, only 7,000-7,500 species are used for their medicinal values by traditional communities. In Indian traditional system of medicine such as Unani and Ayurveda, there is wide use of herbal drugs since ancient times. Unani
uses 700 species, Ayurveda uses about 700 species, in Siddha 600 species; in Amchi and in modern medicine around 300 species are used (Joy et al., 1998). Drugs are prepared from whole plant or different organs like leaves, stem, bark, root, flower, seeds and from excretory plant products such as gum, resin, latex, etc. In fact, now Allopathic system of medicine has also adopted a number of plant derived drugs including important chemical intermediates needed for manufacturing the modern drugs which are obtained from plants. Examples, Diosgenin from *Dioscorea* species, solasodine from *Solanum varicum*, Taxol isolated from the bark of *Taxus baccata* is used as valuable drug for ovarian cancer, while 10- deacetyl baccatine isolated from leaf of the same plant can be used as intermediates to chemically convert into taxol.

In the above context, one of the well known hotspots for medicinal plants in India is the Indian Himalayan Region (IHR) which forms the North Eastern part of the country consisting of eight states of Arunachal Pradesh, Assam, Meghalaya, Manipur, Tripura, Mizoram, Nagaland and Sikkim which can be physiographically categorized into the Eastern Himalayas, Northeast hills (Patkai-Naga Hills and Lushai Hills) and the Brahmaputra and Barak Valley. The region is known for the rich wealth of flora and fauna characterised by high level of endemism. The North Eastern (NE) IHR comprising eight states harbour more than 180 major tribal communities of the total 427 tribal communities found in India (Sajem et al., 2008). Due to geographical remoteness, difficult hilly terrains, heavy dependence on forest resources; the different tribal communities have developed and accumulated valuable knowledge about medicinal values of large numbers of plants. In 2000, the Eastern Himalaya biodiversity ‘hotspot’ (Figure 1.1) was modified to the ‘Indo Burma hotspot’ covering central Nepal to whole of North East India, Andaman and Nicobar
Islands, Hainan island in southern China, Myanmar, Thailand and on to Indochina, then south to Isthmus of Kra in the Malaysian peninsula. This hotspot is the second largest next only to the Mediterranean basin with an area of 2, 20, 60,000 sq km among the 25 hotspots identified globally (Myers, 2000). The Eastern Himalayas are variously considered as the ‘crisis ecoregions’; ‘biodiversity hotspots’; ‘endemic bird areas’; ‘mega diversity countries’; and ‘global 200 ecoregions’ (Brooks et al., 2006). Climate change, deforestation, forest fires, jhum cultivation, etc are the various reasons due to which the biodiversity of this region is in threatened state, which is also affecting the biodiversity of medicinal plants in this region.

1.4 *Rhododendron arboreum*: Potential plant for scientific scrutiny

There is no hard and fast rule to identify medicinal plants for scientific analysis. The most important is traditional knowledge which provides ideas and materials. Plants which are part of traditional knowledge system have proven medicinal values; only the science behind them needs to be explored. Curious observation is another source. For instance, plants which are not devoured by cows, goats and grazing animals are supposed to contain chemicals repulsive for them. Such compounds may be alkaloid, antimicrobial compounds, etc. Certain species of *Dioscorea, Solanum, Hyptis*, etc belong to this category. Plants which grow luxuriously under stressful conditions like high altitude, low temperature, drought condition, etc, where most other plants fail to grow can also be selected for scrutiny. Because they are likely to have some chemical compounds which enable them to withstand stress conditions. One such plant belonging to the last category is *Rhododendron arboreum*. According to latest enumeration (Mao, 2014) in North East India *Rhododendron* species are found in all
the states except Assam and Tripura. Highest diversity is found in Arunachal Pradesh with 109 species followed by Sikkim with 42 species (Figure 1.2).

Figure 1.1 Geographical outline of Eastern Himalayan biodiversity hotspot. (Source: Eklabya NC, Sharma, BS, Thapa R, Bajracharya B, Uddin K, Oli KP, Choudhury D. Biodiversity in the Eastern Himalayas: Status, Trends and Vulnerability to Climate Change Climate Change Impact and Vulnerability in

Figure 1.2 Distribution of *Rhododendron* species in various states in North East India (Numbers indicates the number of species of *Rhododendron* present in each state) (Source: Mao, A.A. (2010): The genus Rhododendron in north-east India. Botanica Orientalis. *Journal of Plant Science*, 7: 26–34)
Rhododendron arboreum belonging to the family Ericaceae, is one of the most stately and impressive Rhododendron species. It is extremely variable in stature, hardness, flower colour and leaf characteristics. The species name arboreum means tree like (Orwa et al., 2009). Originally discovered in North Central India the plant is found in the Himalayas from Kashmir to Bhutan and in the hills of North East India (Chauhan, 1999). It grows at elevations of 4,500 to 10,500 feet & grows up to 40 to 50 feet high sometimes attaining over 100 feet (Rai and Rai, 1994). This is an evergreen much branched tree up to 14 metres in height and 2.4 metres in girth (Chauhan, 1999). Flowering season is from March-April/ June-September bearing deep red or crimson to pale pink flowers. Leaves are oblong-lanceolate, 10-20cm long and 3.6cm wide. Crowded towards the ends of branches, petiole covered with white scales when young (Orwa et al., 2009). It is glossy green, with deeply impressed veins from above white fawn, cinnamon or rusty brown felt is found at the under surface (Rai and Rai, 1994). The flowers of R. arboreum range in colour from a deep scarlet, to red with white markings, pink to white. Bearing up to twenty blossoms in a single truss this Rhododendron is a spectacular sight when in full bloom. It is reported that the bright red forms of this Rhododendron are generally found at the lower elevations (Orwa et al., 2009). Flowers are showy, red in dense globose cymes (Chauhan, 1999); calyx are fine cleft, corolla are tube spotted funnel shaped, stamens are hypozygous declining, filaments are filiform, anthers are ovate, style are capitate (Paxton, 1834), capsule are curved
central column composed of fine lobes, seeds are minute, dark brown, compressed, thin linear having an obvolute membrane (Orwa et al., 2009).

In Homeopathic Materia Medica, the tincture of dried leaves of *R. arboreum* has been used in gout rheumatism (Skidel, 1980). Ayurvedic preparation "Asoka Ristha" containing *R. arboreum* possesses oxytocic, estrogenic, and prostaglandin synthetase-inhibiting activity (Midlekoop and Labadie, 1983). The dried flowers of *R. arboreum* are supposedly highly efficacious in checking diarrhoea and blood dysentery (Laloo et al., 2006). The young leaves are said to be poisonous (causes intoxication in large quantities); but medicinal values are also attributed like its application on the forehead to alleviate headache (Watt, 1892). The fresh and dried corolla that is acid-sweet in nature is used when fish bones get struck in the gullet (Pradhan and Lachungpa, 1990). In hilly areas, the flowers of *R. arboreum* with sweet & sour taste are used in the preparation of squash, jams, jellies and local brew. It is a very common and pleasant drink; drunk once daily as refreshing appetizer and also to prevent high altitude sickness. Fresh petals are used to prepare chutney known as “barah ki chutney”. The juice of the leaves is spread over cots and beds to get rid of bed lice. Wood of the plant is used to make charcoal and fuel. The grained wood of *R. arboreum* is used for making ‘khukri’ handles, packsaddles, gift-boxes, gunstocks and posts (Paul et al., 2005). Flowers and leaves are fitted in long ropes made of munja grass and tied around the houses including temples as decorations (Chauhan, 1999).

*Rhododendron* species are best known in floricultural industry worldwide for their beautiful flowers and foliage. The species also have a great role in ecological stability of ecosystems, as indicators of forest health and for their phenological
sensitivity to climatic changes. There are about 111 *Rhododendron* species on the Indian subcontinent (Mao *et al.*, 2001), with a majority of them in the state of Arunachal Pradesh in the Eastern Himalayas. In a recent report, team of botanical experts carried out explorations in West Siang district’s remote Mechukha valley, few km away from the China border, and found the rare and endemic flower which is blue in colour. In Meghalaya, the prime location of *Rhododendron* is in upper Shillong and on the way to Cherrapunji. In Manipur and Nagaland, the *Rhododendrons* are found from higher subtropical to temperate regions within the states. The major areas from where the *Rhododendrons* are recorded are from Japfu hill ranges of both Manipur and Nagaland. In Manipur, *Rhododendrons* were recorded from subtropical hills of Ukhrul and Senapati districts (1,000-1,500 msl); temperate hills of Siroi, Koubru peak and Mt. Esii (1,600 – 2,500 msl). Similarly in Nagaland, they are found in subtropical hills of Zunheboto and Wakha districts; temperate forests of Mt. Saramati, Mt. Japfu, Jakhama, Khonoma, Puliebadze and Dzulakei hills (Mao and Gogoi, 2012).

Rhododendrons form dominating species all along the cool temperate, subalpine and alpine zones in the Sikkim Himalaya. In Sikkim, *Rhododendron* have distribution from 1,500 metres elevation up to 4,000 metres and most species are found between 3,000-3,500 metres. The 38 species of Rhododendrons in the Sikkim Himalayas show a barrel-shaped altitudinal distribution based on nine different altitudinal distribution ranges categorized between 1,500 and 6,000 meters (Pradhan and Lachungpa, 1990). Maximum abundance of *Rhododendron* is found in Sikkim and among these *R. niveum* is recognised as state tree and flower. One hundred and
twelve locations throughout Sikkim where *Rhododendrons* inhabit were recorded with handheld Garmin GPS (Singh *et al.*, 2009).
Figure 1.3 A: The whole plant (source: www.moef.nic.in); B: The branch (source: www.moef.nic.in); C: Bunch leaves with flowers; D: leaves E: Seeds.
Figure 1.4 Location map of Sikkim, showing general distribution of *Rhododendron* and five protected areas (Source: Singh KK, Rai LK, Gurung B. Conservation of Rhododendrons in Sikkim Himalaya: An Overview World Journal of Agricultural Sciences 5 (3): 284-296, 2009)
With population expansion, urbanisation, unabated deforestation like many other plant species, *Rhododendron* species are also endangered. In the high altitude areas of Arunachal Pradesh, Rhododendrons are indiscriminately cut for firewood by the local people, military establishments and by the people constructing border road as it is the common tree species found in those areas and burns easily. These factors are contributing to the increasing rate of disappearance or extinction of Rhododendrons from the natural habitat. The frequent forest fire during the dry season, particularly in Manipur and Nagaland, is another factor which is threatening the species survival. The taxa found in the states of Manipur and Nagaland has become rare and threatened as the species are only found in few pockets in the states. Of the 11 taxa found in the two states, 5 taxa viz. *R. elliottii*, *R. formosum* var. formosum, *R. formosum* var. inaequale, *R. macabeatum* and *R. wattii* are already red listed under different IUCN categories (Gibbs *et al.*, 2011). Based on the field studies in connection with the present study, the taxa found in the states of Manipur, Nagaland, Meghalaya and Mizoram have become rare and threatened as the species are only found in few pockets in those states. Earlier workers had recorded 46 rare and threatened taxa of *Rhododendron* (Sastry and Hajra, 1983).

1.5 Objectives of the present study

In the backdrop of the above, the objectives of the present study are enlisted below:

1) Collection and cataloguing of plant samples of different plant types occurring in different states of North East India.
2) Preparation of extract with different solvents like methanol, hydroethanol, water and their fractionation. Biochemical analysis for various phytochemicals particularly flavonoids, phenolics etc by standard biochemical methods and HPTLC.

3) *In vitro* anti-oxidant activity of the plant extracts by DPPH reductive test, superoxide anion scavenging activity etc. Analysis for nutritive value of the leave.

4) *In vivo* experimentation for adaptogenic activity with rat model. Biochemical analysis for different parameters like glucose, cholesterol, triglycerides etc and vital enzymes associated with stress tolerance in order to work out the biochemical mechanism involved.

5) Molecular characterization of the plant types by DNA profile amplified with RAPD and ISSR primers.