GENRAL INTRODUCTION

The region of Darjeeling and Sikkim Himalayas is a part of the great chain of the Himalayan Mountains which stretches from the North Western to North Eastern boundaries of the Indian Union. It lies between 26°31' and 28°N latitude and 89°E longitude and occupies a portion where different great areas like China, India, Tibet, Nepal and Bhutan are very close together. After independence and the birth of Indian Republic the hills of Darjeeling was converted to Darjeeling district of the state of West Bengal and Sikkim was merged wtih the Indian Union as the 22nd state in the year 1975.

The characters of vegetation in the region is influenced by the strong laden monsoon wind from the South. The ramifying outer spurs have a heavy rainfall and are densely clad by moist forest of tropical and sub-tropical genera. The central portions of the gorges and valleys have a lesser rainfall and tend to bear a drier type of forest. During the last few decades Darjeeling and Sikkim Himalayas have been subjected to drastic deforestation like all other parts of the country. As a result, the soil of the region has been affected tremendously to cause overinsolation, leaching of mineral and soil erosion. In general, soil of the region is acidic in nature and very poor so far as its fertility status is concerned. It is thus necessary to survey the nature of the soil in relation to distribution of various species of plants including *Diascorea sp.* which are still existing after being adopted to drastic ecological conditions. Moreover, due to rapid growth of human population in the area, the growth of different plant species is being antagonised. It is for these reasons the ecological study in the region with special emphasis on soil conditions, drainage, geographical formation, rainfall, temperature, topography, physiology, human population, vegetation and land use have been felt desirable.

The prehistoric men and women as they advanced towards civilization in the lap of time became dependent on plants. Like the wild animals living in the forests, human being too in the day of yore used plants intuitively for food, shelter and even curing their many a malady and thereby kept their health in perfect state of fitness and lived a long life unlike the human folk of the present day trouble ridden world.

Such is the situation with the region of Darjeeling and Sikkim Himalayas. Many folklore are also known to the hill tribes about the miraculous cure and are being cultured by herbalists, medicine men, Lepchas and Lamas living more or less a secluded life in the region.

Sir J.D.Hooker the world renouned explorer of Darjeeling and Sikkim Himalayas however seemed not to have laid much stress on the healing properties of the indegenous medicinal plants in his monumental work namely, "Himalayan Journal" or "Notes on a Naturalist in Bengal, Sikkim and Nepal Himalayas", although he mentioned in glorious terms about the Lepcha's wonderful knowledge of plants in the region. Before the advent of the western medicines and their supply reached the hill people - the hill tribes for centureis mainly depended upon the indigenous plants for curing their diseases.

Gradual introduction of European system of treatment of diseases and discovery of synthetic drugs and antibiotics and with the advancement of Western medical science which are now reaching gradually even into the interior of the hill ranges, the primitive system of treatment of diseases practised in older times by the medicinemen and herbalists of the different tribes in this region is fast diasppearing. Moreover, due to inherent secretive nature of these herbalists and medicine-men the knowledge and use of some of the really efficaceous vegetable drugs are dying out with the old veterans and head man of the villages who are gradually superstitious and cherish on inherent belief that if the secrets handed down to them from generation to generation about the wonderful uses of plants and given out to unauthorised person, the efficacy of the plants will be reduced. It becomes therefore extremely difficult to extract authentic information from the hill folk on the use of the medicinal plants used in the treatment of various diseases by the hill men.

The last thirty years or so, witnessed a resurgence of interest in the traditional medicines and drugs all over the world. This is because of the fact that many undesirable side effects are seen in modern synthetic drugs, the cost of which again is very high now-a-days. Besides about 10-15 years of effort is required to develop clinically acceptible drugs. Moreover, it has

been felt that there is inadequacy of the modern drugs for the so called "Refractory diseases" like hepatities, amoebic dysentry, diabetes and degenerative disorders like rheumatoid pain and as immunomodulators. As a matter of fact plants are almost exclusive source of drugs for the majority of the world population. Even today Vietnam is almost exclusively depended on traditional medicines. China like India, has amulgammated traditional drugs with the modern medicines. Plant products constitute approximately 25% of all the prescribed medicines even in the most advanced countries like U.S.A. Japanese pharmacopoeia (1986) contains 123 drug plants both crude and pure active principles of which only 29 are used in Western medicine (Pakrashi, 1995; Dev, 1995). Very recently, Mangari et al (1998) have given much importance to conserve important threatned medicinal plant like *D. deltoidea* in India, Samanta and Das (2000) also supports the same situation of the plant in Darjeeling district, West Bengal.

The pioneering and most outstanding floristic contributions have come from Hooker (1855). Others were King and Pentling (1898) and Brushl (1926) who worked on orchids of Darjeeling and SikkimHimalayas. Besides the floristic works of Gammie (1894) and Hara (1966) are remarkable. But none of them gave any importance on the use of traditional or tribal medicines inthe region. In this connection the work of Biswas (1956) and Biswas and Chopra (1940) are worth mentioning because they have described 147 common medicinal plants in Darjeeling and Sikkim Himalayas with much emphasis on medicinal properties and various uses of the plants from ethnobotanical point of view.

Ethnobotany has gained importance now-a-days because of the fact that it is both a fascinating and rewarding subject. It includes the fudamental aspects of identification and cataloging of plants and plant products that are used by the traditional societies and tribal communities. Documentation of the fast disappearing of knowledge possessed by them, require a penchant for adventurous field work along with a knack for mixing with people and winning friends among the ones that are initially shy and non-communicative and who might consider the representatives of the urbanished world as aliens.

The aspects of qualitative evaluation of the use and management of the natural plant wealth the experimental assessment of the benefits derived from the plants and utilising the traditional ecological knowledge for biodiversity, conservation and community development have brought recognition to ethnobotaby as an important and crucial area of research.

Though much information about ethnobotanical research in other places in India is available but the report on this aspect especially in connection with Darjeeling and Sikkim Himalayas is meagre. Only a small fraction of ethnomedicinally important plants has been reported from this region so far (Tamang and Yanzone, 1982; Yonzone et al 1984, 1985, Basu, 1990). Very recently ethnomedicinal information of "Tura" a locally available *Dioscoea sp.* in Kyato (Nakamura 1998) has been pointed out but the proper scientific name of the plant has not been mentioned. Besides the ethnoveternary use of *D. bulbifera*and *D. pentaphylla* in Andhra Pradesh, India has also been reported but no specific role of the plants has been mentioned (Reddy et al, 1998). But recently Min et al (1998) has reported that yam of *D. opposita* has a very good role on Myocardial lecta-adrenoceptors of hypothyroid rabbits. In Japan, Haraguchi et al (1999) isolated a phenanthrene derivative to show antifungal agent in *D. delicata* which is generally considered as natural medinice in the region. Very recently Kelmanson *et. al.* (2000) has reported that extract of tuber of *D. sylvatica* and *D. dregane* are active against *Escherichia coli* and *Pseudomonas aeruginosa* respectively similarly Huetal (1999) perinted out that the ethanol extract of the yam of *D. composita* exibited bioactivity against *Pyriculariaoryzae.*

Thus it is very essential to study bioactivity of chemical constituent available in different ethnobotanically important species of *Dioscorea* distributed in the region of Darjeeling and Sikkim Hamalayas.

Proper identification of any medicinal plant or tribal medicine is very essential in connection with its purposeful utilisation. In this respect the knowledge of pharmacognosy should be utilised for the identification of medicinal plant.

It was not until 1815 that the term pharmacognosy was introduced by C.A.Scydler, a medical student in Halle, Germany. The name is termed out of two Greek words "pharmakon", the drug and "gnosis" - knowledge. The most comprehensive idea of the scope of pharmacognosy was presented by Fluckiger who stated that it was the simultaneous application of various scientific disciplines with the object of acquiring knowledge of drugs from every point of view (Tyler et al, 1976). In a restricted sense the definition of pharmacognosy implies a particular knowledge of methods of identification and evaluation of drugs.

As different *Dioscorea sp.* having ethnomedicinal importance, are identified by the local people only on the basis of morphological character, the knowledge of pharmacognosy involving organoleptic, morphological, anatomical and chemical evaluation of drug will be of much help in better understanding of even minor variation within the species. Very little information is available in connection with the pharmacognostic aspect of some common Indian Dioscoreas (Philip et al 1980). But during study, the authors considered different characteristic features of only the yam of the plant but not taking the consideration of leaf, stem and root of the species. The morphology of pollengrains of *D. prazeri* and *D. bulbifera* has been worked out by Samanta and Das (2000).

The detection of phytosterol has a very good role in connection with the identification of plant from chemotaxonomy point of view. Upto date much inportance has not been given in isolating phytosterol in *Dioscorea sp.*. Very recently Savikuri - Fodulovic et al (1998) have studied phytosterol in callus lines of wild *D. balcamea* in Yugoslovia.

Thus during investigation on ethnobotanically important species of *Dioscorea* in the region of Darjeeling and Sikkim Himalayas much emphasis has been given to study the plants from pharmacognosy point of view for botanical and chemical identification of different species of *Dioscorea.*

Immediately after the discovery of Marker et al (1943) that cortisone and other steroid hormones could be synthesised from diosgenin after being

converted to 16-DPA (16-Dehydropregenalone acetate) screening of plants was taken up in search of cheaper raw material for sapogenins.

After an intensive study by Correl et al (1955) it was concluded that species of *Dioscoera* were ideal for the source of diosgenin. DCOMP also started cultivation of *Dioscoera* in 1977 and by 1990 some 100 acres were under the plantation of *Dioscoera.* At the onset, the indegenous species of *D. prazeri* was tried but due to its high resinous principle, thecultivation of the crop was stopped. Two new species (*D. composita* and *D. floribunda*) were introduced in the region from Central America and commercial cultivation of these plants started for the first time in Darjeeling hills since 1980. These two are among the few *Dioscoera* speciee from about 50 Indian and over 600 trans world species that have been identified to contain diosgenin in amounts which are commercially feasible to extract. The DCOMP commissioned a Diosgenin factory during 1985-86 with an annualy installed capacity of 1500-2000 Kg. production of amorphous Diosgenin. Recently Dixit *et. al.* (2000) become successful to achieve genetic improvement of *D. floribunda* through recycling of clonal selection. Average diosgenin content in these clones has been found to be the highest.

In India the estimated requirement of diosgenin is around 100 tonnes peryear. It is estimated that due to emphasis on population control programme, the demand would go upto 150 tonnes during the next few years. The present production of diosgenin produced mostly from *Dioscoera deltoidea* was obtained from the forests of Jammu and Kashmir, Himachal Pradesh, UP and is approximately 20 tonnes annually. The raw material has been collected indiscriminately for thelast few decades and with this speed of exploitation without any definite programme of cultivation and conservation, the raw material is facing the risk of being exhausted within next few years. Because of the magnitude of the dmand and the exhorbitant prevailing cost involved, screeening of *Dioscoera* sp. in the ecological condition of Darjeeling and Sikkim Himalayas should be taken up and technolgy should be worked out so that productivity of *Diosgenin* could be increased in the yams of *Dioscoera* sp.

Biotechnology has slowly evolved during the early fifties and may be said to have come of age in agriculture only in the eighties. The pioneering researches include the discovery of phytochrome and its role in switching plant advances permits splicing and anncaling DNA sequences at will. Now-a-days, the definitions are usually oriented towards processes and industry related products. Thus in 1981 European Fedaration of Biotechnology defined it as "integrated use of biochemistry, microbiology and chemical engineering in order to achieve the technological application in the capacities of microbes and cultured tissues. In 1982, organisation for the Economic Cooperation and Development, defined biotechnology, as the "application of scientific and engineering principle to the processing of materials by biological agents to provide good and services".

Dr. T.B.Kenorey Jr. Administrator, Agricultural Research Service (USDA)1986, defined it as "those biological means used to develop process and products employing organisms or their components" (Dasgupta 1991).

Diosgenin is generally present in yams of *Dioscoera* in two forms of either free or bound. Free diosgenin is produced in low quantity as compared to that obntained after acid hydrolysis. But during acid hydrolysis for 6-7 hours much of the diosgenin content has been observed to be transformed to dicene form which is considered useless in steroidal hormone industry. Thus proper methodology should be achieved to minimise diene form of diosgenin. Diosgenin in fixed form always becomes linked with the membrane of the cell. During heat stressed condition of the yam of *Dioscoera* integrity of membrane structure is always lost thereby liberating diosgenin. Thus proper technology involving high temperature treatment of freshly collected yam of *Dioscoera* may be evolved in connection with the extraction of diosgenin avoiding cumbrous acid hydrolysis.

Methodology so far used for the estimation of diosgenin from plant are gravimetric (Selvraj 1971). Though estimation of diosgenin with the help of gas liquid chromatography (Tang et al 1978; Glyzine et al 1981), densitometric method using TLC scanner (Gunawan et al 1994) are available but these costly intruments are not available in all laboratories. Besides,

during gravimetric method it is very difficult to estimate diosgenin in small amount of plant sample. Thus a suitable method with the help of easily available colorimeter for quantitative determination of diosgenin is felt necessary.

With this background different species of *Dioscoera*, collected from the ecological condition of Darjeeling and Sikkim Himalayas have been studied from ethobotanical, chemical, biotechnological and pharmacogonosy point of view so that the information derived out of the work will be of much help in connection with purposeful utilisation of these plants in the region.