ABSTRACT

The geographical position of Darjeeling and Sikkim Himalayas lies between 26°31' and 28°N latitude and 89°E longitude.It occupies a partof the great chain of the Himalayas. Due to development of dense forests and unique vegetation, the region has long been recognised as a store house of various medicinal and aromatic plants including different species of *Dioscorea*. Since the discovery that diosgenin could be utilised as precursor for the synthesis of cortisome and steroidal hormones required for the preparation of contraceptive pills, a number of commercially important species of *Dioscorea* are being cultivated for the production of diosgenin. In order to meet high demand of diosgenin, the raw material has been collected indiscriminantly during the last few decades and with the speed of exploitation without definite programme of cultivation and conservation the raw material is facing the risk of being exhausted within the next few years and for this reason there is a need to search alternative source of diosgenin. During survey in the region of Darjeeling and Sikkim Himalayas it has been observed that different species of *Dioscorea* are being used by various ethnic population for different purposes. Altogether eight different species of *Dioscorea* such *D. alata* L, *D. kamoonensis*Kunth, *D. arachidna* Prain & Burkill, *D. sikkimensis*. Prain and burkill, *D. sativum* L and *D. prazeri* Prain & Burkill which are locally named as Ghartarul, Bhyagur, Bharlang, Niltarul, Bantarul, Sutuni, Githa and Cucurtarul respectively have been taken in to consideration.

With this background it has been the objective of the present work to study some chemical and botanical aspects of these species of *Dioscorea* for their purposeful utilisation in the region and for which the results and observations have been represented in seven chapters. Chapter I deals with the review of literature related to chemical, biochemical and botanical aspects of plants with special emphasis on *Dioscorea* sp., Chapter II deals with the ecological survey in Darjeeling and Sikkim Himalayas with special interest on altitudinal distribution of different species of *Dioscorea* at different places. Informations in connection with topography, physiography, drainage, geological formation, climate, human population, land use in the region have been collected. During survey different types of forests according to altitude and their vegetation have

been noted. Soil samples of twelve places at different altitudes have been examined to know different criteria i.e. pH, organic matter, average contents of K.P., Fe., Zn., Mn and Cu. It has been observed that percentage of organic matter increases with the increase of altitude. The available concentration of potassium and phosphorous has been estimated to become low to medium, Zinc and manganese are low in amount and others are normal. Available iron has been observed to increase with the increase of organic matter. Regarding distribution of the species, *D. alata* has been observed to be distributed from 0-9000 ft, *D. kamoonensis*, 3000-above 9000 ft, *D. arachidna* and *D. esculenta*, from 0-3000 ft, *D. sikkimensis and D. sativum,* from 3000-6000 ft but *D. arachidna* and *D. prazeri* from 0-6000 ft. Chapter III deals with the pharmacognostic study, of all the species to include botanical and chemical identification of all the species and materials and methods and results of which have been represented in two sections i.e. Section A includes observations on organoleptic, macrascopic and microscopic study of different parts of the plant. The report in connection with identification work on the species so far reported includes only the macroscopic and microscopic characters of the yam only. But in this part of work organoleptic study involving different criteria like shape, colour, markings, feeling to touch, taste and odour of leaf, stem and yam of different species, the macroscopic and microspic characters of root have been taken into consideration along with those of leaf stem and yams. On the basis of various characteristic features of different plant parts, a key has been prepared for quick identification of the species so that all the species which resemble so closely maybe identified with the help of low cost instruments. As some of the species of *Dioscorea* understudy are toxic to human health, key that has been worked out will be of much help to identify edible and medicinally inportant species. During the study on quantitative microscopy lowest value of stomatal index (19) and Pallisade ratio (30) have been observed in *D. prazeri* and *D. esculenta* respectively whereas the highest value for stomatal index (30) and pallisade ratio (59) have been observed in *D. sikkimensis,* and *D. sativum* respectively. Section B, includes results in connection with chemical investigation of the yam of different species of *Dioscorea*. During investivation, isolation, purification and characterisation of phytosterol and diosgenin have been taken into consideration. Following conventional method of phyto chemical analysis, dried powder of yams of

Dioscorea sp. have been subjected to soxhlet extraction with pertoleum ether for isolation and identification of sterols.The petroleum ether extract has been subjected to column chromatography for purification of phytosterol. For isolation of diosgenin, powdered yam has been made to paste after addition of water and subjected to acid hydrolysis followed by petroleum ether extractions. Stigmasterol has been observed to be present in four species of *D. alata, D. sikkimensis, D. bulbifera and D. sativum,* lanosterol in *D. kamoonensis* and sitosterol in *D. esculenta* and *D. prazeri.* It is very intersting to note that cholesterol is present in all the species including *D. arachidna.* As regards diosgenin *D. alata* and *D. esculenta* have been observed to contain 0.07 and 0.06 percent in the yam on dry wt. basis respectively. The percentage yield of diosgenin in other species are *D. kamoonensis* (0.50%). *D.sikkimensis* (0.60%), *D. arachidna* (0.70%), *D. bulbifera* (0.75%), *D. sativum* (1.35%) and *D. prazeri* (2.30%).

The isolated products have been identified after studying comparative behaviour of isolated natural product with the authentic sample in relation to m.p., mmp, P.C., T.L.C., and IR spectrum and chemical tests.

Chapter IV deals wtih the establishment of a new and rapid colorimetric method for quantitative determination of diosgenin in the yam of different species of *Dioscorea.* Most of the authors has so far been utilised costly instruments like GLC, TLC, Scanners etc. in connection with quantitative estimation of diosgenin. Sometimes gravimetric method has been used but it is not suitable for quantitative estimation of the same on microscale from a large number of samples generally occur during investigation on the different species of *Dioscorea.* Besides the value obtained from the sample of small amount inconnection with gravimetric estimation of diosgenin, does not show much accurate result. Moreover, purification of diosgenin has not been adopted in the methodology reported earlier. Thus a colorimetric method has been standardised on the basis of a chemical test for diosgenin after purification in presence of sulphuric acid and rosorcinol. The reaction mixture consists of 1 ml of diosgenin, dissolved in glacial acetic acid mixed with 1 ml of resorcinol (10,000 ppm) and 0.2 ml of conc. H_2SO_4. The absorption maxima of the colour of the solution has been worked out to be 510 nm. Diosgenin solution having the concentration

from 100 to 10,000 ppm has been observed to obey Becr's law. The proposed method is claimed to be a new one as the application of conc. Sulphuric acid and resoreinol has not been done earlier. Moreover, it is considered as a rapid one because it takes a very small duration of time for estimation of diosgenin in large number of samples. The colour becomes stable after standing the reaction mixture for 25 minutes and becomes stable for 40-50 minutes. The proposed method is supposed to be advantageous because of the fact that diosgenin can be determined from a solution having very low concentration. Only a few milligramme of dried plant material is sufficient for estimation of diosgenin. With the help of this method free diasgenin in the yam of different species of *Diascorea* have been estimated. The yam of *D. alata* and *D. esculenta* which are being used as food by the local people do not show any presence of free diosgenin as compared to others. The free diosgenin content in others species are *D. kamoonensis* (0.20%), *D. arachidna (0.30%), D. sikkimensis (0.25%), D. bulbifera (0.06%), D. sativum (0.40%) and D. prazeri (0.60%).* After separation of free diosgenin the respective sample of yam of each species of *Dioscorea* has been subjected to acid hydrolysis treatment to estimate bound form of diasgenin in *D. alata* (0.07%), *D. kamoonensis* (0.28%), *D. arachidna* (0.35%), *D. sikkimensis* (0.35%), *D. bulbifera* (0.74%), *D. esculenta (0.06%), D. sativum* (1.00%) *and D. prazeri* (1.60%).

Chapter V deals with the isolation and characterisation of antifungal constituent in the yam of cultivated species of *D. alata*. While workings with the isolation of chemical constituent in the yam of different species of *Dioscorea*, the crude natural product isolated from water soluble part of yam of *D. alata* and kept for a long time in laboratory condition no contamination of microorganism has been observed as compared to other isolated products kept under the same condition but having with dense fungalmat. The fungus maintained in P.D.A. culture medium has been identified as Aspergillus niger.

In order to identity the chemical nature of the inhibitor present in the water extract of the yam of *D. alata*, phytochemical analysis has been performed. The pure crystals (mp 287°C) isolated from methanol chloroform mixture, has been identified as saponin due to its characteristic frothing while boiling in water. It has been identified as Dioscin on the basis of super impossible IR spectrum

having characteristic peaks λ max 3350 (Broad) 1640, 1375, 1175, 1050, 850, 820, 720 cm^{-1} of the isolated product with those of authentic sample. Besides, their chromatographic behaviour has been observed to be the same. That the isolated product is dioscin has been further confirmed after identification of the aglycone part as diosgenin on the basis of of the same m.p. IR spectrum and chromatographic behaviour as observed in the isolated product and authentic sample. The sugar part has been confirmed as Rhamnose and glucose on the basis of comparing the chromatographic behaviour of the isolated sugars with the authentic ones. Chapter VI deals with the different ethnic use of the yam of *Dioscorea*. For a long time the yam of *Dioscorea* has attracted the attention of all people as a source of diosgenin and for this reason the consideration of other use of *Dioscorea* has been neglected. During survey it has been observed that different species of *Dioscorea* are still being used by different tribal communities as ethnomedicine and other purposes. All the eight species of *Dioscorea* have been taken into consideration and intensive field work has been done among 15 different types of tribal populations available in 22 different villages in Darjeeling and Sikkim Himalayas. Out of eight different species of *Dioscorea* the yam of six species such as *D. alata, D. kamoonensis, D. sikkimensis, D. bulbifera, D. esculenta and D. sativum* have been observed to be edible but *D. arachidna and D. prazeri* are non edible. In connection with use of yam of *D. esculenta* it has been observed that though local people eat yams but the cultivation of the species is restricted only to the Lepchas. Ethno religious use by the Nepali people has been observed to be restricted mainly to *D. alata, D. sikkimensis D. bulbifera and D. esculenta* on the day of "Makar Sankranti" in the month of January in each year. Ethnospiritual use of *D. arachidna* by Limbus and Rais peole has been observed to be conducted as per recommendation of Phedangmas and Bizuwas commonly named for the doctor of Limbus and Rais people respectively. As regards ethnomedicinal use of *Dioscorea, D. sativum, D. prazeri and D. bulbifera* are considered to be important. In connection with ethno vetarnary use of different species, yam of *D. kamoonensis* is being used as a remedy against "Bhyagute" disease of cattle, the scientific name of which is "Haemorrhagia septimae" caused by *Pasteurella boviseptica*. In modern therapy Sulphamezithin (5 gm/day) is generally used. Similarly yam of *D. sativum* is being used by the tribal people as a remedy against parasitic disease of cattle

caused by *Fasiola hepatica*. It is very interesting to note that whenever the yam of different species of *Dioscorea* is used as edible material either as food or as herbal medicine, the fresh yam is generally cut into pieces and boiled with water. The water extract is generally discarded and which is observed to be traditional method of use. But at time of use of yam against any infection on the external surface of human body the yam is generally crushed into a paste to apply externally as an ointment. In order to identify the biologically active natural product available in the water extract of the yam of *Dioscorea*, phytochemical investigation has been carried out and water soluble dioscin a rhamnogluside of diosgenin has been identified. On the basis of lytic property of "Rhamnoglucose", the chacotriose in dioscin, the saponin in the water extract of *D. alata* and other *Dioscorea* is being claimed as toxic factor in the edible species but having with antimicrobial activity in non edible ethnomedicinal species of *Dioscorea*. Chapter VII deals with the investigation in connection with the effect of high temperature on the yam of *Dioscorea prazeri* to increase production of free diosgenin. Normally the yam of the plant has 0.60% of free diosgenin out of total diosgenin content of 2.2% on dry wt basis. When the small species of fresh yams have been subjected to 45°C for 50 minutes the free diosgenin has been observed to increase maximum of 1.20% on dry weight basis. It is being claimed that glycosidase enzyme may be released during deterioration of tissue during high temperature treatment and becomes active to initiate hydrolysis of saponin, the glycoside of diosgenin to release free diosgenin accumulated in the tissue.

The enzyme has been extracted by acetate buffer prepared after adding 8.3 ml of 0.2 M acetic acid and was made upto 100 ml with 0.2 M sodium acetate solution to maintain pH at 4.0. The optimum time required for maximum production of free diosgenin has been worked out. It is being claimed that there is a possibility to utilise glycosidase to release diosgenin from the saponin i.e. glycoside of diosgenin replacing acid hydrolysis during which 30% of diosgenin is generally converted to pharmaceutically unimportant diene form of diosgenin.

So there is enough scope of isolation and purification of large scale glycosidase for its utilisation in enzyme technology for the production of diosgenin in pharmaceutical industry.