PART - I

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REVIEW OF LITERATURE

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1. Importance of Diosgenin in steroid Industry : Russel Marker of Pensylvania State University discovered that the tubers of wild Mexican plant (Dioscorea mexicana), contain a steroidal sapogenin in large amount. Marker and his co-workers (1943) discovered that wild Mexican species of Dioscorea yield basic diosgenin. Marker (1943) published the process of breakdown of diosgenin to number of sex-hormones, including progesterone and testosterone. In a few years, this discovery led to increased availibility of these drugs and their prices were greatly reduced. However, the use of diosgenin was limited to the production of only sex hormones as its moleculle did not contain keto or hydroxy group in position 11 or 12 and hence could not be used to synthesize cortisone which was clinically more important. In 1949, an important discovery was made based on which, hydroxy group could be introduced in position 11 of the diosgenin molecule by micro-biological technique. This discovery led to transform diosgenin into a valuable starting material for cortisone or progesterone. Subsequently, cortisone was replaced by more advanced and more active corticosteroids like hydro-cortisone and prednisolone.

2. Importance of various plants with special emphasis on different Dioscorea sp. as the source of diosgenin : With the advancement of steroid technology there was a great demand of wild growing Dioscorea sps. in Mexico, and steroid drug was ~~started~~ produced, mainly in Mexico and in the United State in early fifties. During mid fifties, Dioscorea sp. containing diosgenin in appreciable amount became the main source for manufacture of various staroid hormones in different part of the world. There are various sources of diosgenin which can be obtained from different plant species listed in the Table 1.

Table No. 1. List of plants being used or under investigation as source of diosgenin.

Name of the plant.	Percentage of diosgenin.	Habitat & Cycle.
1. *Dioscorea deltoidea* Wall.	3 - 5	Growing wild in the lower foot hills of Sub-Himalayan region; organised cultivation partially successful in some parts of India. Cycle 3 to 5 years (Chatterjee, 1978).
2. *D.prazeri* Prain & Burk.	Upto 2.5	Naturally occuring in North Eastern Himalayan region and presently under cultivation in Darjeeling Hills. Cycle 3 years (Chatterjee *et al*. 1980).
3. *D.floribunda* Mart & Gall.	2 - 5	Central American species ; grows well in peninsular region and North-East Indian low altitude locations. Cycle 2 to 3 years (Randhawa, 1975, Husain, 1977).
4. *D. composita* Memsl.	2 - 4.5	Central American species. Can grow in low altitude of West Bengal and peninsular India. Cycle 2 to 4 years (Chatterjee & Nandi, 1977).

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Name of the plant.	Percentage of diosgenin.	Habitat & Cycle.
5. Costus speciouş (koeng.) Sm.	1.5 -3	Grows wild all over India especially in foot hills. Cycle 1 to $1\frac{1}{2}$ years (Panda & Chatterjee, 1980).
6. Trigonella foenumgraceum Linn.	0.7-1.7	High sapogenin yielding under experimental cultivation. Cycle 3 to 6 years (Bluden et al, 1975, Khanna & Jain, 1975).
7. Kallastroemia pubescence (G.Don) Dandy.	0.5-1.5	Tropical American, naturalised in many parts of India. Cycle 6 months (Chakravarty et al) 1976
8. Balanities segyntiaca (Linn.) Delile	1 - 2.5	Perinial spring plant ; found mostly in semi-arid Zones (Hardman & Sofoworn, 1972).

To date almost all the supplies of *Dioscorea* raw materials come from the wild sources. The major producing countries are Mexico, Guatemala, India and China. In India species like *D. deltoidea*, *D. floribunda*, *D.composita* and *D. prazeri* are principally used for diosgenin extraction. However, the wild resources are getting rapidly depleted. Due to ever growing demand for steroid drugs (and for diosgenin), coupled with dwindling wild sources of this precursor and continued sprout in its price, research efforts were intensified all over the world to develop new sources of diosgenin with a special attention to find out alternate precursore with better route of production and cheaper end products.

3. Cultivation of *Dioscorea* sp. in India : At a symposium held in Lucknow in the year 1952, Dr. R.N.Chakravarty of the School of Tropical Medicine, Calcutta, was the first to suggest the possibility of exploitation of diosgenin containing Indian *Dioscorea* sp. He mentioned that *D. deltoidea* and *D. prazeri* growing wild in North-Western and North-Eastern Himalayan regions respectively contained appreciable amounts of diosgenin. The results made known in 1956, generated considerable interest in some pharmaceutical firms. An Indian Company CIPLA entered the steroid field and began producing diosgenin in 1961 and Wyeth in 1962. Subsequently, CIBA-Geigy, Searly and Organon also started manufacturing sex hormones. Glaxo (India) switched over to local diosgenin and subsequently to its intermediate for producing Beta-methasone. In 1966-67 CIMAP (CSIR) entered the commercial production of diosgenin in its drug factory, Jammu, utilising the wild *D.deltoidea*. It is for the first time that in Eastern India the Directorate of Cinchona & Other Medicinal Plants, Govt. of West Bengal has started on a commercial scale, production of diosgenin and also has entered to produce down stream products of diosgenin.

India depended almost entirely on the wild D.deltoidea. tubers for its diosgenin require. The large scale collection of tubers has resulted in depletion of forest resources and in some areas complete eradication of the wild plant is well noticed. The natural regenaration of this species required more than 7 years and it has been felt necessary to bring it under cultivation. Successful cultivation of D. floribunda and D. composita in Bangalore, Jammu, Goa and other parts of the country is reported from 1973 onwards.

Cultivation of Dioscorea in India has been studied by Bammi and Randhawa. The wild sources of D. deltoidea are dwindling in India and supplies of wild material with optimum diosgenin content are becoming more and more scarce and expensive. In West Bengal D. prazeri a ~~wild~~ native species, which was under experimental cultivation at the foot-hills of the Eastern Himalayan region like in Darjeeling Terai, appeared less promising both in terms of tubers and diosgenin content. As such the emphasis has shifted to cultivation of Central American species, viz. D.composita and D. floribunda but D.floribunda is mostly prefered ~~for the~~ due to maximum return.

4. Biosynthesis, mobilization, estimation and factors affecting production of diosgenin in plant parts of Dioscorea sp. : The natural diosgenin (sapogenin) is obtained from the tuber of Dioscorea and extensively used ~~as~~ for production of several steroidal hormones including cortico-steroids, sex hormones and antifertility compounds.

The sapogenin was first discovered by Fuji and Matsukawa (1936) which remained dormant for some years, until Marker and his associates (1943) revealed the synthesis of cortisone and other drugs. Kennard and Noris (1956) developed cultural practices of D. floribunda who showed that plants on wire trellis with vines gave significantly higher yield of tuber than plants growing on poles only or without support. Bennett and Heffmann (1965) traced the bio-synthesis of sapogenine of *Dioscorea* by incorporating the radio active mevalonic acid. Martin and Delpin (1965) estimated the sapogenin production and agronomical potential of *D.speculiflora* and Baker, Martin and Wilson (1966) observed that diosgenin occured in the cotyledon-endosperm of the dormant seeds of *D.deltoidea* and *D. sylvatica* and in the tubers, roots, stems and leaves of those plants from the seedling stage onwards. Actively growing aerial tissues, such as leader shoots appeared to be the sites of formation, from which the diosgenin could be transmitted to the tubers. Martin and Deplin (1967) reported that soil and climatic factors governed the sapogenin production in *D. composita* and *D. floribunda* and suggested that the plants propagated from stem cuttings gave better results in *D. floribunda*.

Karnic (1975) reported that *D.prazeri* and *D. deltoidea* from different locations in India yielded varying percentage of sapogenin content at various stages of growth. Sapogenin concentration increased with the age of the tuber. The optimum sapogenin was found when the plants were just shedding leaves i.e. in dormant stage, which appeared to be the best for commercial exploitation.

Bammi and Randhawa (1975) reported germination of stem cutting of *D.floribunda* by usual horticultural methods. Chaturvedi (1975) demonstrated the possibilities of rapid clonal propagation of *D. floribunda* through tissue culture by achieving 100 % rooting of single leaf-cuttings.

Nandi and Chatterjee (1977) pointed out that *D.prazeri* containing 2.0 to 4.0 percent diosgenin had been growing wild in Eastern Himalayan hills and Chatterjee (1980) described the detailed cultural practices of the plant. The effect of chemicals on three species of *Dioscorea* had been analysed by Nandi and Chatterjee (1977). Shelvaraj *et al* (1977) showed a co-rrelation between diosgenin content and dry matter production.

In 1979, Chatterjee et al. pointed out distinct effects of external and and internal factors on productivity patterns of active principles in some medicinal plants including Dioscorea and such increase of productivity was found to be functions of tographical, ecological, environmental as well as of some biochemical factors. Shelvaraj and Subhas Chander (1977) reported that fresh tubers contained 4 - 6 percent more diosgenin than dry tubers.

Tal and Goldberg (1981) described the application of HPLC in quantitative analysis of diosgenin and stigmasterol from D. deltoidea.

5. Planting time and Type of planting materials, as factors affecting yam and diosgenin yield in Dioscorea sp. : Enyi, BAC (1970) found that there was a positive co-rrelation between rainfall and vine growth, vine weight, and tuber yield in D. cayanensis which required a long growing season for maximum production ; whereas in D. alata, D. rotundifolia and D. esculanta, much shorter growing season was required. According to Gooding (1970), rainfall below 100 cm during the 8 months growing season appeared the limiting factor in D. alata. Wilson and Mapother (1969-70) concluded that in D. deltoidea plants raised from seedlings and set out in May, tuber yields were maximum at 17 to 19 months. Diosgenin percentage augmented during the first Year after which there was little change.

Seale (1960-61) claimed that D. floribunda gave highest yield when harvested at 31 months after planting. In this connection Selvaraj et al. (1972) found the diosgenin content was correlated with their dry matter content where the relationship was closer with 2 years old tubers in D. floribunda and D. deltoidea. Mehta and John reported that diosgenin was translocated into the tubers after biosynthesis principally in the aerial parts. Cruzedo et al (1965) claimed in their experiments that in Dioscorea floribunda the sapogenin concentration increased more rapidly than _D. composita_ but due to more rapid tuber growth, _D. composita_ out yielded other species in total content during 3rd season field growth. Decrease in sapogenin content during spring growth and increase in summer and fall were noted. _D. floribunda_ could be harvested after 3 seasons of growth whereas 4 or 5 seasons of growth might increase average yearly yield of _D. composita_. Both species should be harvested during the winter season when sapogenin content was stable.

Shelvaraj _et al_ (1972) investigated that the distribution of diosgenin in one year old tubers of three sapogenin bearing species viz. _D. composita_, _D. floribunda_ and _D. deltoidea_. The intact tuber was separated into three different portion used generally for propagation i.e., Crown, Median and Tips. The sapogenin content was found to be more in the dorsal portion in the tuber than the ventral portion in _D. composita_ and _D. deltoidea_ on fresh weight basis. However in _D. floribunda_ the ventral portion had the maximum content. Tip portion of the tuber in _D. composita_, the median portion of the tuber in _D. floribunda_, tuber portion without any sprouted buds in _D. deltoidea_ had the maximum sapogenin content. They also reported that the diosgenin content of _D. floribunda_ tuber have closer correlation with the dry matter production of 2 years old tuber. Randhawa _et al_ (1968) claimed that _D. floribunda_, planted in April in Bangalore region, was best as it gave quicker sprouts and higher tuber yields. They also claimed that two year crop of _D. floribunda_ in the same region yielded 60 MT/ha by using medians and tip portions of tuber as planting materials (which otherwise gave lesser yield during first year).

Randhawa _et al_ (1975) also found in their experiments in IIHR (Bangalore, India) that diosgenin percentage on dry matter basis in _D. floribunda_ tubers were not affected by the agronomic treatments as it was a purely inherited character. The yield of diosgenin therefore depended on tuber yields. Diosgenin content varied from 0.3 to 6.77% on the dry weight basis in 612 samples of D. floribunda. They did not found any correlation between girth of tuber and diosgenin content but a positive correlation was obtained between available phosphorus in soil and diosgenin content in tuber, whereas the level of available potassium in the soil was negetively correlated with diosgenin content. The relationship obtained between dry matter and diosgenin ~~contents~~ in one year old tubers of D. floribunda was significantly varied but with low prediction index. The relationship improved substantially in two year old tubers. Regarding the distribution of diosgenin in tubers, they found that the 30 days after planting contained less diosgenin compared to madian and tip portion. Regarding the economics of commercial cultivation they reported a net profit of about 10,000/- to Rs. 12,500/- per hectare for one year crop and Rs. 25,000/- to Rs. 30,000/- per hectare for two years crop.

Soderholm (1968) reported in the experiments that $2\frac{1}{2}$ years crop of D.composita, gave the yield of 21 MT of dry tubers and 1049 lbs of crude sapogenins.

6. Size of planting materials as factor affecting yam and diosgenin yield in Dioscorea sp. : Randhawa et al. (1975) suggested that 40 to 60 gms pieces of D. floribunda tuber as a planting ~~materials~~ were more economical. They also suggested in their experiments in IIHR that as the Crown portion of the tuber contained less sapogenin compared to median and tip, the crown portions could be used as planting materials (having the maximum survival percentage) to get the maximum return.

Gregory (1968) claimed that D. floribunda propagated from segments of the dormant tuber rootstock resulted uneven sprouting with high loss from rot ; whereas stimulation was obtained by using rootstocks from active growth. A 31°C temperature significantly stimulated bud development in the D.floribunda selections.

Sharma et al (1985) and Saha & Chatterjee, (1985 & 1989) claimed in their experiments that D. floribunda could be selected for commercial cultivation ~~of~~ due to less cost of maintenance and cultivation as compared to D. composita. They also found that D.floribunda planted in the middle of June with the Crown and tip portion as ~~a~~ planting materials having 50 gms pieces and uniform spacing was best as it gave quicker sprout, higher relative leaf growth rate, crop growth rate and tuber yield per hectare in 2½ years crop.

7. Spacing trial in connection with productivity of yam in Dioscorea sp.

Baker (1964) found that the yield response to population change was approximately linear and the slopes of the curves for different plant populations were themselves in linear relationship to the plant population per unit. Randhawa et al, (1968) observed that a spacing of 60 cm X 60 cm and 60 cm X 30 cm was optimum for one year and two years crop of D.floribunda respectively. Saha & Chatterjee (1985) claimed that the planting density with uniform fertilizer dose in D.floribunda would give highest yield. In other planting densities, the variation of yield was not significant.

8. Use of Fertilizers in connection with the production of yam in Dioscorea sp. : Nutritional conditions effect the general growth of the plants as well as actual formation of active principles. Braun and Jorg (1964) reported that sieve tube bundles of the Dioscoreaceae showed high acid phosphatase localisation and which was argued for active transport of assimilates.

Cruzedo et al (1965) claimed that complete fertilization in D.composita increased diosgenin content. The fertilization affects had also been studied by Ferguson and Hynes in *D.esculanta* and *D.alata* (1970). Nandi & Chatterjee (1975) showed the effect of phosphorus fertilization to enhance extension growth of *Dioscorea.* The same authors (1978) observed an enhancement in diosgenin content by phosphate and its decrease by nitrogen fertilizers in *D.bulbifera* var. *pulchella*, *D.pentaphylla* and in *D. composita*. Khan *et al*. (1978) observed that application of fertilizers NP and N gave higher Yield (2.27, 2.24 % respectively) as compared to P fertilizer (2.07 %).

Robinovich (1975) reported that in pot experiments with *D. deltoidea*, 60 kg of N + 100 kg of P_2O_5 + 60 kg of K_2O/ha and 100 kg of P_2O_5 + 60 kg of K_2O/ha stimulated growth and development of root system, the later increased by 63 % compared with the control. Liming aided *D. deltoidea* 's development. Two years old seedlings showed 1.8 % - 2.2 % diosgenin content. Recently, Singh *et al*. (1981) observed significant interaction between N & P, N & K and N with P & K on diosgenin yield. The same authors also noted that the tuber yield/plant and tuber production per unit area of *D.floribunda* to be increasing significantly with increase in N & P dose.

Chatterjee *et al* (1985), found that 250 kg of N / ha yielded positive responses, while P & K had no significant responses in yield. Saha & Chatterjee (1989) reported that *D. floribunda* would have a good prospect for commercial cultivation around Darjeeling district on marginal land, having impact of Socio-economic condition of the hilly people.