SEASONAL VARIATION OF DIOSGENIN IN *DIOSCOREA DELTOIDEA* WALL

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Summary: *Diosgenin was isolated from the yam samples of* Dioscorea deltoidea *collected during May-November; and the data accumulated from the analysis at random collections from various sites carried out for three years in different localities. October collection gave 5.5-6% diosgenin as compared with other periods by summing up the results of three years (1977-80) of the two localities in the temperate forest of Kaghan and Galis Forest Division. Next higher yield was observed in the month of August.*

Introduction: Saponins occur in plants only in the form of glycosides called steroidal saponins. The analytical procedure to determine the content involves an acidic hydrolysis of saponins to yield steroidal sapogenins. However, the actual saponins content of yams is larger as compared to sapogenins. Since sapogenin is the useful form, the values are interpretted and used as a basis in the synthesis of steroid harmones. So much so that a large industry is engaged in Mexico in the isolation of sapogenins from plants, and their further conversion to other steroids (3, 4).

Dioscorea spp has been recognised alongwith some other species, a useful and economic source of diosgenin. *Dioscorea deltoidea* grows wild in hilly forests. In view of the importance of this genus, the isolation of diosgenin being the starting material for harmones and cartisones, a search was made to explore the possiblity of commercially exploiting the indigenous yams, naturally growing in different forest area i.e., Galis, Kaghan, Azad Kashmir, Swat, Dir and Chitral. Month-wise collection from Kaghan and Galis Forest Divisions was made and analysed.

Review of literature: A marked change in the steroidal sapogenins due to age of the plant was found in seedlings of *Dioscorea tokoro Makino* when the whole plant was analysed. (4, 7).

Marker et.al. found that the predominant sapogenin isolated from young mature, old and flowering plant of Agave, have successively fewer hydroxyl groups. In the case of yucca, which does not die after flowering unlika agave, they isolated complex mixture of sapogenin or only one sapogenin possessing few hydroxyl groups, from the plants at the flowering period (4). Dadiwar and

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Fayas recognised the same tendency among the sapogenins of Agave sisalana perenne. However they found the reappearance of gitogenin in the flowering stalk of this plant and considers it a result of the mechanism by which the plants dies away and then prepare to give a new life (5,8).

The concentration of sapogenin is very much lower in the dorment seeds suggested the possibility of the synthesis of diosgenin at the actively growing parts of the shoots and the translocation of this sapogenin to the rhizomes in their study on *Dioscorea deltoidea* wall. Because the climatic data recorded between April and October, 1967 at Absatre farm did not greatly deviate from average of that during the first 16 years. It is estimated that this marked Dioscorea tokoro in the field even through some parts of this change may be due to climatic factors (1, 4). Variation of different sapogenins is observed in smilagenin, hecagenin, tegogenin in wood, leaves flowers and seeds of Joshua tree. The *dominant* concentration of sapogenin in the seed of Joshua tree was observed in the month of October, and the site and altitude did not have any significant effect on the yield of sapogenin in this tree (8).

Collection of material: Rhizome of *Dioscorea deltoidea* were collected monthly from Kaghan and Galis Forest Division during the months of June to Nov. 1977 to 1980. The most important factors were to be carefully taken into consideration during the collection of the rhizomes were (i) Aspects (ii) altitude (iii) slopes (iv) time of collection and (v) age of the plant. In most cases, consideration of soil texture and moisture were also tried to have rough similarity of places from where the collection of rhizomes were carried out. The rhizomes of the plant were collected, cleaned, dried and stored in cloth sample bags. Preliminary cleanliness, dryness, of the samples are essential, if it was not done the samples before reaching the laboratory get deteriorated due to fungal attack.

Method: The sliced rhizomes after drying, were powdered with the help of mill and sieved through 80-100 mesh sieve. Samples of the rhizomes were hydrolysed with 2N hydrochloric acid, for 3 hrs. The hydrolysed product was washed with distilled water, till the washing became neutral to blue litmus paper. After that it was washed with 1% sodium carbonate solution till the washing became colourless. The same was again washed so that the washing became neutral to red litmus paper. It was then dried in an oven at 80-100°C. The dried sample were kept in a thimble and were subjected to soxhlet extraction with hexane. The extracted material of hexane was then purified (purity was ascertained through m.p. 201° — 203°C and chromatography) and weighed.

Results and Discussion: Three years results as shown in the table 1-6, were analysed statistically. The October yield is significantly higher from all other months followed by August, July and June yield (medium group), and the third group is September which gives the lowest yield. This holds good for all the

three years as indicated in the tables. In Joshua tree, the sapogenin yield during the month of October is higher, followed by September. The final lowest group are the remaining months of the years (8). It seems that in both species October, yield is higher while the difference of September and other months of the year may be due to the distribution of sapogenin in other part, i.e., in Joshua tree the yield of sapogenin in the seed is higher as compared to the remaining parts of the tree (5). But in *Dioscorea deltoidea*, a creeper, the leaves and stem containing negligibly low yield of sapogenins.

Barker et.al., suggested the possibility of the synthesis of diosgenin at the actually growing parts of the shoots and the translocation of this sapogenin in the rhizomes in their study of *Dioscorea deltoidea* wall. Hence it gives clear indication from the study of Barker. et.al., that the sapogenin may translocate from shoots to rhizome in the growing period of the year (3). In Joshua tree too, the growing season for the tree is September and the concentrations of sapogenin in the seeds are more as compared to the other parts of the tree. In the *Dioscorea deltoidea* the fruit and seed formation may have occurred in the months of August and Sept. But in this case the sapogenin might have shifted from the growing parts to the rhizome in more concentration as compared to other parts of the plant.

From the study it could not be possible to establish the effect of aspects and altitude on the yield of diosgenin. The third observations recorded to study the effect of locality, selected for the collection of the plant rhizomes, but it too on the yield of diosgenin also did not give any conclusive result. The study was undertaken, keeping in view the important role played by steroidal sapogenins in the synthesis of harmones and to provide starting materials for the establishment of industries in the country. The genus is widely distributed in Hazara, Azad Kashmir, Swat and Dir Forests. The extraction of this plant for commercial exploitation may be made during suitable period of the year, the rhizomes give higher yield of diosgenin when collected in the month of October.

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Conclusion: It is concluded that the assayed of diosgenin from *Dioscorea deltoidea* rhizomes collected from two localities and at different interval of time gave highest yield in the month of October, next higher yielding period is August.

The localities do not have any significant effect on the yield of diosgenin from *Dioscorea deltoidea* rhizomes, collected from Kaghan and Galis Forest Divisions. Similarly, we have studied the effect of aspect and altitude on the yield of diosgenin which too, do not have any effect on the yield. It is suggested that the collection of yams during the month of October may be carried out for the assay of diosgenin on the smaller level as well as on commercial level.

Table 1

Diosgenin PC from *Dioscorea deltoidea* = 1977-78 (Dungagali)
(PC)

Rep	Elev	June	July	Aug	Sept	Oct	Nov	Total
I	6000—6500	4.61	4.95	5.07	4.89	5.66	4.36	29.54
II	6500—7000	4.84	4.95	5.11	4.46	5.36	4.61	29.33
III	7000—7500	5.36	4.95	5.14	4.31	5.57	4.32	29.65
IV	8000—8500	4.85	4.95	5.10	4.46	5.65	4.46	29.47
	Mean:—	4.85	4.95	5.10	4.57	5.65	4.46	147.95

Least significant difference table

	June	July	Aug	Sept	Oct	Nov
November	0.39*	0.49*	0.64*	0.11	1.19	—
October	0.80*	0.70*	0.55*	1.80*	—	—
September	0.28	0.38*	0.53*	—	—	—
August	0.25	0.15	—	—	—	—
July	10	—	—	—	—	—
June	—	—	—	—	—	—

LSD at .05 level = 0.32

Table 2

Diosgenin PC from *Dioscorea deltoidea* wall = 1977-78 (Shogran)
(PC)

Rep	Elev	June	July	Aug	Sept	Oct	Nov	Total
I	6000—6500	5.03	5.14	4.99	4.80	5.85	4.61	30.42
II	6500—7000	4.78	5.14	4.42	5.05	5.85	5.54	29.78
III	7000—7500	4.60	5.24	4.99	4.28	6.18	4.29	29.58
IV	7500—8000	4.46	4.93	5.30	5.23	5.71	4.60	30.23
V	8000—8500	4.72	5.26	5.24	4.43	5.65	4.51	29.81
Mean:—		4.72	5.14	4.99	4.76	5.85	4.51	

Least significant difference table

	June	July	Aug	Sept	Oct	Nov
November	21	.63*	.48*	.25	1.34*	—
October	1.13*	.71*	.86*	—	—	—
September	0.04	.38*	.23	1.09*	—	—
August	0.27	.15	—	—	—	—
July	0.42*	—	—	—	—	—
June	—	—	—	—	—	—

LSD at .05 level = .37

Table 3

Diosgenin PC from *Dioscorea deltoidea* = 1978-79 (Dungagali)
(PC)

Rep	Elev	June	July	Aug	Sept	Oct	Nov	Total
I	6500--6500	4.16	3.53	3.63	4.11	4.96	3.37	23.76
II	6500—7000	4.16	3.53	4.63	4.02	4.96	3.37	24.67
III	7000—7500	3.72	3.16	4.14	4.05	4.96	4.34	23.37
IV	7500—8000	4.61	4.01	4.45	3.98	4.96	3.14	25.42
V	8000--8500	4.16	3.41	5.30	4.39	4.96	3.37	25.49
Mean:—		4.16	3.53	4.43	4.11	4.96	3.37	

Least significant difference table

	June	July	Aug	Sept	Oct	Nov
November	0.79*	0.16	1.06*	0.74*	1.59*	—
October	0.80*	0.57*	0.53*	0.85*	—	—
September	0.05	0.58*	0.32*	—	—	—
August	0.27	0.90*	—	—	—	—
July	0.63*	—	—	—	—	—
June	—	—	—	—	—	—

LSD at .05 level = 0.31

Table 4

Diosgenin PC from *Dioscorea deltoidea* = 1978-79 (Shogran)
(PC)

Rep	Elev	June	July	Aug	Sept	Oct	Nov	Total
I	6000—6500	3.80	5.93	4.44	4.23	4.97	3.36	26.73
II	6500—7000	4.05	4.16	4.00	4.22	4.79	3.25	27.37
III	7000—7500	4.51	3.66	4.35	2.65	5.70	3.20	24.07
IV	7500—8000	3.39	3.48	4.35	3.81	5.12	3.17	23.32
V	8000—8500	3.26	3.31	4.61	4.06	5.01	3.64	23.89
Mean:—		3.802	4.108	4.350	3.794	5.188	3.324	

Least significant difference table

	June	July	Aug	Sept	Oct	Nov
November	0.48	0.79*	1.03*	0.47	1.80*	—
October	1.32*	1.01*	0.77*	1.33*	—	—
September	1.01	0.32	0.56	—	—	—
August	0.55	0.24	—	—	—	—
July	0.31	—	—	—	—	—
June	0.31	—	—	—	—	—

LSD at .05 level = .76

Table 5

Diosgenin PC from *Dioscorea deltoidea* = 1979-80 (Dungagali)
(PC)

Rep	Elev	June	July	Aug	Sept	Oct	Nov	Total
I	6000—6500	4.68	4.26	4.78	3.77	5.31	3.34	26.14
II	6500—7000	5.55	4.25	4.78	3.77	5.31	3.34	27.00
III	7000—7500	4.26	4.26	4.78	3.77	5.31	3.15	25.53
IV	7500—8000	4.24	4.08	4.78	4.37	5.31	3.53	26.31
V	8000—8500	4.68	4.45	4.78	3.17	5.31	3.34	25.73
Mean:—		4.682	4.260	4.78	3.77	5.31	3.34	

Least Significant differemce table

	June	July	Aug	Sept	Oct	Nov
November	1.34*	0.92*	1.44*	0.03	1.97*	—
October	0.63*	1.05*	0.53*	1.54*	—	—
September	0.91*	0.49*	1.01*	—	—	—
August	0.10	0.52*	—	—	—	—
July	0.42*	—	—	—	—	—
June	—	—	—	—	—	—

LSD at .05 level = .40

Table 6

Diosgenin PC from *Dioscorea deltoidea* = 1979-80 (Shogran)
(PC)

Rep	Elev	June	July	Aug	Sept	Oct	Nov	Total
I	6000—6500	4.18	4.15	4.46	3.97	5.05	3.40	25.21
II	6500—7000	4.38	4.98	4.24	4.62	5.12	3.61	26.95
III	7000—7500	4.58	5.30	4.78	3.79	5.63	3.68	27.73
IV	7500—8000	4.37	3.21	4.49	4.49	5.26	3.56	25.84
V	8000—8500	4.37	4.41	4.49	4.33	5.26	3.56	26.42
Mean:—		4.368	4.410	4.492	4.332	5.264	3.562	

Least significant difference table

	June	July	Aug	Sept	Oct	Nov
November	0.81*	0.85*	0.93*	0.77*	1.70*	—
October	0.89*	0.85*	0.77*	0.93*	—	—
September	0.04	0.08	0.16	—	—	—
August	0.16	0.08	—	—	—	—
July	0.04	—	—	—	—	—
June	—	—	—	—	—	—

LSD at .05 level = .54

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