Screening of *Dioscorea* Species for Diosgenin from Southern Western Ghats of India

KI Asha[1] and GM Nair[2]
[1]*National Bureau of Plant Genetic Resources Regional Station, Vellanikkara, Thrissur-680 656 (Kerala)*
[2]*Tropical Botanical Garden and Research Institute, Palode, Thiruvananthapuram (Kerala)*

Dioscorea deltoidea Wall. is the major species exploited in India for diosgenin obtained from rhizomes. The natural stock of *D. deltoidea* being exhausted due to large-scale collection from forest areas and very poor rate of regeneration, leads to apprehensions that this important raw material may completely be lost to the industry if steps to conserve the stocks are not taken up immediately. Preliminary screening of different species locally available to identify new sources of diosgenin identified leaf samples to show presence of diosgenin in all the twelve species while tuber samples of only 4 species viz., *Dioscorea hispida, D. hamiltonii, D. spicata* and *D. pubera* revealed its presence. Of these, the maximum diosgenin yield was recorded in *D. pubera* (1220 µg/g d. wt.) followed by *D. spicata* (305 µg/g d. wt.), *D. hispida* (57 µg/g d. wt) and *D. hamiltonii* (3 µg/g d. wt.).

Key words: *Dioscorea*, Diosgenin, Yams

Diosgenin is the most important and versatile precursor of steroidal drugs obtained from rhizomes of various species of *Dioscorea*, which account for about 50% of the total steroidal drug output in the world. Diosgenin occurs naturally in a number of plant genera such as *Dioscorea* (Dioscoreaceae), *Costus* (Zingiberaceae), *Trillium, Paris* (Liliaceae), *Balanites* (Simarubiaceae) and *Trigonella* (Leguminosae). Correll *et al.* (1955) reported that species of *Dioscorea* were the ideal source of diosgenin.

Dioscorea deltoidea Wall. is the major species exploited for diosgenin in India. The raw material comes exclusively from forest areas as the plant has shown discouraging performance under domestication. The natural stock of *D. deltoidea* is gradually being exhausted due to large-scale collections from these forest areas accompanied with a very poor rate of regeneration which leads to apprehensions that this important raw material may be completely lost if steps to conserve the species are not taken up immediately.

Under the above circumstances, a preliminary screening of the different species locally available becomes necessary, to identify new sources of diosgenin for commercial use. In the present study, the leaf and tuber samples of field grown plants of 12 wild species, originally collected from the Southern Western Ghats, grown under uniform conditions were screened for diosgenin, adopting the standard methodology.

Materials and Methods

Materials selected for the study includes one accession each from 12 species of *Dioscorea* procured from NBPGR Regional Station, Thrissur and grown in the greenhouse of Department of Botany, University of Kerala, Thiruvananthapuram, Kerala (Table 1). The extraction and analysis of diosgenin in the different *Dioscorea* species was based on the procedures adopted by Li *et al.* (2002) in *Dioscorea zingiberensis*. Preliminary screening for the presence of diosgenin was carried out using leaf samples taken during the intensive vegetative growth period. Further, tuber samples of these species, grown *in vivo*, harvested in January having uniform stages of growth were also subjected to screening and quantification for diosgenin. Leaf samples were air dried and powdered. The tuber samples after through washing and blotting were sliced and oven dried at 60°C for 48 hours and then powdered. 1g each of the powdered leaf / tuber sample was extracted with 20 ml 2N HCl for 3½ hours by periodical stirring and cooled to room temperature and filtered. The filtrate thus obtained was neutralized with 2N NaOH and allowed to dry by evaporation. The pelleted out filtrate was extracted with 5 ml chloroform and stored. The residue thus obtained was washed thoroughly in water to remove the acid content completely and dried at 78°C for 4 hours in an oven and was extracted with petroleum ether (40-60°C bp) at room temperature and filtered. The filtrate was evaporated and pelleted out. The pellet thus obtained was extracted with 5ml chloroform and the two chloroform extracts were mixed thoroughly and analyzed by TLC and HPLC using the reference sample of diosgenin from Sigma Aldrich, USA.

Thin Layer Chromatography (TLC) (Li et al., 2002)

TLC plates were prepared using silica gel (G-60 Merck) as the absorbent. Slurry of silica gel with distilled water, 1:2 (w/v) ratio, was spread on 5 mm thick glass plate

*Author for correspondence, e-mail: ashakarthy@rediffmail.com

Table 1. Materials analyzed

Species	IC Number	Locality of collection
Dioscorea bulbifera L.	202349	Peechi, Thrissur, Kerala
Dioscorea pentaphylla L.	202367	Peechi, Thrissur, Kerala
Dioscorea hispida Dennst	202370	Nilambur, Malappuram, Kerala
Dioscorea tomentosa Heyne	202379	South Canara, Karnataka
Dioscorea pubera Bl.	202382	Begur, Wynad, Kerala
Dioscorea intermedia Thw.	202384	Santhanpara, Idukki, Kerala
Dioscorea spicata Roth.	202383	Thirthahally, Coorg, Karnataka
Dioscorea oppositifolia L.	202386	Valpara, Palghat, Kerala
Dioscorea wightii Hook. f.	214855	Mundanthurai, Tamil Nadu
Dioscorea belophylla Voight	248181	Devakolly, Coorg, Karnataka
Dioscorea wallichii Hk. f.	202312	Courtallum, Tirunelveli, Tamil Nadu
Dioscorea hamiltonii Hk.	202328	Vadakkancherry, Thrissur, Kerala

(20 x 20 cm) with the help of a TLC applicator, which was adjusted to a thickness of 0.5 mm. The plates were dried at room temperature and activated at 100°C for 30 minutes in a thermo regulated hot-air oven.

100 µl each of the different samples (leaf and tuber) were spotted on to the activated silica gel TLC plates along with authentic reference sample of diosgenin at 2 cm from the bottom, using calibrated micro syringes and the spots were allowed to dry. Meanwhile, the required quantity of solvent system *i.e.* Chloroform:Petroleum ether (40-60°C bp) 4:1 (v/v) was taken in the chromatographic chamber, closed tightly and kept for half an hour in order to create a saturated condition inside the chamber. The plates with the sample spots, after drying, were kept in a slanting position inside the chromatographic chamber and sealed tightly. After sufficient solvent percolation and when the solvent front reached three fourth of the total length of the glass plate, the chromatograms were taken out, solvent front was marked and allowed to dry. The air-dried plates after sufficient solvent percolation were sprayed with 20% sulphuric acid (v/v) and heated at 100°C for 25 min. resolved purple coloured spots, which corresponds to diosgenin. Presence of diosgenin in the samples was identified as pink spots corresponding to the *Rf* value of the standard diosgenin.

The retention factor was calculated as:

$$Rf = \frac{\textit{Distance travelled by the spot}}{\text{Distance travelled by the solvent system}}$$

High Performance Liquid Chromatography (HPLC)

HPLC analysis of the tuber samples was carried out in a Shimadzu LC-10AS liquid chromatography system equipped with UV detector and a Shimpac C-18 column (Shimadzu, Japan). The mobile phase used was methanol:water (95:5) with a flow rate of 1 ml / min. The samples were filtered through a nitrocellulose filter (20 micron). 20 µl each of the filtered samples were injected one at a time into the machine using a micro injector. The absorbance of diosgenin was detected at 210 nm using the UV-visible detector (Shimadzu SPD-IDA, Japan). The chromatogram was analysed using e-R7Ae Plus (Shimadzu, Japan) Chromatopac System. The quantity of diosgenin in each sample was calculated and compared with the authentic sample of diosgenin.

Identification and Quantification of Diosgenin

Diosgenin content in the crude extracts of leaf and tuber samples of different species grown in the field under identical conditions was subjected to TLC and HPLC. After preliminary screening of the leaves by TLC, the tuber samples were subjected to TLC and then HPLC for quantification.

Results and Discussion

Detection of diosgenin in the samples by TLC

Preliminary screening of leaf samples showed diosgenin in all the twelve species under investigation. Amongst these, the presence of diosgenin in tubers was revealed only by 4 species namely *D. pubera, D. spicata, D. hispida* and *D. hamiltonii* as purple coloured spots at an *Rf* value of 0.37.

Quantification of diosgenin in samples by HPLC

The quantification of diosgenin in the tuber samples of the 12 species of *Dioscorea* revealed the presence in only 4 species *viz. D. hispida, D. hamiltonii, D. spicata* and *D. pubera.* The retention times (RT) of the different species that contain diosgenin namely *D. pubera, D. spicata, D. hispida* and *D. hamiltonii* were 8.089, 8.12, 8.146 and 8.126 minutes, respectively (Table 2), which corresponded with the RT of diosgenin standard 8.094 minutes (Table 2). Of these, the maximum diosgenin yield was recorded in *D. pubera* (1220 µg/g d. wt.) followed by *D. spicata* (305 µg/g d. wt.), *D. hispida* (57 µg/g d. wt.) and *D. hamiltonii* (3 µg/g d. wt.). The other 8 species lack diosgenin in tubers as revealed by both TLC and HPLC. These species contained a number of unidentified compounds, as revealed in the HPLC profile (Table 2).

Earlier studies on the growth of tubers and diosgenin content in *Dioscorea deltoidea* (Shah *et al.*, 1998; Chauhan, 1999), *D. zingiberensis* (Li *et al.*, 2002) and *D. composita* (Zullo *et al.*, 1987) revealed contents to be directly proportional to age.

An interesting correlation between morphological characters and diosgenin content has been elucidated based on the studies of the Japanese *Dioscorea* species (Akahori, 1965). In this, analysis of 3 of the Japanese species of *Dioscorea*, which did not contain sapogenin revealed that species with alternate leaves, stems twining to the left and no bulbils contained diosgenin, whereas, the species with opposite leaves and stems twining to the right with edible roots lacks diosgenin. Contrary to these result, *D. bulbifera*, which though has stems twining to the left and globose tuber and forms bulbils, was not containing diosgenin (Hegnauer, 1963; Kaul and Staba, 1968 and Quigley, 1978).

Correlation studies on diosgenin content with flesh colour revealed lower contents for white flesh tubers in *D. floribunda* and *D. deltoidea* (Singh *et al.*, 1999) while the later for *D. deltoidea* showed higher concentrations for white flesh of tubers (Bindroo, 1988; Kant *et al.*, 1998). The present analysis involving the different species of *Dioscorea* showed higher diosgenin content for coloured fleshed tubers (*D. pubera*, orange yellow tuber) than the white ones. Hence, utilization of tuber flesh colour as an indicator for higher diosgenin content as suggested by Kant *et al.* (1998) may not be dependable in visual screening of different *Dioscorea* species.

From the present investigation it can be established that the South Indian species of *Dioscorea* contain diosgenin. The lower diosgenin content presently detected in the species may be due to the interaction of environmental factors or may be due to genetic make up of the parent clones.

Out of the 12 species studied, only 4 showed the presence of diosgenin in their tubers. In *Dioscorea* species, site of synthesis is reported to be the leaves and the metabolites are then translocated by the stem to the tubers for long-term storage. Bennett *et al.* (1963) suggested that diosgenin is biosynthesized principally in the aerial parts of *D. spiculiflora* and is then rapidly translocated to the tubers. Blunden *et al.* (1968) found that the total sapogenin yield was 6-40 times greater in leaves than the stems in *D. sylvatica*, *D. prazeri*, *D. floribunda*, *D. composita* and *D. deltoidea*. Contradictory to this, Kant *et al.* (1998) found that no diosgenin occurs either in

Table 2. HPLC profile of various compounds present in different species of *Dioscorea*

Diosgenin/Plant species	Retention time	Concentration
Diosgenin (standard)	**8.09**	**8.466**
Dioscorea bulbifera	1.37	12.014
	2.29	2.232
	3.06	7.129
	3.35	72.614
	4.41	1.833
	6.57	2.677
Dioscorea pentaphylla	1.41	13.129
	3.41	70.798
	4.48	6.116
	5.58	2.531
	6.01	1.612
	6.63	4.047
Dioscorea hispida	2.1	13.096
	3.35	65.270
	4.20	2.920
	4.40	7.138
	5.32	1.282
	5.49	1.036
	5.93	4.402
	6.58	4.703
	8.146	**0.0397**
Dioscorea tomentosa	2.22	14.722
	3.36	61.128
	4.42	16.612
	5.94	2.323
	6.57	4.373
Dioscorea pubera	3.43	80.822
	4.49	7.870
	6.71	5.542
	8.09	**1.189**
Dioscorea intermedia	0.90	15.859
	3.43	77.226
Dioscorea spicata	2.52	14.255
	3.24	6.205
	3.45	47.779
	3.83	7.379
	5.35	8.028
	8.12	**0.208**
Dioscorea oppositifolia	1.95	15.135
	3.39	65.115
	4.45	5.904
	4.62	9.642
Dioscorea wightii	2.48	12.298
	3.27	5.709
	3.47	58.564
	4.58	6.216
	4.72	12.584
Dioscorea belophylla	1.43	14.463
	3.44	53.634
	3.83	9.506
	4.50	9.669
Dioscorea wallichii	2.58	16.423
	3.35	69.657
	4.39	7.089
Dioscorea hamiltonii	2.35	12.585
	3.35	67.656
	5.31	14.286
	8.13	**0.022**

the leaves or stems of *D. deltoidea*, though yamogenin in traces has been found in the stem and leaves of the species *D. deltoidea*. But the presence of diosgenin in leaf samples of all the 12 species and its absence in the tubers of 8 species indicated that the genus *Dioscorea* is characterized by the presence of the secondary metabolite diosgenin, but the species show distinct variation in translocation of the molecules from the site of synthesis to the site of accumulation.

In short, the present investigation reveals the presence of diosgenin in the species from Southern Western Ghats, which in future can be exploited by the pharmaceutical industry to meet the ever-increasing demand of diosgenin.

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