

Short communication

Genetic variability of *chakkarakolli* (*Gymnema sylvestre* R. Br.) in Kerala assessed using morphological and biochemical markers

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Abstract

Gymnema sylvestre R. Br. is a native medicinal plant valued for its antidiabetic property. The major bioactive constituents of *Gymnema* are a group of saponins. In the present study, morphological and biochemical markers were employed for characterizing 93 germplasm accessions of *Gymnema* representing different geographical regions of Kerala. Seven vegetative traits and total saponin concentrations in the leaves were studied on three-year-old plants. The results indicate high variations in morphological and biochemical characters. Saponin concentration ranged from 0.6% for 'Pambadi' to 5.4% for 'Kottayi'.

Keywords: Antidiabetic plant, Saponins, Gymnemic acid.

Gymnema sylvestre R. Br. (Asclepiadaceae) grows in the tropical forests of central and southern India. The leaves of this plant have been used for over 2000 years to treat diabetes, giving it a prominent place in the indigenous system of medicines in this country. Fresh leaves when chewed exhibit a remarkable property of temporarily paralysing the sensory perception of sweet and bitter tastes (Warrier et al., 1995); hence, the Hindi name *gurmar* and the Malayalam name *chakkarakolli*. Administration of *Gymnema* lowers the blood glucose level in diabetic patients (Shanmugasundaram and Paneerselvam, 1981) and the alcoholic extracts of *Gymnema* have been shown to increase the release of insulin from pancreatic β -cells (Shanmugasundaram et al., 1990). The quantity of gymnemic acid, the active principle in *Gymnema* leaves is, however, variable among accessions from different ecoclimatic regions (Yokota et al., 1994). Considerable variations also exist among the morphological traits of *Gymnema* accessions from Tamil Nadu and Kerala (Thamburaj et al., 1996). However, detailed information on the extent of variability

in the *Gymnema* populations of Kerala is not available. Hence, a study was undertaken to characterize the morphological and biochemical variations among the *Gymnema* germplasm accessions from diverse ecoclimatic regions of Kerala. This is of particular significance in the current scenario where demand for plant-based medicines is increasing and over-exploitation of the wild resource is endangering its genetic diversity in the natural habitat (FRLHT, 1997). Documenting the genetic variations will provide an efficient tool for identifying useful genotypes that could be used as cultivars for extraction of standard drugs.

Ninety-three accessions of *Gymnema* collected from various geographical regions of Kerala during 2001 and maintained at Vellanikkara (Table 1) were used for the present study. The plants were grown under open conditions at 2 x 2 m spacing and the study was conducted three years after planting. The vegetative characters mentioned in the descriptor for *Gymnema sylvestre* provided by the National Bureau on Plant Genetic

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Table 1. Total saponin concentration of 93 *Gymnema* accessions maintained at Vellanikkara estimated by TLC densitometry.

Accession	Saponin (%)	Accession	Saponin (%)
'Adapurutti'	1.5	'Mathur 121'	3.0
'Chekkampuzha'	1.5	'Mathur 123'	3.8
'Dhoni'	4.9	'Mathur 124'	2.5
'Erumayoor 63'	2.0	'Mathur 127'	2.8
'Erumayoor 68'	2.3	'Mundur'	2.3
'Erumayoor 69'	5.1	'Nelliampathi'	2.5
'Erumayoor 70'	4.7	'Odakkali'	3.4
'Erumayoor 71'	4.5	'Pambadi 112'	2.0
'Erumayoor 74'	4.0	'Pambadi 113'	2.9
'Kannadi 128'	3.4	'Pambadi 114'	1.7
'Koduvayoor 75'	3.2	'Pambadi 116'	2.0
'Koduvayoor 76'	3.5	'Pambadi 117'	1.6
'Koduvayoor 78'	2.0	'Pambadi 118'	2.8
'Koduvayoor 79'	2.3	'Pambadi 119'	2.1
'Koduvayoor 82'	3.5	'Pambadi'	0.6
'Koduvayoor 83'	2.9	'Pambadumpara'	2.9
'Koduvayoor 87'	4.2	'Panniyur'	3.3
'Kollangode'	2.1	'Pazhayannur'	1.6
'Kongad'	1.5	'Peerumedu'	1.1
'Kottakkal'	1.8	'Peringottukurushi 135'	2.7
'Kottayi 145'	4.9	'Peringottukurushi 136'	3.9
'Kottayi 146'	4.4	'Peringottukurushi 137'	3.9
'Kottayi 148'	5.1	'Peringottukurushi 138'	4.4
'Kottayi 149'	5.4	'Peringottukurushi 140'	2.8
'Kozhinjampara 51'	3.3	'Peringottukurushi 141'	2.8
'Kozhinjampara 52'	2.8	'Pudussery 100'	3.4
'Kozhinjampara 53'	3.7	'Pudussery 99'	4.6
'Kozhinjampara 56'	4.3	'Thenkurushi 31'	3.2
'Kozhinjampara 59'	2.0	'Thenkurushi 32'	2.9
'Kozhinjampara 60'	2.7	'Thenkurushi 35'	4.1
'Kozhinjampara 61'	2.7	'Thenkurushi 36'	2.4
'Kuthannoor 101'	5.2	'Thenkurushi 37'	2.6
'Kuthannoor 104'	2.1	'Thenkurushi 38'	2.1
'Kuthannoor 106'	2.8	'Thenkurushi 40'	2.5
'Kuthannoor 107'	2.7	'Todupuzha'	2.9
'Kuthannoor 108'	4.8	'Valiyathovala'	1.6
'Kuthannoor 109'	3.2	'Walayar 01'	3.0
'Kuthannoor 110'	3.3	'Walayar 02'	3.5
'Kuzhalmannom 89'	2.8	'Walayar 03'	2.1
'Kuzhalmannom 90'	2.3	'Walayar 04'	2.1
'Kuzhalmannom 91'	2.4	'Walayar 05'	2.4
'Kuzhalmannom 94'	3.3	'Walayar 06'	4.1
'Kuzhalmannom 95'	2.9	'Walayar 08'	2.5
'Kuzhalmannom 96'	3.8	'Walayar 09'	2.7
'Kuzhalmannom 97'	2.2	'Walayar 10'	2.9
'Kuzhalmannom'	2.1	'Walayar'	2.2
'Mannarkkad'	3.4		

Resources, New Delhi such as habit, leaf length, leaf width, leaf shape, pubescence on leaf, leaf base shape, and leaf tip shape were used for morphological characterization. Total saponin concentrations in the leaves estimated by TLC-densitometry technique were used as the biochemical marker. Leaf samples for saponin estimation were collected during April and May 2004, which is the period of active vegetative growth. Leaves were collected from each plant such that the sample contained both tender and mature leaves. Saponin was extracted with 100% ethanol (v/v) and silica gel G was used to remove the pigments efficiently without affecting the saponins. Precoated TLC alumina sheets (Silica gel 60 F₂₅₄) were used for the assay and the samples were spotted using micropipettes. On each plate, along with the samples, two spots of reference standard, 28.77% gymnemic acid (Chemilioids, Vijayawada) were also spotted to give a concentration of 23µg and 52µg of saponins. The running solvent system developed by Golba (2000) was used with suitable modifications for eluting the saponins. An initial run in a pure solvent was included to elute the non-saponins to the solvent front. The developed chromatographic plates were removed from the developing chamber and dried with a spray of hot air from a drier to remove the solvents. Using vanillin-H₂SO₄ spray reagent, pink coloured saponin spots were obtained (Fig. 1) and densitometric quantification was used to determine the percentage of saponin present in each accession with reference to standard spots using SPOTDENSO tool of Alpha Imager. Image of TLC

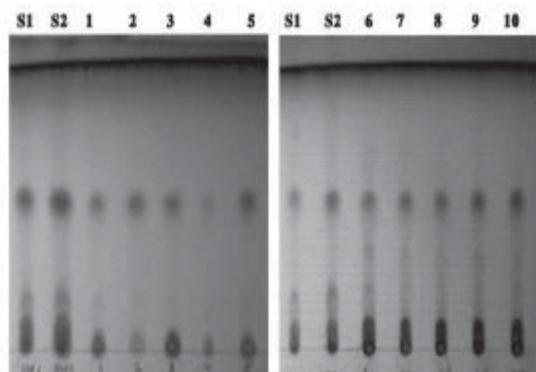


Figure 1. TLC Plates showing saponin spots: S1 and S2 are standard gymnemic acid at two concentrations and 1 to 10 are samples.

plates was documented and stored in the gel documentation system (Alpha Imager).

Results show that all the accessions studied exhibited the habit of woody climbers. Warriar et al. (1995) also described *Gymnema* as a large, much branched woody climber. All other characters, however, showed great variations. For instance, leaf shapes included elliptic-oblong, ovate, ovate-lanceolate, lanceolate, and cordate. Average leaf length ranged from 1.84 cm for 'Pambadi 116' to 7.14 cm for 'Panniyur' and the average leaf width varied from 0.82 cm for the accession 'Thenkurussi 38' to 5.78 cm for 'Valiyathovala'. Out of the 93 accessions studied, 49 were non-hairy. Others like 'Pambadumpara', 'Valiyathovala' and 'Peerumedu' from high range areas were, however, very hairy. The leaf base shape was mostly sub-cordate, but truncate, rounded, and obtuse were also seen. The leaf tip shape in *Gymnema* was either acute or acuminate. However, a few accessions showed cuspidate shape. Similar observations were made earlier by Thamburaj et al. (1996) in a study involving 12 germplasm accessions. They observed lanceolate and ovate shapes with the leaf tip being either blunt or pointed and 50% of their genotypes were highly pubescent while the others were non-hairy.

The saponin fraction obtained from *Gymnema* leaves is a complex mixture of several constituents. Its densitometric quantification revealed that the saponin concentration in the leaves of different accessions varied between 0.6% for 'Pambadi' to 5.4% for 'Kottayi 149' (Table 1). Other accessions from 'Pambadi 112', 113, 114, 116 and 119 also had relatively lower saponin concentrations (around 2%). Conversely, accessions from 'Kottayi' had higher saponin concentrations of 4.4% or more. The accessions from 'Walayar' had wide variations in saponin concentrations (2 to 4%). They were also showing high variations in their morphological characters. 'Peerumedu' and 'Valiyathovala' from the high range areas had lower saponin levels. In a study on the quantitative analysis of gymnemic acid, Yokota et al. (1994) observed that the gymnemic acid concentration in the leaves of *Gymnema* collected from India varied between 3.9 and 4.6%. The saponins being secondary metabolites are often influenced by the environmental

and seasonal factors. From the results, it is evident that there is wide variation in the morphological and biochemical characters of *Gymnema* accessions, which can be further exploited to popularise the useful genotypes for extraction of drugs.

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