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Research Article

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Variation in gymnemic acid content in *Gymnema* germplasm from different geographical regions of Gujarat

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ABSTRACT

Gymnema sylvestre an antidiabetic plant, is a large tropical liana, native to central, southern and western India and is also found growing in Africa and Australia. It possesses antimicrobial, antihypercholesterolemic, hepatoprotective and sweet suppressing activities. Gymnema leaf contains more than 20 saponin glycosides. The major saponin fraction comprises of gymnemic acid which is a complex mixture of at least nine closely related acidic glycosides. The herb's bioactive ingredient, gymnemic acid, extracted from leaves and roots, helps to lower and balance blood sugar levels. Gymnemic acid is estimated through estimating the content of gymnemagenin. In order to identify the accession with the highest gymnemic acid content, eight different Gymnema sylvestre clones viz; GS1, GS2, GS3, GS4, GS5, GS6, GS7 and GS8 were collected from medicinal garden of Indian Institute of Advanced Research, Gandhinagar; Indrora Park, Gandhinagar; Botanical garden, Gujarat University, Ahmedabad and Directorate of Medicinal and Aromatic Plant Research, Anand. Gymnemic acid was extracted from leaves and estimated through HPLC technique. The bioactive agent, gymnemic acid content (4.14 %) is found to be highest in Gymnema sylvestreGS2 clone. All the clones studied exhibited the habit of woody climbers. However, they showed variation in morphological character and bioactive agent content.

Keywords: *Gymnema sylvestre*, Gymnemic acid, bioactive agents, antidiabetic agent, Gujarat

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INTRODUCTION

Gymnema sylvestre (Retz.) R. Br. ex Sm., a well-known antidiabetic plant since past 2000 years [1], is a vulnerable, slow growing twinning shrub belongs to family Asclepiadaceae [2].It is native to tropical or sub-tropical Asia, South Africa, Oceania, China and Australia [3]. In India, it is commonly found in Western Ghats and some central states viz. Madhya Pradesh, Uttar Pradesh, Bihar and Orissa [4]. It is known in Sanskrit-*Meshashiringi, Madhunashini (Madhu*=sugar, *nashini*= destroy*)*, in Hindi-*Gurmar*. The Latin name of *Gymnema sylvestre* means "sugar destroyer" and is considered as herbal remedy for high blood sugar. It is considered to be a very effective antidiabetic plant in Indian indigenous system of medicine. Besides this, in prevailing systems of medicine, the plant is used in the treatment of dyspepsia, constipation [5],jaundice, haemorrhoids [6], renal and vesicle calculi [7], cardiopathy, asthma [8] bronchitis, amenorrhoea and leucoderma [9]. In addition to this, the plant has also shown antimicrobial[10]anti inflammatory(11) and free radical scavenging [12] activities. *Gymnema* leaf contains more than 20 saponin glycosides. The major saponin fraction comprises of gymnemic acid (the anti-sweet principle) which is a complex mixture of at least nine closely related acidic glycosides [13]. The herb's bioactive ingredient, *gymnemic acid*, extracted from leaves and roots, helps to lower and balance blood sugar levels [14].The gymnemic acid contain several acylated (tigloyl, methylbutyroyl etc.,) derivatives of deacylgymnemic acid (DAGA)which is 3-

O-b-glucuronide of gymnemagenin (3b, 16b, 21b, 22a, 23, 28-hexahydroxy-olean-12-ene). Gymnemagenin is not present in free form in plant; it is a common genin of gymnemic acids which can be produced only after acidic and basic hydrolysis. Therefore, gymnemic acid is estimated through estimating the content of gymnemagenin (aglycone of gymnemic acid). Since the gymnemic acid fraction is allegedly beneficial for treating diabetes and obesity, preparations containing *Gymnema* plants is used in several commercial formulations viz. Madhu Rakshak, Nature care Gymnema (Dabur India Ltd., New Delhi, India), Dolabi (Hamdard Laboratories, New Delhi, India), Blood sugar (Nutrasanus), Glucose Support (Vitabse, Monroe), Nutrilite (Amway Pvt. Ltd.) Dibecon, Ayurslim, Meshashringi (Himalaya Drug Co., Bangalore, India) and many others [15; 16].

The quantity of gymnemic acid, the active principle in *Gymnema* leaves is, however, variable among accessions from different ecoclimatic regions [17]. Considerable biochemical and morphological variations have been reported in *G.sylvestre* accessions from Tamil Nadu and Kerala [18].Recent researches revealed that the concentration of chemical constituents in *Gymnema* also varies in their different parts, growth stages and in different seasons [19; 20].

Hence, a study was undertaken to characterize the morphological and biochemical variations among the *Gymnema* germplasm from diverse geographical regions of Gujarat. 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.

EXPERIMENTAL SECTION

Collection of plant material

The leaves of eight accessions of *Gymnema sylvestre* germplasm were collected from different locations of Gujarat state of India. The details and geographical distribution of the collected sample materials is shown in Table 1. The leaves were collected in new polythene bags and surface sterilized with 1% mercuric chloride solution. The leaves were chopped separately into small pieces and shade dried at room temperature for seven days.

Table 1: Details of *Gymnema sylvestre* accessions used for genetic diversity analysis

Sl. No.	Sample Code	Location		Place of Collection
		Latitude	Longitude	
1.	GS1	23°13′0′′N	72°41′0′′E	Indian Institute of Advanced Research (IIAR) garden, Gandhinagar
2.	GS2	23°13′0′′N	72°41′0′′E	IIAR garden, Gandhinagar
3.	GS3	23°13′0′′N	72°41′0′′E	IIAR garden, Gandhinagar
4.	GS4	23°2′0′′N	72.35′0′′E	Botanical Garden, Gujarat University, Ahmedabad
5.	GS5	23°13′0′′N	72°41′0′′E	Indroda Nature Park, Gandhinagar
6.	GS6	23°13′0′′N	72°41′0′′E	Indroda Nature Park, Gandhinagar
7.	GS7	22°34′0′′N	72°56′0′′E	Directorate of Medicinal and Aromatic Plants Research (DMAPR), Anand
8.	GS8	22°34′0′′N	72°56′0′′E	DMAPR, Anand

Morphological characterization

Plant habit, leaf length, leaf width, leaf shape, pubescence on leaf, leaf base shape, and leaf tip shape were studied for morphological characterization.

HPLC analysis of bioactive agents

Extraction of gymnemagenein

500 mg of powdered leaves of *G. sylvestre* was weighed accurately into a round-bottom flask, 25 mL of 50% (v/v) methanol was added. To 10 ml of this solution, add 2 ml of 12 % KOH and the mixture was refluxed on a water bath for 1 h. The mixture was cooled and the whole solution was again refluxed for 1 h after adding 5.5 ml of 4 N HCl. The resulting solution was cooled, adjusted to pH 7.5—8.5 with potassium hydroxide solution, made up to a volume of 50 mL with 50% (v/v) methanol and filtered through Whatman no. 1 filter paper, and finally by 0.45µm pore size filter. The filtrate containing saponin content in the form of gymnemagenin present in the leaves was estimated by HPLC [21].

Preparation of standard stock solutions

Standard gymnemagenin was obtained from M/s Natural Remedies, Bangalore, India. All chemicals and reagents used were of HPLC grade. Standard stock solutions of gymnemagenin were prepared separately by dissolving 10 mg

of gymnemagenin in 10 mL of methanol to get concentration of 1000 µg/mL (Stock solution). The solution was stored in refrigerator and found to be stable for one month.

HPLC system conditions

The HPLC system (Shimadzu) consists of LC-20AD solvent delivery pumps plus intelligent pump along with manual injector (20 µL loop capacity per injection) and SPD-M 20A photodiode array detector. Lab Solution Shimadzu software was used for analysis. The chromatographic analysis was carried out on Achromolith C_{18} reverse phase HPLC column, 4.6 × 250 mm ID at a 210 nm wavelength. The separation was achieved with a binary gradient program (Table 2) for pump B–Acetonitrile and pump A –Water. The mobile phase composition was methanol: water, pH 2.8, adjusted with orthophosphoric acid (92:08, v/v) with 1.0 mLmin-1 flow rate. Analysis was performed at a flow rate of 1.0 ml/min throughout the gradient run and the data acquisition was performed at a wavelength of 210 nm. The HPLC was calibrated till the base line was established. The area under respective peak was recorded and it was used to calculate the percent content of gymnemagenin in the *G. sylvestre* sample.

Table.2. Binary gradient program

Time (min.)	B. Conc (Acetonitrile)	A. Conc (Water)
0.01	25	75
20.00	55	45
25.00	60	40
30.00	60	40
35.00	25	75
40.00	25	75
40.01	Stop	

RESULTS AND DISCUSSION

All the clones studied exhibited the habit of woody climbers. At leaf morphological level, among the populations collected, variation was recorded (Table 3) in terms of leaf shape, leaf apex shape, leaf base shape and leaf pubescence. For instance, leaf shapes included ovate, oblong, and narrow. Average leaf length ranged from 3.40 cm for GS7 to 6.58 cm for GS2 and the average leaf width ranged from 1.80 cm for GS3 to 3.48 cm for GS2. The leaf base shape was mostly rounded. The leaf tip shape in the collected accessions varied from acute to acuminate. Leaf positions in *Gymnema* clones were either opposite superimposed or opposite decussate. Both hairy and non hairy clones were there.

Table 3. Morphological characterization of the *Gymnema sylvestre* clones from various locations of Gujarat

S.no.	*Gymnema sylvestre* genotype	Collected Site	Leaf length (cm)	Leaf Width (cm)	Leaf Shape	Pubescence on Leaf	Leaf base shape	Leaf tip shape	Leaf position on branch
1.	GS1	IIAR garden, Gandhinagar	6.10	3.30	Oblong, Narrow	Non Pubescent	Round	Acute to Acuminate	Opposite, Decussate
2.	GS2	IIAR garden, Gandhinagar	6.58	3.48	Ovate	Non Pubescent	Round	Acute	Opposite, Decussate
3.	GS3	Medicinal garden IIAR, Gandhinagar	4.77	1.80	Oblong narrow	Non Pubescent	Heart shaped	Acute	Opposite, Decussate
4.	GS4	Botanical Garden, Gujarat University, Ahmedabad	4.73	2.74	Oblong	Pubescent	Heart Shaped	Acute to Acuminate	Opposite, Superimposed
5.	GS5	Indroda Nature Park, Gandhinagar	3.50	2.08	Ovate	Non Pubescent, leathery	Round	Acute	Opposite, Decussate
6.	GS6	Indroda Nature Park, Gandhinagar	4.50	2.58	Oblong narrow	Pubescent, velvety	Heart Shaped	Acute	Opposite, Superimposed
7.	GS7	Directorate of Medicinal and Aromatic Plants Research (DMAPR), Anand	3.40	2.01	Oblong narrow	Non Pubescent	Round	Acute	Opposite, Decussate
8.	GS8	DMAPR, Anand	4.60	2.10	Oblong narrow	Pubescent	Round	Acute	Opposite, Superimposed

HPLC analysis of the saponins extract also showed variation in the gymnemagenin content in the different *Gymnema* clones. The saponins being secondary metabolites are often influenced by the ecogeographical and genotypic conditions. The gymnemagenin was found to be highest in GS2 (4.14 %) lowest in GS7 (0.25 %).

Gymnemic acid is calculated by estimating the content of gymnemagenin (aglycone of gymnemic acid). Gymnemagenin is not present in free form in plant, it is a common genin of gymnemic acids which can be produced only after acidic and basic hydrolysis**.** The gymnemic acid content is found to be highest in GS2 (4.14 %).

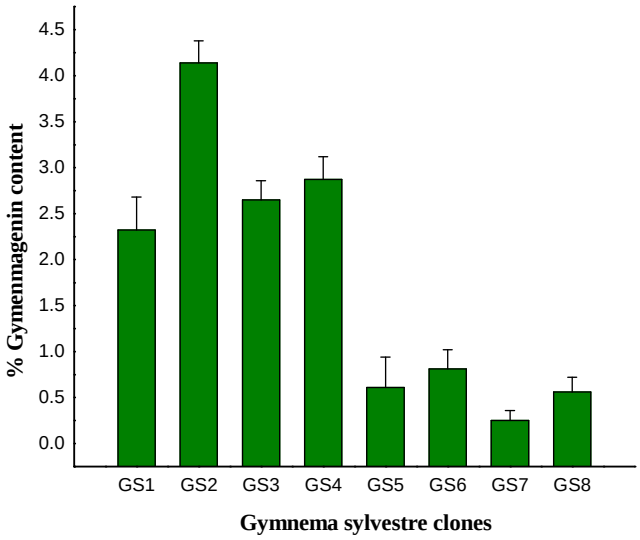

Fig.1. Amount of Gymnemagenin in *Gymnema sylvestre* clones estimated by HPLC

CONCLUSION

From this study, it is evident that there is wide variation in the morphological and biochemical (saponin concentrations) characters of *Gymnema sylvestre* clones from different geographical regions of Gujarat locations. The dynamics of gymnemagenin content is possibly associated with the expression of different genes at different developmental stages of the plant or to the environmental factors arising from seasonal variations. Further the existence of considerable variations at the molecular level in these genotypes could be established by performing RAPD analysis. Documenting the genetic variations will provide an efficient tool for identifying useful genotypes that could be used as cultivars for extraction of standard drugs.

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