

## Chemical variability in gymnemagenin, deacyl gymnemic acid, lupeol and stigmasterol of *Gymnema sylvestre* R. Br. populations in India

Madan M Pandey<sup>a</sup>, Subha Rastogi<sup>a,\*,#</sup>, Kanchana Vaishnav<sup>b</sup>, Baleshwar Meena<sup>b</sup>, Devendra Singh<sup>c</sup> & Tikam S Rana<sup>b</sup>

<sup>a</sup>Pharmacognosy, Phytochemistry & Product Development Division,

<sup>b</sup>Plant Diversity, Systematics & Herbarium Division,

<sup>c</sup>Plant Conservation and Agrotechnology Division, CSIR-National Botanical Research Institute, Lucknow

\*E-mail: srastogi@niscpr.res.in

Received 28 January 2021; revised 30 March 2022; accepted 13 May 2022

Variability in chemical composition of *Gymnema sylvestre* R.Br. (Apocynaceae) sampled from different biogeographical regions of India was investigated. The impact of geographic conditions on gymnemagenin, deacylgymnemic acid, lupeol and stigmasterol content variability was studied by using chromatographic analysis. HPLC analyses showed gymnemagenin and deacylgymnemic acid content to vary between 10.19-23.38  $\mu\text{g}/\text{mg}$  and 4.70-35.42  $\mu\text{g}/\text{mg}$  (w/w dry weight plant material) respectively, while lupeol and stigmasterol were found to range from 0.57 to 10.8  $\mu\text{g}/\text{mg}$  and 0.25 to 15.6  $\mu\text{g}/\text{mg}$  (w/w in methanolic extract) respectively, by HPTLC. Taking into account the importance of geographical origin and climatic conditions that significantly affect the production and accumulation of secondary metabolites, the selected accessions/germplasms of *G. sylvestre* with high concentration of biologically potential phytoconstituents could be useful to industries for harvesting optimum levels of bioactive antidiabetic agent gymnemic acids and other sterols. These elite accessions/germplasms could be promoted and used for mass propagation and cultivation to ensure sustained supply of quality raw material for herbal drug industries, companies and entrepreneurs involved in the profession of medicinal plants and as secondary source of income generation to farmers.

**Keywords:** Deacylgymnemic acid, Geographical variation, *Gymnema sylvestre*, Gymnemagenin, Lupeol, Stigmasterol

**IPC Code:** Int Cl.<sup>23</sup>: A61K 36/00, A61K 36/185

*Gymnema sylvestre* R.Br. (family Apocynaceae), a reputed herb in the Ayurvedic system of medicine, is commonly known as “Gurmar” or sugar destroyer for its distinct anti-sweet property. It is a slow growing perennial woody climber native to tropical and subtropical forests and is common in humid and hilly regions of evergreen forests of southern and central India, Sri Lanka, Tropical Africa and Australia. Leaves are opposite, usually elliptic or ovate (1.25-2.0 inch $\times$ 0.5-1.25 inch), inflorescence lateral umbel in cymes; calyx lobes are long, ovate, obtuse and pubescent; corolla pale yellow, valvate, campanulate with single corona with 5 fleshy scales; follicles terete and lanceolate, up to 3 inches; carpels 2, unilocular ovules may be present, anther connective produced into a membranous tip<sup>1-3</sup>. The propagation is through

seeds sown in the months of November-December and harvested from September to February<sup>4</sup>.

*Gymnema sylvestre* exhibits a broad range of therapeutic properties and has been extensively used as an effective natural remedy for diabetes for over 2000 years<sup>5</sup>. Leaves of *G. sylvestre* possess several therapeutic properties such as antidiabetic, hypolipidemic, stomachic, diuretic, refrigerant, astringent and tonic<sup>6-8</sup>. In addition, it also possesses antimicrobial, anti-osteoporosis, hepatoprotective and anti-saccharine activities<sup>9</sup>. Various *in vitro* studies suggested that the purified gymnemic acids possess potent antihyperglycemic, normoglycemic and antihyperlipidemic activities<sup>10</sup>. Its antidiabetic and antihypertensive effects have also been clinically tested<sup>11</sup>. It exhibited antihyperglycemic effect by regeneration of pancreatic cells, stimulating insulin release and inhibiting glucose absorption in blood<sup>12</sup>. Randomized controlled clinical studies for evaluating the safety and efficacy of a polyherbal Unani

\*Corresponding author

<sup>#</sup>Present address: CSIR-National Institute of Science Communication and Policy Research (NIScPR), New Delhi 110 012

formulation containing *G. sylvestre* have also been carried out<sup>13</sup>. Trials have also shown that consuming *G. sylvestre* reduces the desire for high-sugar sweet foods<sup>14</sup>. Since the gymnemic acid rich fractions of *G. sylvestre* are very helpful for treating type II diabetes as well as obesity, they are used in various therapeutic herbal products and its powder as well as extract is being used in several commercial formulations. A number of preparations based on *G. sylvestre* are available in the form of tablets and food supplements in USA (Blood Sugar by Nutrasanus and Glucose Support by Vitabase) and India (Dibecon, Ayurslim, Meshashringi by Himalaya Drug Co.; Madhu Rakshak, Nature Care Gymnema by Dabur India Ltd.; Nutrilite Madhunashini Shunti & Twak by Amway Pvt. Ltd.; Pancreofly by Butterfly Ayurveda; Dolabi by Hamdard Laboratories; BGR-34 by AIMIL Pharmaceuticals<sup>15</sup>).

The major bioactive constituents responsible for the antidiabetic or antihyperglycemic activity of *G. sylvestre* are a group of triterpenoid glycosides of oleanane and dammarene classes known as gymnemic acids. Gymnemic acids are acylated (tigloyl, methylbutyryl) derivatives of deacylgymnemic acid (DAGA) which is a 3-O- $\beta$ -glucouronide of gymnemagenin. Gymnemagenin is an aglycone of gymnemic acids which is produced after acidic and basic hydrolysis, while deacylgymnemic acid (DAGA) can be produced after only basic hydrolysis of the gymnemic acids. Another antidiabetic compound from *G. sylvestre* is a 35 amino-acid peptide-gurmarin. Besides, flavones, hentriacontane, anthraquinones, phytin, resins, pentatriacontane, formic acid, tartaric acid, butyric acid, lupeol, stigmasterol,  $\beta$ -amyirin related glycosides and calcium oxalates have also been reported from *G. sylvestre*<sup>16</sup>.

It is well established that plants of the same species growing in different phytogeographic regions or under different environmental conditions show significant differences in production and accumulation of the primary and secondary metabolites<sup>17-20</sup>. Till date, *G. sylvestre* has not been investigated systematically and only stray information on estimation of gymnemic acids<sup>21,22</sup> and morphological, biochemical, genetic and anatomical variability is available<sup>15,23-26</sup>.

Considering *G. sylvestre* as a potential antidiabetic agent useful in production of various herbal formulations, this species is proven adequate object of the present work. Due to the rising utilisation of *G. sylvestre* as a potential hypoglycaemic agent, it has turned out to be much progressively critical to fulfil raw material demand for the herbal drug industries<sup>27</sup>. No

efforts have so far been made to explore a large number of accessions representing major diversity areas in India in order to identify elite germplasm(s) having high concentration of potential bioactive compounds. Considering the diverse agro-climatic conditions and regional topographic variability in the country, present study was undertaken to estimate the gymnemic acids among *G. sylvestre* samples collected from different geographic regions of India, and to identify elite germplasm of this potential antidiabetic plant resource for mass production of raw materials to cater the fast growing need of herbal drug industries. Since direct estimation of gymnemic acids is very difficult, they being a complex mixture of several closely related compounds that are not available commercially as reference compounds, therefore they were quantified with reference to gymnemagenin or DAGA obtained on hydrolysis. Besides this, the contents of two non-polar compounds, lupeol, an anti-inflammatory and anti-cancer triterpenoid<sup>28</sup> and stigmasterol, a potential antiosteoarthritic and antihypercholesterolemic phytosterol<sup>29,30</sup>, were also determined during the present investigation.

## Material and Methods

### Standards and reagents

Gymnemagenin, Deacylgymnemic acid (DAGA), Lupeol and Stigmasterol were procured from Sigma-Aldrich (USA). HPLC-grade acetonitrile, methanol, water, phosphoric acid and all the other chemicals and reagents were bought from Merck (Darmstadt, Germany).

### Plant material

A total of 53 accessions of *G. sylvestre* were collected in their natural habitats from different locations of India in the month of August-November during 2018-2019. The leaves were collected in sterilized dry polythene bags. The samples were separately chopped, shade dried below 40°C and properly stored at 25°C away from sunlight in order to avoid material contamination or degradation. Each shade dried sample was ground and pulverized separately to a powder using a grinder having a size of 0.2 to 0.5 mm and by sieving using mesh No. 40. The geographic distribution and collection details of all samples are provided in Figure 1 and Table 1, respectively.

### Physicochemical evaluation

All the samples (dried and powdered leaves) of *G. sylvestre* were evaluated for their physico-chemical

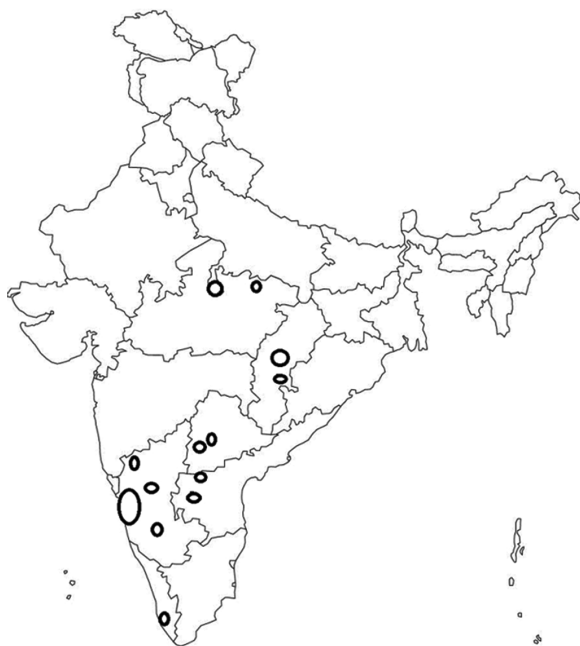


Fig. 1 — Phytogeographical locations of *G. sylvestre* samples

parameters. Alcohol and water soluble extractives as well as total and acid insoluble ash content were estimated as per the methods described in the Ayurvedic Pharmacopoeia of India<sup>31</sup>.

#### Extraction and sample preparation

##### Extraction

In order to estimate the gymnemagenin and deacylgymnemic acid, the leaf samples were extracted with 50% aqueous methanol. Each powdered leaf sample (5 g/sample) was refluxed separately for 30 min with 50 mL of 50% (v/v) aqueous methanol, cooled and filtered through a Whatmann No. 1 filter paper. The residues were further refluxed with 2x50 mL of 50% aqueous methanol, cooled and filtered. All the three filtrates of each sample were combined separately and concentrated under vacuum to 25 mL. These concentrated samples were further subjected to hydrolysis for estimation of gymnemagenin and deacylgymnemic acid using HPLC.

Similarly, lupeol and stigmasterol were estimated in methanolic extracts, and the samples were prepared by ultrasound-assisted extraction process. Powdered leaf samples (10 g each) were extracted with methanol (100 mL each) using ultrasonic bath equipment. The bath was programmed at 40 kHz, 100 W, 40°C and 30 min of sonication. The extracts were finally filtered through Whatman No. 1 filter paper, concentrated at reduced temperature (below 50°C) by rotary evaporation (Buchi, USA) and

lyophilized (Freezone 4.5, Labconco, USA) under high vacuum (133×104 mbar) at (40±2)°C to yield the respective methanolic extracts. These methanolic extracts were stored at 5°C till further analyses.

##### Hydrolysis and preparation of test samples for estimation of Gymnemagenin

Concentrated sample (5 mL) from each accession was taken separately, 5 mL of methanol and 2 mL of 12% potassium hydroxide were added and each refluxed for 1 h. The samples were cooled down and 1.8 mL of 12 M hydrochloric acid was added and then again heated on water bath for 30 min. After cooling, the pH of the samples was adjusted to 7.5 to 8.5 with 12% potassium hydroxide. The final hydrolysed sample solution was diluted to 100 mL with 50% aqueous methanol, filtered using Millex 0.45 µm syringe-driven filter and 50 µL of each resulting test samples were injected for estimation of gymnemagenin by HPLC.

##### Hydrolysis and preparation of test samples for estimation of Deacylgymnemic acid

Concentrated sample (5 mL) from each accession was taken separately, 5 mL of methanol and 2 mL of 12% potassium hydroxide were added and each refluxed for 1 hr. After cooling, the pH 7.0 to 7.5 was adjusted in each sample with 10% hydrochloric acid. The hydrolysed sample solution was further diluted to 100 mL with 50% aqueous methanol and filtered using Millex 0.45 µm syringe-driven filter. 50 µL of each resulting test samples were injected for estimation of deacylgymnemic acid by HPLC.

##### Preparation of test samples for estimation of Lupeol and Stigmasterol

Ten mg of each dried concentrated methanolic extract was dissolved in 1mL methanol, filtered through 0.45 µm membrane filter and 10 µl of each was applied for quantification of lupeol and stigmasterol by HPTLC.

##### Preparation of standards

Accurately weighed 1 mg of each standard of gymnemagenin and deacylgymnemic acid were dissolved in 10 mL methanol while 1 mg of each of reference standard lupeol and stigmasterol were dissolved in 10 mL of chloroform solvent, respectively. The final concentration of all the standards *viz.* gymnemagenin, deacylgymnemic acid, lupeol and stigmasterol was 100 µg/mL.

##### Chromatographic conditions (HPLC)

HPLC analysis was performed according to the method described by Manohar *et al.*,<sup>32</sup> with minor

Table 1 — Collection details of samples of *G. sylvestre*

Sample code	Locality	State	Geo-coordinates		Elevation (m)
			Lat. (N)	Long. (E)	
Gs 1	Chandavar	Karnataka	14.4083	74.4479	18
Gs 2	Honnavar	Karnataka	14.2941	74.4447	50
Gs 3	Karikan	Karnataka	14.327	74.4755	31
Gs 4	Madanigeri Village	Karnataka	14.4424	74.4424	39
Gs 5	Karwar	Karnataka	14.6087	74.3606	41
Gs 6	Sindolli village	Karnataka	15.3474	74.4959	667
Gs 7	Yalagupa	Karnataka	14.2943	74.5082	28
Gs 8	Jog fall	Karnataka	14.2455	74.8069	519
Gs 9	Trivandrum	Kerala	8.7478	77.0239	90.72
Gs 10	Satna	Madhya Pradesh	25.08552	80.82523	203
Gs 11	Kumta	Karnataka	14.44117	74.43743	19
Gs 12	Mirjan	Karnataka	14.48184	74.4315	12
Gs 13	Mugta	Karnataka	14.57942	74.38815	36
Gs 14	Gokarna Junction	Karnataka	14.58482	74.38158	34
Gs 15	Valgalli village	Karnataka	14.41024	74.44627	48
Gs 16	Harodi village	Karnataka	14.40798	74.44846	20
Gs 17	Kujalli village	Karnataka	14.40435	74.45327	27
Gs 18	Aunsalli village	Karnataka	14.36246	74.47766	15
Gs 19	Vandoor village	Karnataka	14.32697	74.47555	32
Gs 20	Kodsani village	Karnataka	14.60308	74.38077	23
Gs 21	Shirur village	Karnataka	14.60854	74.36054	19
Gs 22	Karwar village	Karnataka	14.79091	74.11406	33
Gs 23	Mugali village	Karnataka	14.20704	74.45774	46
Gs 24	Hadinbala village, (Kadakeri)	Karnataka	14.29421	74.50826	39
Gs 25	Yalaguppa hamlet	Karnataka	14.27686	74.51968	30
Gs 26	Dibbanagal hamlet	Karnataka	14.26838	74.53095	38
Gs 27	Mavinollai	Karnataka	14.23246	74.81886	492
Gs 28	Keregaon Range, Dhamtri Forest, Raipur	Chhattisgarh	20.59161	81.67324	365
Gs 29	Keregaon Range, Dhamtri Forest, Raipur	Chhattisgarh	20.59058	81.67326	363
Gs 30	Keregaon Range, Dhamtri Forest, Raipur	Chhattisgarh	20.59048	81.66957	368
Gs 31	Keregaon Range, Dhamtri Forest, Raipur	Chhattisgarh	20.59127	81.67504	369
Gs 32	Keonchi, Gaurila Range, Marwahi Forest Division, Bilaspur	Chhattisgarh	22.59935	81.77199	601
Gs 33	Kakrui, Lalitpur	Uttar Pradesh	24.6438	78.37588	364
Gs 34	Jamunia Khera, Bhikampur, Madawara, Lalitpur	Uttar Pradesh	24.37534	78.86255	365
Gs 35	Guru Gorakhnath Pahad, Mahoba	Uttar Pradesh	25.27615	79.85602	241
Gs 36	Hyderabad	Telangana	17.3132	78.38711	11
Gs 37	Vikarabad	Telangana	17.32901	78.27333	25
Gs 38	Kurnool	Andhra Pradesh	15.80891	78.58196	8
Gs 39	Anantpur	Andhra Pradesh	14.1801	78.1364	16
Gs 40	Yana	Karnataka	14.43927	74.43083	35
Gs 41	Maneer village	Karnataka	14.44248	74.44252	14
Gs 42	Mirjan fort	Karnataka	14.49215	74.42021	15
Gs 43	Gokarna	Karnataka	14.48887	74.41866	9
Gs 44	Opposite to Mirjan fort	Karnataka	14.48802	74.4182	10
Gs 45	Honnavar to Karikan road	Karnataka	14.31582	74.47075	56
Gs 46	Arengadi village	Karnataka	14.32152	74.481	63
Gs 47	Belse village	Karnataka	14.62259	74.34872	19
Gs 48	On the way to Ankola	Karnataka	14.74492	74.25146	69
Gs 49	Kodsani	Karnataka	14.60264	74.37863	18
Gs 50	Apsarkonda	Karnataka	14.23779	74.44742	13
Gs 51	Bhatkal	Karnataka	14.27684	74.51934	25
Gs 52	Mudkani village	Karnataka	14.24704	74.56013	30
Gs 53	Gersoppa	Karnataka	14.25029	74.62085	20

modifications on a liquid chromatography system (Waters, Milford, MA, USA) consisting of 515 pumps and equipped with an online degasser, a Waters PCM (pump control module), a Waters 2707 autosampler, a Waters 2996 photodiode array (PDA) detector, and Waters Empower software. The separation was achieved on RP C18 column Polaris 5 C18-A (5  $\mu\text{m}$ ; 250 $\times$ 4.6 mm) using an isocratic mode. Mobile phase consisting Acetonitrile: Water: Phosphoric acid (30:70:0.1 v/v) for gymnemagenin and Acetonitrile: Water: Phosphoric acid (25:75:0.1 v/v) was employed for deacylgymnemic acid, respectively. The mobile phase was filtered through 0.45  $\mu\text{m}$  Millipore filter and degassed by sonication for 30 min. The flow rate was adjusted to 1.0 mL min<sup>-1</sup> with run time of 40 min. The column temperature was maintained at 25°C. The detection wavelength was 210 nm which was close to absorption maxima for both the compounds. Injection volume for the samples was 50  $\mu\text{L}$ . Comparison of the retention times and spectra of the peaks obtained with the respective reference standards were used for identifying the peaks in the chromatograms of the test samples. Quantification of the compounds was achieved using calibration plots of the individual standard solutions. The concentrations of the standards used for the calibration curves ranged from 0.1  $\mu\text{g}$  to 0.5  $\mu\text{g}$ .

#### Chromatographic conditions (HPTLC)

For HPTLC analysis pre-coated silica gel 60 F<sub>254</sub>, 0.2 mm thickness, aluminium sheets, 20 $\times$ 10 cm (Merck, Darmstadt, Germany) were used. Samples were applied using 100  $\mu\text{L}$  Hamilton syringe using Linomat 5 automatic applicator (Camag, Switzerland) under a flow of N<sub>2</sub> gas. The plates were developed in Camag twin trough glass chamber (20 $\times$ 10 cm) using the standardized solvent system; the experimental condition temperature 25 $\pm$ 2°C and relative humidity 40%. Scanning was performed using a TLC Scanner 3 (Camag, Switzerland) operated by winCATS 4 software (version 3.2.1). 10  $\mu\text{L}$  of each test sample along with standard solutions of lupeol and stigmasterol were applied to the TLC plate and plate was developed to a distance of 9 cm using Toluene: Ethyl acetate (80:20) (v/v) as mobile phase, in a Camag twin-trough chamber, previously saturated with mobile phase vapour for 15 min at 25 $\pm$ 2°C. After development, the plates were completely dried in air at room temperature, sprayed with anisaldehyde sulphuric acid reagent for derivatization and heated at 110 $\pm$ 2°C for 5 min. The densitometric analysis of

developed spots was carried out at the wavelength of 540 nm for lupeol and 580 nm for stigmasterol. The R<sub>f</sub> values and colour of the resolved bands were noted and photographs were taken by means of a Camag Reprostar 3 video documentation unit by illumination under visible light. Calibration curves for lupeol and stigmasterol were prepared over a concentration range of 100-600 ng/band by plotting peak areas vs concentrations and the regression equation was computed. The peak areas of the corresponding peaks of lupeol and stigmasterol were recorded and the amount of these compounds was calculated.

#### Results and Discussion

The aim of the present study was to investigate different samples of *G. sylvestre* collected from various geographic regions in India for their physicochemical parameters as well as to identify the elite germplasms based on the contents of the bioactive compounds. The analysis of physicochemical parameters and estimation of four marker compounds (gymnemagenin, deacylgymnemic acid, lupeol and stigmasterol) revealed high content variability among the 53 samples of *G. sylvestre* analysed in the present study (Fig. 2 and Fig. 3).

The gymnemagenin and deacylgymnemic acid were present in all accessions of the species, except sample no. Gs9 collected from Trivandrum, Kerala. The contents of gymnemagenin and deacylgymnemic acid were estimated using HPLC while lupeol and stigmasterol were quantified using HPLC. The HPLC and HPTLC profiles of the standard compounds along with the samples are shown in Figure 4 and Figure 5, respectively.

The gymnemagenin content varied from 10.19-23.38  $\mu\text{g}/\text{mg}$  (w/w dry weight plant material), while deacylgymnemic acid ranged from 4.70-35.42  $\mu\text{g}/\text{mg}$  (w/w dry weight plant material) (Fig. 6). Notably, the maximum concentrations of gymnemagenin and

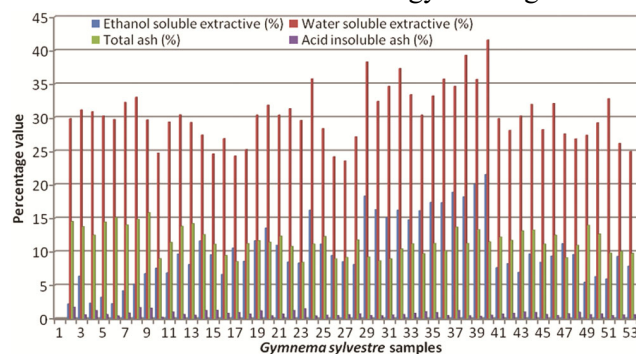


Fig. 2 — Physicochemical parameters of *G. sylvestre* samples

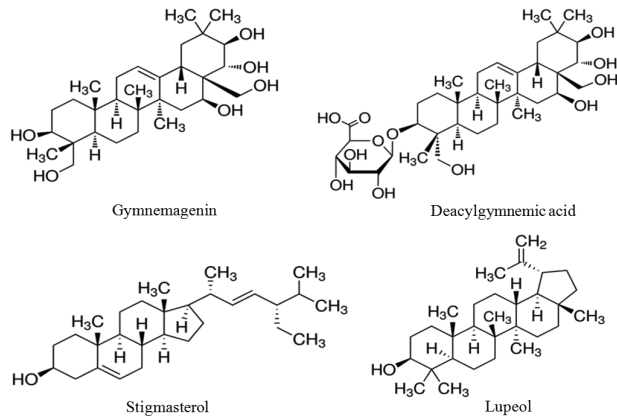


Fig. 3 — Chemical markers for *G. sylvestre*

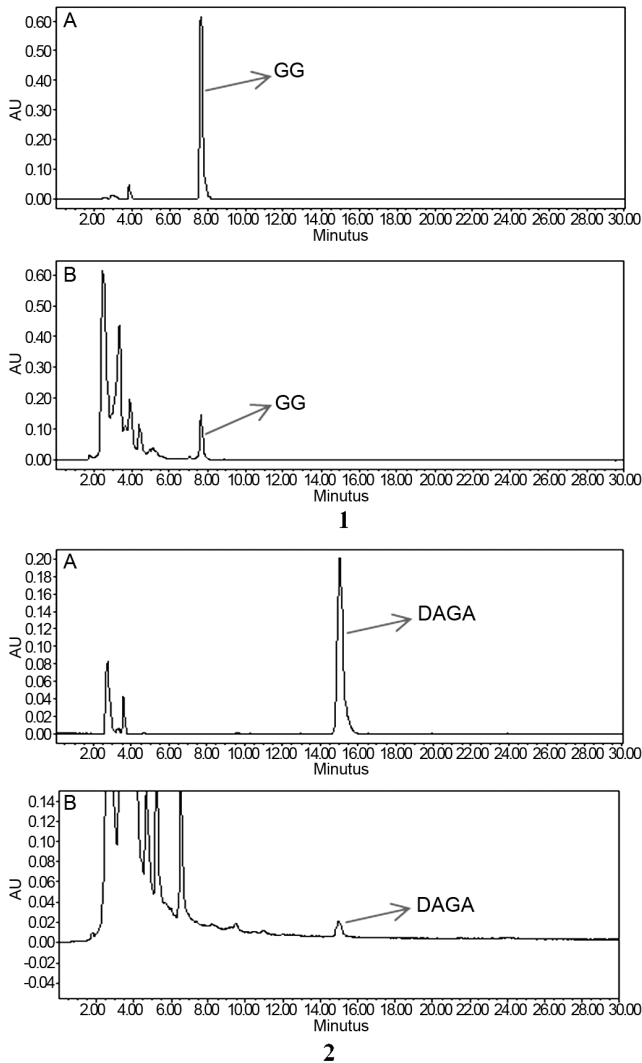


Fig. 4 — HPLC profiles of *G. sylvestre*: 1. A. Standard gymnemagenin (GG), B. *G. sylvestre* sample after alkaline hydrolysis followed by acid hydrolysis & 2. A. Standard deacylgymnemic acid (DAGA), B. *G. sylvestre* sample after alkaline hydrolysis.

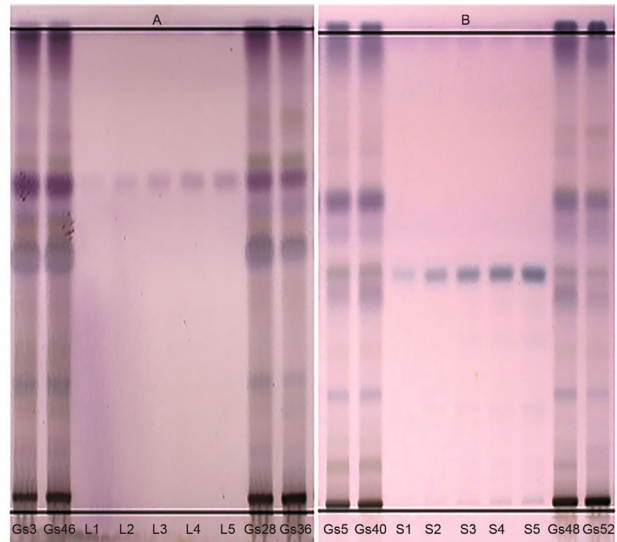


Fig. 5 — HPTLC profiles of *G. sylvestre*: A. Gs 3, Gs 46: *G. sylvestre* samples; L1-L5: standard Lupeol; Gs 28, Gs 36: *G. sylvestre* samples & B. Gs 5, Gs 40: *G. sylvestre* samples; S1-S5: standard Stigmasterol; Gs 48, Gs 52: *G. sylvestre* samples.

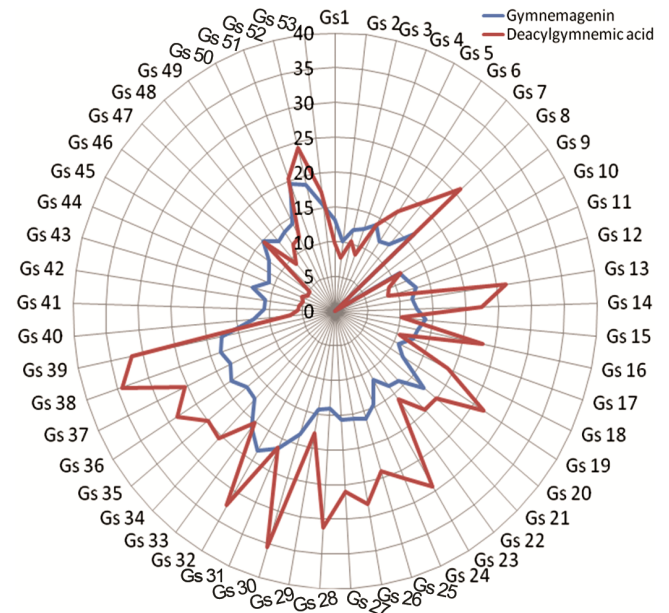


Fig. 6 — Gymnemagenin and Deacylgymnemic acid content in *G. sylvestre* samples

deacylgymnemic acid contents were detected in sample no. Gs32 (23.38 µg/mg) and sample no. Gs30 (35.42 µg/mg), respectively collected from Bilaspur and Raipur districts of Chhattisgarh state. Sample number Gs2 and Gs46 collected from Honnavar and Areangadi villages of Karnataka were found to contain the lowest amount of gymnemagenin (10.19 µg/mg) and deacylgymnemic acid (4.70 µg/mg), respectively.

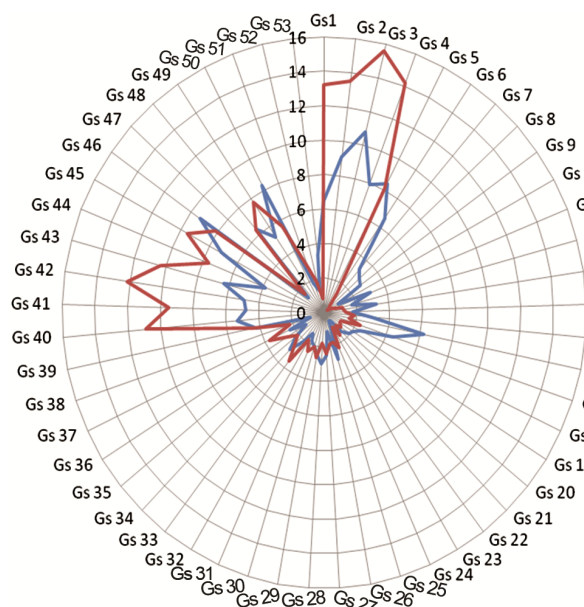


Fig. 7 — Lupeol and Stigmasterol content in *G. sylvestre* samples

The lupeol content in methanolic extracts of different samples ranged from 0.57  $\mu\text{g}/\text{mg}$  to 10.8  $\mu\text{g}/\text{mg}$  (w/w) in sample nos. Gs23 and Gs3 from Mugali and Karikan villages in Karnataka, whereas the stigmasterol content ranged from 0.25 to 15.6  $\mu\text{g}/\text{mg}$  in sample no. Gs9 of Trivandrum, Kerala and Gs3 of Karikan, Karnataka, respectively (Fig. 7).

Earlier reports of gymnemagenin contents (3.43–5.32 mg/g and 0.301–0.335 mg/g) analysing eighteen accessions of *G. sylvestre* collected from southern states including coastal areas and samples from Ahmedabad, Neemach, and Mumbai (western part) of India revealed low levels of gymnemagenin content variability<sup>26,33</sup>. Since the time and season of collection are known to affect the content of the phytoconstituents, it could be the reason for the chemical variability observed. The *G. sylvestre* samples studied by Dhanani *et al.*<sup>26</sup> were collected during the month of July while the present samples were collected during the month of August–November which corresponded with the fully mature stage of the studied species. Our results suggest that in order to obtain higher concentration of the bioactive gymnemic acids the optimal time of harvesting of *G. sylvestre* may be during the month of August–November when it is fully matured. Furthermore, we have also studied the content of lupeol and stigmasterol in the same populations. During the last decade, there has been an unprecedented increase in intake of plant based natural triterpenes. Triterpenes are important components of plant membranes and serve to stabilize phospholipid

bilayers in plant cell membranes just as cholesterol does in animal cell membranes<sup>34</sup>. Triterpenes and phytosterols are natural components of human diets. These compounds also have cholesterol-lowering properties. There are currently more than 10 major commercially triterpene-based products being sold all around the world, for their beneficial activity against inflammation, cancer, arthritis, diabetes, heart diseases, renal toxicity and hepatic disorders<sup>35–37</sup>. Thus, in the present study we have estimated four phytoconstituents- gymnemagenin and deacylgymnemic acid, as well as lupeol and stigmasterol in 53 samples representing seven Indian states with vast physiographic and ecological variability.

It was also observed that *G. sylvestre* populations of Bilaspur and Raipur districts of Chhattisgarh exhibiting the highest content of gymnemagenin and DAGA are products of neutral to slightly alkaline soil (6.9–8.53)<sup>38</sup> whereas the Honnavar populations revealing lowest contents of gymnemagenin and DAGA are growing in acidic soils (pH<6.3)<sup>39</sup>. The general climate of Chhattisgarh is dry humid type and Bilaspur and Raipur forest regions fall under medium to high rainfall areas of the state, whereas coastal areas like Honnavar in Uttara Kannada district, Karnataka has a relative high humidity (>70%) than the plateau side where it goes down to less than 50%. It is evident from the data emanating from this study that the populations growing wild in central plains of Chhattisgarh contain higher concentration of gymnemagenin and DAGA, thereby indicating that the prevailing climatic conditions and soil type in central plains are much more suitable for production and accumulation of gymnemic acids in *G. sylvestre* than that of southern plateaus and coastal regions in India. This indicates that the raw material from this region may be best suitable for production of high quality products for human consumption.

### Conclusions

Since the last few decades, concerns about the side effects and resistance due to long term use of modern medicines are growing. The use of plant-based medicines, phytonutrients and nutraceuticals is increasing rapidly across the globe and with many people now resorting to these products for treatment of various health issues, over-exploitation of plant resources is endangering the genetic diversity in their natural habitat<sup>40,41</sup>. Thus, there is an urgent need to carry out mass production of the potential plant

materials to cater to fast expanding herbal drug industries. Since the biological activity is directly related to the bioactive phytoconstituents present in the plant material, it is essential to use quality raw materials for drug manufacturing. Hence, the present study was undertaken to estimate these phytochemicals among the *G. sylvestre* samples collected from different geographical regions of India to identify the elite germplasm of this potential antidiabetic plant for mass production to cater to the growing need in the herbal drug industries. The outcome of the present study will be helpful in the present scenario of demand and supply of quality raw herbal material for herbal drug industries by cultivation of the elite germplasm. Thus elite germplasm/accessions with high concentration of bioactive compounds needs to be promoted for mass propagation and cultivation through village committees and farmer groups to ensure sustained supply of quality raw materials for herbal drug industries and enhancing rural income.

### Acknowledgments

The authors thank Director, CSIR-National Botanical Research Institute, Lucknow (India) for providing support and encouragements. The Council of Scientific and Industrial Research, New Delhi (India) is greatly acknowledged for the financial support (HCP-10).

### Conflicts of Interest

The authors have no conflicts of interest to declare.

### Authors' Contributions

MMP, SR & TSR: Conceptualization; MMP & SR: Investigation, Methodology, Formal analysis, Writing, Review and Editing; KV, BM & DS: Investigation

### References

- Rapini A, Chase M W, Goyder D J & Griffiths J, Asclepiadeae classification: evaluating the polygenetic relationships of New World Asclepiadoideae (Apocynaceae), *Taxon*, 52 (2003) 33-50.
- Gurav S, Gulkari V, Duragkar N & Patil A, A systemic review: pharmacognosy, phytochemistry, pharmacology and clinical applications of *Gymnema sylvestre* R. Br., *Pharmacogn Rev*, 1 (2007) 338-343.
- Potawale S E, Shinde V M, Anandi L, Borade S, Dhalawat H, et al., *Gymnema sylvestre*: A comprehensive review, *Pharmacology OnLine*, 2 (2008) 144-157, DOI: 10.13040/IJPSR.0975-8232.IJP.5(3).136-43
- Reddy P S, Gopal G R & Sita G L, *In vitro* multiplication of *Gymnema sylvestre* R.Br., an important medicinal plant, *Curr Sci*, 75 (8) (1998) 843-845.
- Nandkarni A K, Indian Materia Medica Vol. 1. In: *Gymnema sylvestre*, (Popular Prakashan, Bombay), 1992, p. 596-597.
- Kirtikar K R & Basu B D, Indian medicinal plants—III. International, (Book Distributors, Deharadun, India), 1988, p. 1625.
- Kapoor L D, Handbook of Ayurvedic medicinal plants, (CRC Press, Boca Raton, FL), 1990, p. 200-201.
- Vidyasagar G M & Siddalinga M S M, Medicinal plants used in the treatment of *Diabetes mellitus* in Bellary district, Karnataka, *Indian J Tradit Know*, 12 (4) (2013) 747-751, DOI: <http://hdl.handle.net/123456789/22193>
- Nadkarni A K & Nadkarni K M, Indian Materia Medica, Vol. 1. (Popular Prakashan, Bombay) 1976, p. 569.
- Daisy P, Eliza J & Farook K A, A novel dihydroxy gymnemic triacetate isolated from *Gymnema sylvestre* possessing normoglycemic and hypolipidemic activity on STZ-induced diabetic rats, *J Ethnopharmacol*, 126 (2) (2009) 339-344, DOI: 10.1016/j.jep.2009.08.018
- Hassan M, Niazi A T, Khan S & Gul F, Antidiabetic and antihyperlipidemic effects of *Artemisia absinthium* L., *Citrullus colocynthis* (L.) Schrad. And *Gymnema sylvestre* (Retz.) R.Br. ex Sm. on type II diabetes hyperlipidemic patients, *Indian J Tradit Know*, 17 (2) (2018) 233-239, DOI: <http://nopr.niscair.res.in/handle/123456789/43657>
- Ahamad J, Saima A & Showkat R M, Simultaneous quantification of gymnemic acid as gymnemagenin and charantin as  $\beta$ -Sitosterol using validated HPTLC densitometric method, *J Chromatogr Sci*, 53 (2015) 1203-1209, DOI: 10.1093/chromsci/bmu166.
- Ahmad N, Nawab M, Kazmi M H & Fatima S H. Evaluation of efficacy and safety of a polyherbal Unani formulation in diabetes mellitus type 2 (Zayābīṭus Sukkari Qism Sāni) - A randomised controlled clinical study, *Indian J Tradit Know*, 20 (1) (2021) 15-20, DOI: <http://nopr.niscair.res.in/handle/123456789/56308>
- Turner S, Diako C, Kruger R, Wong M, Wood W, et al., Consuming *Gymnema sylvestre* reduces the desire for high-sugar sweet foods, *Nutrients*, 12 (4) (2020) 1046, DOI: 10.3390/nu12041046
- Maheshwari N V & Nadgauda R S, Variation in gymnemic acid content in *Gymnema* germplasm from different geographical regions of Gujarat, *J Chem Pharm Res*, 8 (3) (2016) 37-41.
- Tiwari P, Mishra B N & Sangwan N S, Phytochemical and pharmacological properties of *Gymnema sylvestre*: An important medicinal plant, *Biomed Res Int*, (2014) Article ID 830285, DOI: <http://dx.doi.org/10.1155/2014/830285>
- Edreva A, Velikova V, Tsonev T, Dagnon S, Gürel A L, et al., Stress-protective role of secondary metabolites: diversity of functions and mechanisms, *Gen Appl Plant Physiology*, 34 (2008) 67-78.
- Oh M M, Trick H N & Rajashekar C B, Secondary metabolism and antioxidants are involved in environmental adaptation and stress tolerance in lettuce, *J Plant Physiol*, 166 (2009) 180-191, DOI: 10.1016/j.jplph.2008.04.015
- Pavarini D P, Pavarini S P, Niehues M & Lopes N P, Exogenous influences on plant secondary metabolite levels,



- Anim Feed Sci Technol*, 176 (1-4) (2012) 5-16, DOI: <https://doi.org/10.1016/j.anifeedsci.2012.07.002>
- 20 Gutbrodt B, Dorn S, Unsicker S B & Mody K, Species-specific responses of herbivores to within-plant and environmentally mediated between-plant variability in plant chemistry, *Chemoecology*, 22 (2012) 101-111, DOI: 10.1007/s00049-012-0102-1
- 21 Toshihiro Y, Kenzi M, Kenzo O & Osamu T, Quantitative analysis of gymnemic acids by High Performance Liquid Chromatography, *Nippon Shokuhin Kagaku Kaishi*, 41 (3) (1994) 202-205, DOI: <https://doi.org/10.3136/nskk1962.41.202>
- 22 Puratchamani V & Jha S, Standardization of *Gymnema sylvestre* R.Br. with reference to gymnemagenin by high-performance thin-layer chromatography, *Phytochem Anal*, 15 (3) (2004) 164-166, DOI: 10.1002/pca.763.
- 23 Thamburaj S, Subbaraj D, Kasturi S & Vijayakumar M, Evaluation of germplasm accessions of *Gymnema sylvestre* R.Br., *South Indian Horticulture*, 44 (5/6) (1996) 174-176.
- 24 Nair S & Keshavachandran R, Genetic variability of Chakkarakolli (*Gymnema sylvestre* R.Br.) in Kerala assessed using morphological and biochemical markers, *J Trop Agric*, 44 (2006) 64-67.
- 25 Shah Nawaz M, Zanan R L, Wakte K V, Mathure S V, *et al.*, Genetic diversity assessment of *Gymnema sylvestre* (Retz.) R.Br. ex Sm. populations from Western Ghats of Maharashtra, India, *Genet Resour Crop Evol*, 59 (2012) 125-134, DOI: <https://doi.org/10.1007/s10722-011-9757-7>
- 26 Dhanani T, Singh R, Waman A, Patel P, *et al.*, Assessment of diversity amongst natural populations of *Gymnema sylvestre* from India and development of a validated HPLC protocol for identification and quantification of gymnemagenin, *Ind Crop Prod*, 77 (2015) 901-909, DOI: <https://doi.org/10.1016/j.indcrop.2015.09.076>
- 27 Parthasarathy R & Sathiyabama M, Gymnemagenin-producing endophytic fungus isolated from a medicinal plant *Gymnema sylvestre* R.Br., *Appl Biochem Biotechnol*, 172 (6) (2014), 3141-3152, doi: 10.1007/s12010-014-0754-0
- 28 Saleem M, Lupeol, A novel anti-inflammatory and anti-cancer dietary triterpene, *Cancer Lett*, 285 (2) (2009) 109-115, DOI: 10.1016/j.canlet.2009.04.033
- 29 Gabay O, Sanchez C, Salvat C, Chevy F, Breton M *et al.*, Stigmasterol: a phytosterol with potential anti-osteoarthritic properties, *Osteoarthritis Cartilage*, 18 (1) (2010) 106-116, DOI: <https://doi.org/10.1016/j.joca.2009.08.019>
- 30 Chandler R F, Hooper S N & Ismail H A, Antihypercholesterolemic studies with sterols: beta-sitosterol and stigmasterol, *J Pharm Sci*, 68 (2) (1979) 245-247, DOI: 10.1002/jps.2600680235
- 31 Anonymous, The Ayurvedic Pharmacopoeia of India, Part I, (Government of India, Ministry of Health & Family Welfare, Department of AYUSH New Delhi), 2008.
- 32 Manohar S H, Naik P M, Praveen N & Murthy H N, Distribution of gymnemic acid in various organs of *Gymnema sylvestre*, *J For Res*, 20 (3) (2009) 268-270, DOI: <https://doi.org/10.1007/s11676-009-0046-7>
- 33 Trivedi P D, Pundarikakshudu K & Shah K, A validated reverse phase liquid chromatographic method for quantification of gymnemagenin in the *Gymnema Sylvestre* R.Br. leaf samples, extract and market formulation, *Int J Appl Sci Eng*, 9 (1) (2011) 25-31, DOI: 10.6703/IJASE.2011.9(1).25
- 34 Liby K T, Yore M M & Sporn M B, Triterpenoids and rexinoids as multifunctional agents for the prevention and treatment of cancer, *Nat Rev Cancer*, 7 (2007) 357-369, DOI: 10.1038/nrc2129
- 35 Moreau R A, Whitaker B D & Hicks K B, Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses, *Prog Lipid Res*, 41 (2002) 457-500, DOI: 10.1016/s0163-7827(02)00006-1
- 36 Fernández M A, de las Heras B, García M D, Sáenz M T & Villar, A, New insights into the mechanism of action of the anti-inflammatory triterpene lupeol, *J Pharm Pharmacol*, 53 (2001) 1533-1539, DOI: 10.1211/0022357011777909
- 37 Sudhakar V, Kumar S A, Sudharsan P T & Varalakshmi P, Protective effect of Lupeol and its ester on cardiac abnormalities in experimental hypercholesterolemia, *Vasc Pharmacol*, 46 (2007) 412-418, DOI: 10.1016/j.vph.2006.12.005
- 38 Singh S S, Kunui K, Minj R A & Singh P, Diversity and distribution pattern analysis of cyanobacteria isolated from paddy fields of Chhattisgarh, India, *J Asia Pac Biodivers*, 7 (4) (2014) 462-470, DOI: <https://doi.org/10.1016/j.japb.2014.10.009>
- 39 Amara D M K, Patil P L, Kamara A M & Saidu D H, Assessment of soil fertility status using nutrient index approach, *Int J Food Agric Veterinary Sci*, 6 (3) (2016) 1-15.
- 40 World Health Organization, WHO Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems, (Geneva, Switzerland: World Health Organization), 2004.
- 41 Syedy M & Nama K S, *In vitro* propagation of shoots and callus induction of *Gymnema sylvestre* R.Br. "an important anti-diabetic plant", *Int J Curr Pharm Res*, 10 (3) (2018) 60-64, DOI: <https://doi.org/10.22159/ijcpr.2018v10i3.27340>