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Pharmacognostical Evaluation of *Gymnema sylvestre* R. Br.

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Abstract – In India, *Gymnema sylvestre* due to the unique property of the plant to antagonize the sweet taste is known as 'Gur-mar'. It has several ethnomedicinal values as various tribals/traditional communities and rural peoples of India find diverse medicinal uses viz. antidiabetic, stomachic, diuretic, and is useful in cough and throat troubles. Besides, it has strong effect on reducing blood sugar. The present communication deals with the detailed pharmacognostical evaluation of the aerial parts of *G. sylvestre* collected from three places of the country-Varanasi (U.P), Panchmarhi (M.P), Salem (Tami Nadu) and commercial sample procured from local market. The botanical and physico-chemical parameters of all the samples were quite similar though little variations were observed in foaming index, alcohol and water soluble extractives of local sample. The microscopic characteristics of the drug are horse shoe shaped petiole with 3 amphicribal vascular bundles, sieve tubes well developed; anomocytic stomata only on the abaxial surface of the leaf, the fan shaped amphicribal vascular bundle, presence of intraxylary phloem. The TLC fingerprint profile of all the samples was more or less similar only the quantity of some of the compounds varied.

Keywords – *Gymnema sylvestre*, Gur-mar, Gymnemic acid, Pharmacognosy, Antidiabetic

Introduction

Gymnema sylvestre R. Br. is a stout, large, woody, climber, belonging to the family Asclepiadaceae. It is distributed in the tropical and subtropical regions of the world (Hooker, 1885). The leaves of this plant have been used as hypoglycemic, diuretic, and are useful in cough (Kirtikar and Basu, 1935). Plant claimed strong reducing effect on blood sugar concentration. In addition, this plant is famous for its fascinating ability to antagonize the sweet taste of sugar thus known as Gurmar in 'Hindi' (Sahu *et al.*, 1996; Ueno 1996; Yoshioka *et al.*, 1996; Srivastava *et al.*, 1985; Khare *et al.*, 1983; Bishayee *et al.*, 1991; Shanmugasundaram *et al.*, 1990; Gupta and Seth 1962; Gupta 1963; Gupta and Variyar 1964; Mitra *et al.*, 1975; Shanmugasundaram *et al.*, 1983; Srivastava *et al.*, 1986; Prakash *et al.*, 1986; Shanmugasundaram *et al.*, 1988; Baskaran *et al.*, 1990); The active constituent gymnemic acid is also useful for the prevention of the formation of dental plaque and caries (Rastogi and Mehrotra, 1995).

G. sylvestre has great ethnobotanical importance as various tribal/traditional communities and rural people of India find diverse medicinal use of this plants. The Jungle Irulas inhabitants of the Nagari hills of the North Arcot district are in the habit of chewing a few green leaves in the morning to keep the urine clear and for reducing glycosuria. Similarly, the Bourgeois classes of Bombay and Gujarat also chew the fresh leaves for the same effect. The root is used as a local and internal remedy in snake-bites. It also possess emetic and expectorant properties. In the Konkan, the dried and powdered leaves are used as an errhine (Kirtikar and Basu, 1935).

The pharmacopoeial standards of the leaf of this plants have been recently published by Indian Council of Medical Research, New Delhi, India (Anonymous, 2003), but in the present communication detailed pharmacognostical studies of whole aerial parts have been undertaken as in most of the herbal drug markets of the country the leaves along with the aerial parts are being sold as Gurmarbuti. This study includes macro and microscopic features, physico-chemical value along with TLC profile of the aerial parts of *Gymnema sylvestre,* collected from different regions, in order to check the adulteration and substitution in commercial samples.

Materials and Methods

The aerial parts of the *G. sylvestre* were collected from

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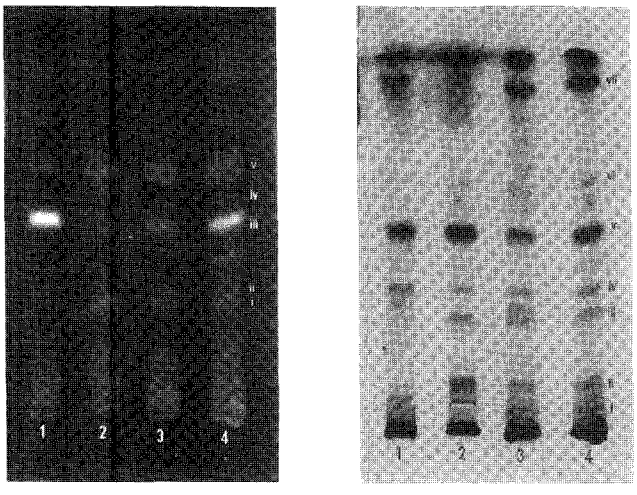
different agrogeographical zones of the country viz. Salem (Tamil Nadu), Varanasi (Uttar Pradesh), Panchmarhi (Madhya Pradesh) and a commercial sample was procured from local market. Microscopic studies were done on transverse and longitudinal sections by using saffarin as the staining agent (Johansen, 1940). Histochemical studies were performed for the presence of lignin, suberin, mucilage and type of crystals present. The quantitative analysis viz. the total ash, acid insoluble ash, total alcohol and water soluble extractives were also assayed by I. P methods (Anonymous, 1966), foaming index (Anonymous, 1998) and the behaviour of powder drug with different chemicals was also studied (Chase and Pratt, 1949). The percentage of sugar, starch (Gomery, 1957) and tannins (Anonymous, 1984) were calculated by usual methods. For TLC 2 g each of the powdered material was refluxed separately with 25 ml methanol on a water bath for 30 min. consecutively 3 times the extract was filtered and taken as a test solution. The 20 µl of the test solution applied on precoated silica gel GF_{254} (Merck) TLC plates with the help of Camag Linomat IV applicator. The plate was developed in a solvent system toluene : ethylacetate (80:20) to a distance of 8.5 cm at room temperature 32°C. The TLC plate were sprayed with anisaldehyde sulfuric acid reagent and then heated for 10 min at 110°C. The photographs were taken by Desaga Video Documentation Unit III under UV 366 before spraying and under visible light after derivatization.



A B

TLC Finger-print profile of methanolic extract of *Gymnema sylvestre* R.Br. (1-4 samples collected from different geographical zones of India)

Derivatization

(a) Under UV 366 nm.

(b) Under visible light after spraying with detecting reagent and heating at 120°C for 10 minutes.

Macroscopic

Stem hairy, light brown, finely longitudinally ridged. Leaf about 2 to 6 cm long and 1 to 4 cm broad, yellowish brown on adaxial and dark green on abaxial side. The leaves are simple, entire, opposite, petiolate, base cordate, margin entire, apex acute, reticulate venation, pubescent on both the surfaces, however, the dorsal surface is highly pubescent. The texture of leaf is papery and is bitter in taste. It also possesses remarkable property of paralysing the sense of taste for sweet substances for few hours. No characteristic odour.

Microscopic (Anonymous, 2003)

Petiole (Fig. 1)

Transverse section of petiole is horse-shoe shaped. The epidermis is barrel shaped single layered, thick walled covered with uniseriate, multicellular, thick walled, nonglandular trichomes. The cortex is collenchymatous. The vascular bundles are amphicribal and 3 in number - two lateral, and one median. Phloem well developed,

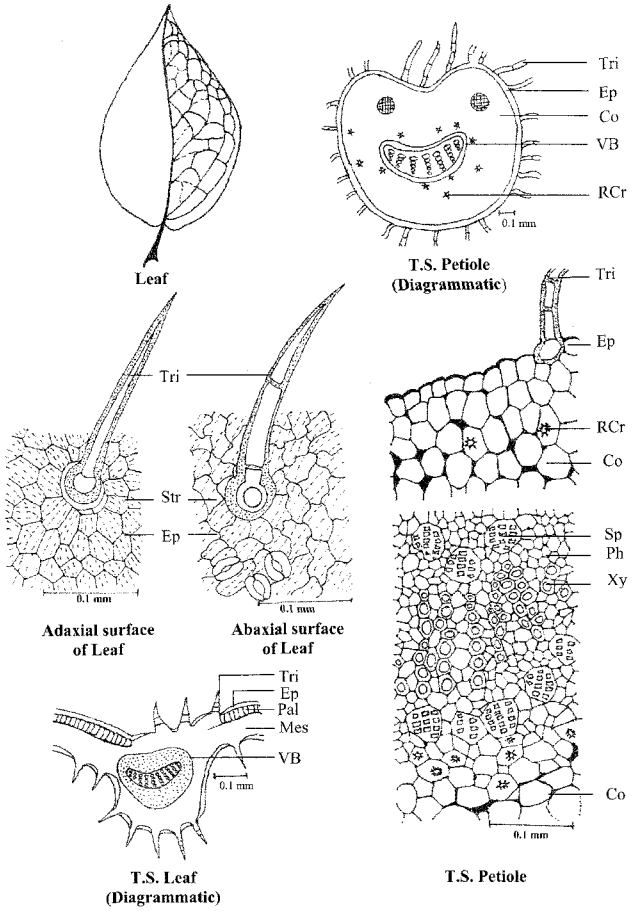


Fig. 1. Macro and microscopy of *Gymnema sylvestre* Leaf.

Table 1. Quantitative microscopy of leaves of *Gymnema sylvestre*

Leaf region	Leaf surface	Stomatal index*	Stomatal number*	Trichome number*	Palisade Ratio* (beneath 4 epidermal cells)	Vein Islet number*	Vein termination number*
Apex	Adaxial surface	–	–	20 Range (15-25)	19 Range (16-23)	9 Range (6-11)	24 Range (17-29)
	Abaxial surface	11.89 Range (11.11-13.49)	95 Range (90-110)	15 Range (15-20)			
Middle	Adaxial surface	–	–	15 Range (10-20)	20 Range (18-24)	8 Range (6-10)	27 Range (17-29)
	Abaxial surface	15.21 Range (14.59-16.09)	110 Range (100-120)	20 Range (15-25)			
Base	Adaxial surface	–	–	20 Range (15-20)	20 Range (18-22)	9 Range (6-11)	23 Range (17-34)
	Abaxial surface	14.97 Range (12.05-17.20)	90 Range (75-120)	20 Range (10-25)			

*Average of 27 readings.

consisting of sieve tubes, companion cells and phloem parenchyma. The xylem consists of vessels, tracheids and tracheidal fibres. The rosette crystals of calcium oxalate are present more towards the center. The starch grains are polygonal, simple or compound in two to many groups, the hilum is indistinct.

Lamina (Fig. 1)

The striated cuticle is present on both the surfaces. The adaxial epidermal cells are hexagonal and the abaxial cells are slightly wavy in surface view. The trichomes are uniseriate, multicellular with 2 to 5 celled, present in abundance on both the surfaces. Anomocytic stomata are present only on abaxial side. In the quantitative microscopy of leaves stomatal index, stomatal number, trichome number, palisade ratio, vein islets and vein termination numbers are presented in Table 1.

The transverse section of lamina shows thick cuticle with single layered thick walled epidermal cells on both the surfaces interrupted by trichomes. Single layered closely arranged palisade cells are present just below the adaxial epidermis. The mesophyl, 3 – 5 celled thick with large intercellular spaces. The vascular bundles are amphicribal and fan shaped.

Stem (Fig. 2)

The transverse section of stem is circular in outline and showing secondary growth. The epidermis is barrel shaped and thick walled. Trichomes are multicellular and uniseriate (185 to 485 μ long and 9 to 25 μ broad).The cork arises in the sub epidermal region and are 3 to 5 layered thick. The cortical cells are latterly elongated and

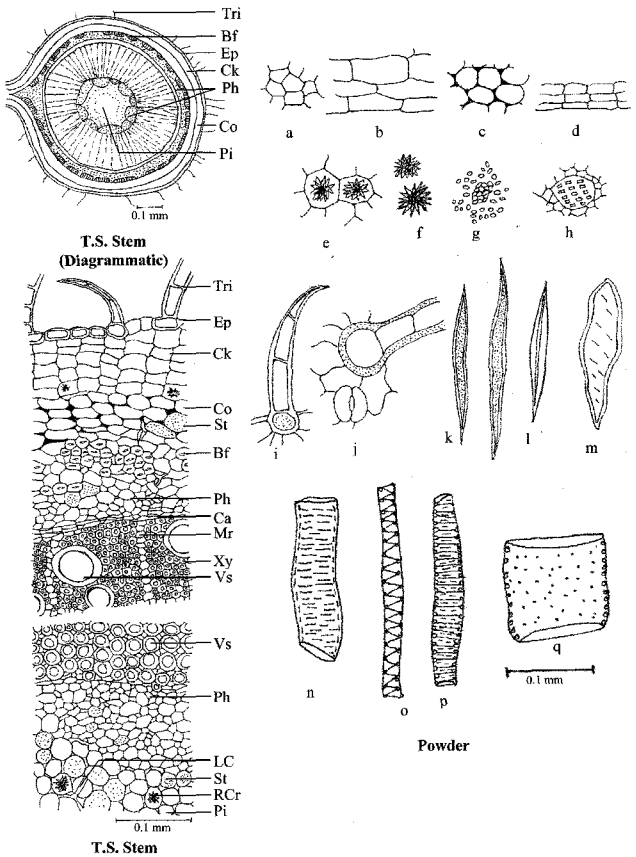


Fig. 2. Microscopy of *Gymnema sylvestre*.

collenchymatous. The endodermis is conspicuous, the pericycl is broad and is represented by patches of fibres. The phloem well developed consists of large sieve plates, companion cells and phloem parenchyma. The cambium is 2 or 3 layered. The xylem is in the form of a continuous

cylinder traversed by narrow medullary rays. The large vessels mostly solitary and pitted, xylem parenchyma filled with starch grains. Intraxylary phloem present at the periphery of the pith in the form of separate strands showing the remanant of primary bicollateral vascular bundles. Pith is collenchymatous. Rosette crystal of calcium oxalate and laticiferous canals are present in cortical, phloem and pith regions. On maceration-vessels, tracheidal fibre, tracheids and fibres are 132.07 – 330.19 × 18.87 – 122.67 µm, 226.41 – 584.90 × 9.43 – 28.30 µm, 87.74 – 377.36 × 9.43 – 47.17 µm and 226.02 – 499.99 × 9.43 – 28.30 µm respectively.

Powder (Fig. 2)

Powder is green in colour, bitter in taste with pleasant aromatic odour. On microscopic examination, it shows thick walled, uniseriate multicellular trichomes, anomocytic stomata, idioblast with rosette crystals of calcium-oxalate, starch grains, remnants of collenchymatous and parenchymatous cells; vessels, tracheids, tracheidal fibres, bast fibres and sieveplates. When powder is treated separately with 1 N aqueous NaOH and 50% KOH, shows green fluorescence under UV 254 nm and orange yellow colour with 50% HNO_3 in daylight.

Phytochemical studies

The percentage of total ash, acid insoluble ash, alcohol soluble and water soluble extractives are tabulated in Table 2. Besides, sugar, starch, tannins and foaming index were also calculated and the results are presented in Table 3. The TLC fingerprint profiles of all the samples are almost similar. The different bands are observed under UV 366 nm before spraying and after spraying with anisaldehyde sulphuric acid reagent. The R_f values and colours of the bands are presented in the Table 4.

List of Abbreviations

Bf-Bast fibres, **Ca**-Cambium, **Ck**-Cork, **Co**-Cortex, **Col**-Collenchyma, **Ep**-Epidermis, **LC**-Laticeferous Canal, **Mes**-Mesophyll, **MR**-Medullary Ray, **Pal**-Palisade cells, **Ph**-Phloem, **Pi**-Pith, **RCr**-Rosette Crystals of calcium oxalate, **Sp**-Sieve plate, **St**-Starch, **Sto**-Stomata, **Str**-Striations, **Tri**-Trichome, **VB**-Vascular Bundle, **Vs**-Vessel, **Xy**-xylem.

Abbreviations of powder

a & b- parenchyma, **c**-collenchyma, **d**- cork cells, **e**-

Table 4. R_f values and colours of the band in methanolic extract of *Gymnema sylvestre*

Visible (After spraying)		UV- 366	
R_f Values	Colour of bands	R_f Values	Colour of bands
0.074	Grey	–	–
0.130	Green	–	–
–	–	0.287	Brick red
0.333	Grey	0.333	Brick red
0.380	Greyish blue	–	–
–	–	0.425	Brick red
0.518	Purple	0.518	Brick red
–	–	0.574	Brick red
0.657	Green	0.675	Brick red
0.888	Purple	–	–

Table 2. Physico chemical parameters of different samples of *Gymnema sylvestre*

Sample	Total ash %		Acid insoluble ash %		Alcohol soluble extractive %		Water soluble extractive %	
	Mean*	SD	Mean*	SD	Mean**	SD	Mean**	SD
Varanasi	8.55	0.378	0.65	0.041	21	0	30.5	0
Panchmarhi	10.58	0.235	1.68	0.268	19.65	0.217	35.83	0.204
Salem	10.63	0.275	1.5	0.05	20.83	0.25	30	0.295
Lucknow Market	10.42	0.151	1.69	0.135	10	0	26	0

*Average of 3 readings. **Average of 6 readings.

Table 3. Physico chemical parameters of different samples of *Gymnema sylvestre*

Sample	Tannins %		Sugar %		Starch %		Foaming index**
	Mean*	SD	Mean*	SD	Mean*	SD	
Varanasi	1.640	0.018	6.092	0.112	37.107	1.963	333.3
Panchmarhi	1.201	0.099	5.22	0.216	37.35	0.254	333.33
Salem	1.731	0.005	3.852	0.298	37.045	2.335	333.33
Lucknow	1.87	0.009	3.298	0.791	43.627	0.790	270.27

*Average of 3 readings.

**The foaming index was calculated by the formula 1000/a.

Where a= the volume of decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed.

parenchyma with rosette crystal, **f**- rosette crystal of calcium oxalate, **g**- starch grains, **h**- sieve plate, **i**- trichome, **j**- stomata and trichmoe base, **k**- tracheidal fibres, **l**- fibre, **m**- tracheid, **n-q**- vessels.

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