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Original Research Article

Pharmacognostical and Physicochemical Standardization of Multiple Samples of Gurmar Buti Leaves (*Gymnema sylvestre* R.Br.) having Immense Therapeutic Values

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Abstract

A comparative screening was undertaken to standardize the pharmacognostical and physicochemical characteristics of the Unani single drug Gurmar Buti (*Gymnema sylvestre* R. Br.; Family: Asclepiadaceae). Gurmar Buti (leaves) used in Unani and Indian Systems of Medicine for the treatment of increased blood glucose level, dyslipideamia and other metabolic disorders. Three samples of crude drugs were procured from raw drug vendors of Delhi, Hyderabad and Central Research Institute of Unani Medicine, Hyderabad. Macroscopical studies revealed the presence of petiolate, acute apex, reticulate venation, pubescent on both the outer surface of leaves. Microscopical studies showed the presence of paracytic stomata, epidermis, primary cortex, vascular bundles, phloem, companion cells, phloem parenchyma, xylem, fibres starch grain and rosette crystals of calcium oxalate. Lamina epidermal cells with convex wall and 2-5 cells present in abundance. The paracytic stomata in transverse section of leaves were also revealed. The physicochemical data showed that the drug samples contained foreign matter, moisture and. HPTLC fingerprinting of aqueous and alcoholic extractives showed various spots at 366nm (UV region) which might be in rich bioactive constituents. The quality control results revealed the absence of toxic and hazardous contamination among the drug samples. The obtained research data of pharamacognostical and physicochemical parameters and comparative screening provides the referential support information in the identification and reinvestigation of the study drug in accordance with the quality assurance and pharmacovigilance.

Keywords

Comparative screening
Gurmar Buti
Gymnema sylvestre
Pharamacognosy
Quality assurance

Introduction

The drug from *Gymnema sylvestre* R. Br. is commonly known as Gurmar buti. In Unani system of medicine, it is reported to have anti-diabetic, anti-itching, anti-eczematic, anti-thrust and anti-cough properties and useful in *Ziabetus shakari* (*Diabetes mellitus*), leprosy and eczema. It is also reported to have anti-toxic and de-addictive properties and used in scorpion bite and in addiction of drug like opium (Chopra et al., 1966; Kirtikar and Basu, 1984). In Ayurveda, the drug is also known as *Meshashring* which means sugar destroyer, so it has been used to regulate blood sugar level. Leaf powder or dry extract is used alone and in composition of compound drug as an anti-diabetic agent (Chopra et al., 1966; Kirtikar and Basu, 1984). Gurmar buti acts well in body weight management programme because it complements exercise and dietary reform by permoting blood glucose balance and obesity control also helpful in the treatment of type-I and type-II diabetes. As it is clear that high dietary fat consumption is a primary cause in the development of obesity and obesity associated type-II diabetes, and in hypercholestreamia and cardiomyopathy (Sagar, 2013; Sagar et al., 2015).

G. sylvestre is a precious herb belonging to the family Asclepiadaceae, a much branched, large, more or less pubescent, climbing shrub with young stem and branches terete. Leaves subcoriaceous 2-4cm long, ovate or elliptic, acute or shortly acuminate, cuneate rounded or cordate at the base, often glabrous above, more or less pubescent beneath, especially on the veins, shortly petioed. Flowers are small, yellow, in axillary and lateral umbel in cymes; Calyx-lobes are long, ovate, obtuse and pubescent. Corolla is pale yellow campanulate, valvate, corona single, with 5 fleshy scales. Scales adnate to throat of corolla tube between lobes; Anther connective produced into a membranous tip, pollinia 2, erect, carpels 2, unilocular; locules many ovuled. Follicles are terete and lanceolate, up to 3 inches in length.

The leaves contain hentriacontane, pentatriacontane, chlorophylls (a and b), phytin, resins, tartaric acid, formic acid, butyric acid, anthraquinone derivatives, inositol and d-quercitol. Anti-diabetic principles are *Gymnema* saponins I-IV and gymnemic acids I-IX. Flavonol glycosides, kaempferol and quercetin, tritepenoid saponins, gymnemasins, sapogenin and gymnemic acids-A, B, C and D (Chakravarthi, 1981; Yoshikawa et al., 1992; Sahu et al., 1996; Sairam et al., 1998; Liu et al., 2004). The leaves also contain betaine, choline, gymnemine alkaloids, hydrocarbons such as nonacosane, tritriacontane, tatric acid, formic acid, butyric acid, amino acids such as leucine, isoleucine, valine, alanine and γ-butyric acid. Flavonol glycosides, kaempferol and quercetin have been isolated from the aerial parts of the plant. Three new oleanane-type triterpene glycosides and six oleanane-type saponins were also isolated (Ye et al., 2000 and 2001; Liu et al., 2004; Anonymous, 2006).

Minimum concentration of *G. sylvestre* extract at 4 microg/ml concentration shows immense antiviral activities, required to completely inhibit viral cytopathic effect (CPE), i.e., MIC 100 values, anti-MCV (Mouse Corona Virus) and anti-HSV (Herpes Simplex Virus) effective activities at a low concentration of extract (Vimalanathan et al., 2009). *G. sylvestre* was collected from ten locations Tirunelveli hills (Virudhachalam, India) and genetic variability was investigated using RAPD-PCE as well as RAPD-PCR finger prints and the population showed high percentage of polymorphism was selected and considered to be the superior genotypes (Britto et al., 2010; Sagar, 2013; Sagar et al., 2015). The extracts of *G. sylvestre* (GS) have been used for the treatment of Type 2 diabetes mellitus (T2DM) in India for centuries. The effect of a novel high molecular weight GS extract, Om Santal Advasi (OSA; 1 g/ day, 60 days) induced significant increase in circulating insulin and C-peptide, which were associated with significant reduction in fasting and post-prandial blood glucose, *in vitro*. Effects of OSA on insulin secretion from human beta-cells are consistent with a mode of action through enhancing insulin secretion. These observations suggest that OSA may provide a potential alternative therapy for the hyperglycemia associated with T2DM (Romaiyan et al., 2010). *G. sylvestre* based herbal formulation composed of *G. sylvestre*, cinnamon herbal extract, hydroxyl propyl cellulose, magnesium striate, calcium phosphate, gelatine and iron and titanium oxide exhibited improved control of blood glucose levels and Type 2 diabetes when compared to the placebo group in clinical trials of patients (Anonymous, 2012). The antioxidant activity of the *G. sylvestre* leaf extract showed ferric reducing power and the free radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH). The study suggests that *G. sylvestre* has significant antioxidant activity (Sagar, 2013; Rahman et al., 2014; Sagar et al., 2015). In the present study, pharmacognostical and physicochemical standardization of multiple samples of *G. sylvestre* leaves have been carried out.

Materials and methods

Procurement of the plant material

Raw drug samples were procured from raw drug national vendors of Old Delhi region (GB1) and CRIUM Pharmacy store, Hyderabad region (GB2) and CRIUM Herbal garden region (GB3) samples. The collected drug samples identification and their botanical authentication were carried out by CRIUM, Hyderabad SMP Unit research botanists and DSRU Pharmacognosists. The required dried crude drug samples were taken as each 500g.

Pharmacognostical studies

Powder microscopy: During these studies the small quantity of powder leaf drug material placed on the slide and treated with chloral hydrate solution. Finally it is subjected to microscopical studies with trinocular microscope using 10X and 40X objective lenses and photographed.

Sectioning: Transverse sections (T.S.) were made with a fine blade of microtome by cutting with the help of potato. All the fine dissections were kept in watch glass along with water. The fine and thin selected sections were stained with a single stain-phloroglucinol + HCl (Johansen, 1940; Chopra et al., 1966; Deokule and Pokhakar, 2010). The slide containing transverse sections of leaf were observed under trinocular microscope using 10X and 40X objective lenses.

Phytochemical screening

Phytochemical screening of the phytocomoponents as shown in Table 1, were carried out using standard procedures described below.

Test for phenols

To 0.5 g each of the extract, 2 ml of ferric chloride was added. A reddish brown coloration at the interface indicated the presence of phenols.

Test for terpenoids (Salkowski test)

To 0.5 g each of the extract, 2 ml of chloroform was added. Concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration at the interface indicated the presence of terpenoids.

Test for flavonoids

Three methods were used to test for flavonoids. First, diluted ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract followed by addition of concentrated sulphuric acid (1ml), a yellow coloration that disappears on standing indicated the presence of flavonoids. Second, few drops of 1% aluminium solution were added to a portion of the filtrate, a yellow coloration indicated the presence of flavonoids. Third, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min, the mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of diluted ammonia solution, a yellow coloration indicated the presence of flavonoids.

Test for saponins

To 0.5 g of extract 5 ml of distilled water was added in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously again and after some time an emulation formed which indicated the presence of saponins.

Test for tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration which indicated the presence of tannins.

Test for alkaloids

The extract (0.5 g) was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate 2 ml of diluted ammonia was added followed by addition of 5 ml of chloroform and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one part of the extract and Dragendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Dragendorff's reagent) was considered as positive for the presence of alkaloids.

The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the following reagent and chemicals: Alkaloids with Dragendorff's test,

Hager's test, Wagner's test, Mayer's test reagents, flavonoids with Shinoda's test reagent and Mg, HCl; tannins with ferric chloride and potassium dichromate solution and saponins with ability to produce stable foam and steroids with Liebermann-Burchard test, Salkowski reaction reagents. Carbohydrates and reducing sugars were tested using Anthrone test and Fehling's test reagents respectively. Glycosides were tested using Molisch test reagent. Triterpenoids was tested using Liebermann-Burchard test reagent.

Physicochemical screening

Physicochemical screening was carried out under the following parameters like foreign matter %, total ash captained % at 450°C, acid insoluble ash % at 550°C and loss on drying at 105°C, water and alcohol soluble extractive matter % were carried out as per IPC approved standard methods (Sagar et al., 2015).

HPTLC (TLC) analysis

Preparation of extract: Five gram of each powdered drug sample (GB1, GB2 and GB3) was extracted using continuous Hot extraction by Soxhlet refluxing and Syphon work technique- Laboratory Model (Borosil complete Soxhlet assembly) upon water bath and hot air oven at constant temperature not more 65ºC which was used for alcohol extraction and aqueous extraction at specified heating mantle temperature range of 70°C to 90°C for 6 to 8h was used, with suitable alcohol and aqueous solvents. The extractives were filtered through Whatman No.1 filter paper and concentrated made up to 5ml in standard flask separately. HPTLC Instrument used was Desaga Sarstedt Gruppe (Germany).

TLC developing method

Alcohol and aqueous extracts were applied on pre-coated aluminium plates silica gel $60F_{254}$ TLC plate (E-Merck, KgaA, Germany) as absorbent stationary phase and developed the plates using the solvent systems Toluene: Ethyl acetate: Methanol (7:2:1) as mobile phase respectively (Wagner, 1984).

Quality control

Quality control was done as per WHO, AOAC guidelines and IPC approved format (WHO, 1998; AOAC, 2000; Anonymous, 2006; Anonymous, 2007).

Table 1. Preliminary phytochemical screening of *G. sylvestre* leaf samples.

Phytochemical test	Methanol extract	Ethanol extract	Aqueous extract
Alkaloids			
a) Dragendorff's test	++ve	++ve	++ve
b) Mayer's test	++ve	++ve	++ve
Carbohydrates			
a) Anthrone test	+ve	+ve	+ve
b) Fehling's test	+ve	+ve	+ve
Flavanoids			
Shinoda's test	++ve	++ve	++ve
Flavones			
Shinoda's test	+ve	+ve	+ve
Glycosides			
Molisch test	+ve	+ve	+ve
Triterpenoids			
Liebermann-Burchard test	++ve	++ve	++ve
Phenols	++ve	++ve	++ve
Saponins	++ve	++ve	++ve
Steroids			
a) Liebermann-Burchard test	+ve	+ve	+ve
b) Salkowski reaction	+ve	+ve	+ve
Tannins	-ve	-ve	-ve
Proteins	+ve	+ve	+ve
Amino acids	+ve	+ve	+ve
++ Highly active; + Moderately active; +- Trace; - Absent.			

Results and discussion

Leaves of *G. sylvestre* R.Br. are widely used in Indian traditional system of medicines including Unani, for the treatment of diabetes and urinary disorders and in most of herbal drug market of the country as Indian proprietary medicines, leaves are being sold along with the aerial parts as Gurmarbuti (*G. sylvestre*), physicochemical parameters, High Performance Thin Layer Chromatography (HPTLC) analysis, quality control, quality assurance parameters of the leaves of *G. sylvestre* are described and discussed respectively below.

Physicochemical parameters

Alcohol soluble extractive matter (12.75, 12.94 and 12.98%) as well as water soluble extractive matter (25.87, 26.57 and 26.73%) indicated the presence of active polor phyto-constituents such as gymnemines alkaloids, gymnemic acid A, B, C and D, *Gymnema* saponins I-IV, flavonol, glycosides, tritepenoid saponins, kaempferol and quercetin (Table 1) similar to the report of Chakravarthi (1981), Sagar (2013) and Sagar et al. (2015). Foreign matter (0.24, 0.28 and 0.12%) and ash (9.78, 9.96 and 9.71%) were found within permissible limits as indicated by the absence of any mixed foreign adulterative materials in both the samples (Table 2).

The HPTLC studies of alcoholic and aqueous concentrated leaf extracts of the samples GB1, GB2 and GB3 showed the similarity of the drug, however, the sample GB3 (Delhi Vendor) showed slightly decrease in the concentration of active constituents in the separated spots. It may be due to poor storage and aliened tempt conditions by the supplier. The R*f* values of both the crude herb extracts which have been taken from various regions are shown in Figs. 1 and 2 and Tables 3 and 4.

Fig. 1: HPTLC view of alcoholic leaf extract of *G. sylvestre* samples (GBHG1 as GB1, GBHG2 as GB2, GBHG3 as GB3). Detector developed observation at 366 nm UV region (Solvent System - Toluene: Ethyl acetate: Methanol in 7:2:1 ratio).

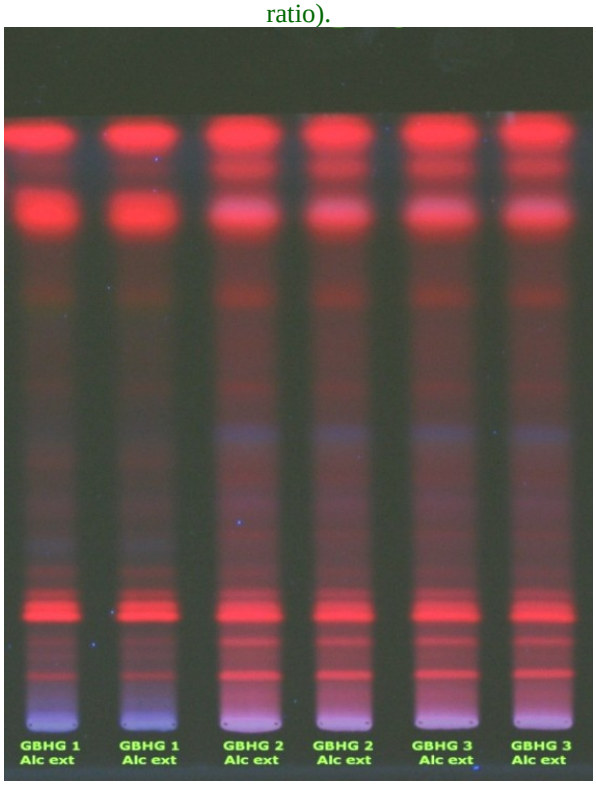


Table 2. Physicochemical parameters standardization *G. sylvestre* samples.

Parameters analyzed	Sample 1 - GB1 (Mean value)	Sample 2 - GB2 (Mean value)	Sample 3 - GB3 (Mean value)
Foreign matter (%, w/w)	0.24	0.28	0.12
Moisture (%, w/w)	4.24	4.98	5.20
Alcohol soluble ext. matter (%, w/v)	12.75	12.94	12.98
Water soluble ext. matter (%, w/v)	25.87	26.57	26.73
Total ash contained (%, w/w)	9.78	10.29	9.71
Acid insoluble ash (%, w/w)	1.82	2.04	1.60
The drug samples represented were procured from the regions: GB1 = Delhi Vendor; GB2 = Hyderabad Vendor; GB3 = CRIUM Herbal garden, Hyderabad.			

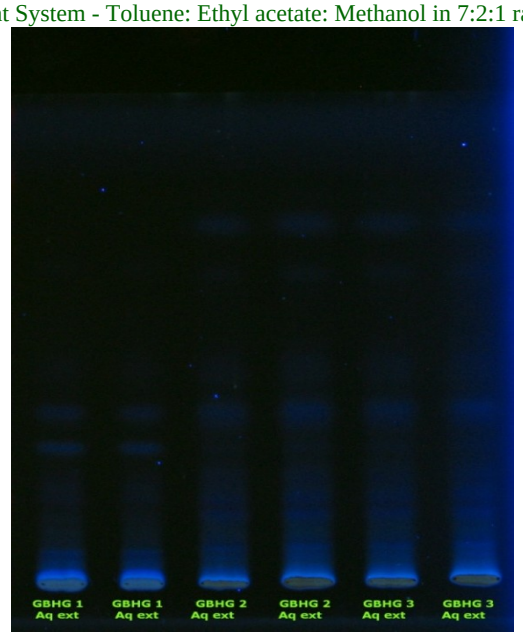
Table 3. R*f* values of leaf alcoholic extract of *G. sylvestre* samples.

R*f* values of separated spots		
UV 366nm, GB-1	**UV 366nm, GB-2**	**UV 366nm, GB-3**
0.09 Red	0.09 Red	0.09 Red
0.11 Red	0.14 Red	0.14 Red
0.14 Red	0.18 Red	0.18 Red
0.18 Dark red	0.20 Red	0.20 Red
0.20 Red	0.21 Red	0.21 Red
0.21 Red	0.26 Red	0.26 Red
0.26 Red	0.30 Red	0.30 Red
0.30 Blue	0.36 Red	0.36 Red
0.36 Red	0.44 Red	0.44 Red
0.44 Red	0.49 Blue	0.49 Blue
0.55 Red	0.55 Red	0.55 Red
0.71 Red	0.71 Red	0.71 Red
0.85 Dark red	0.85 Red	0.85 Red
0.98 Dark red	0.93 Red	0.93 Red
	0.98 Red	0.98 Red
GB1 = Delhi Vendor; GB2 = Hyderabad Vendor; GB3 = CRIUM Herbal garden, Hyderabad.		

Table 4. R*f* values of leaf aqueous extract of *G. sylvestre* samples.

R*f* Values of separated spots		
UV 366nm, GB-1	**UV 366nm, GB-2**	**UV 366nm, GB-3**
0.08 Blue	0.08 Blue	0.08 Blue
0.29 Blue	0.18 Blue	0.18 Blue
0.36 Blue	0.36 Blue	0.36 Blue
	0.46 Blue	0.46 Blue
	0.64 Blue	0.64 Blue
	0.75 Blue	0.75 Blue
GB1 = Delhi Vendor; GB2 = Hyderabad Vendor; GB3 = CRIUM Herbal garden, Hyderabad.		

Fig. 2: HPTLC view of aqueous leaf extract of *G. sylvestre* samples (GBHG1 as GB1, GBHG2 as GB2, GBHG3 as GB3). Detector developed observation at 366 nm UV region (Solvent System - Toluene: Ethyl acetate: Methanol in 7:2:1 ratio).



Quality control and quality assurance parameters

The quality control and quality assurance studies performed using WHO, IPC, UPC and AOAC standard methods are given in Tables 5 and 6. The microbial load (cfu/g) and heavy metals concentration were found within the permissible limits (ppm).

The aflatoxins toxic contamination estimation showed no detectable levels of aflatoxins in the drug samples tested in the present study (Table 7). The pharmacognostical observations also reveal the same (Fig. 3).

Further the estimated foreign matter, moisture content and ash values indicate the absence of any adulterants as well as free from contamination. Similar reports have also been reported in various samples (WHO, 1998; Anonymous, 2006; Anonymous, 2007; Sagar et al., 2015).

Fig. 3: Pharamacognostical standardization of *G. sylvestre* leaves.

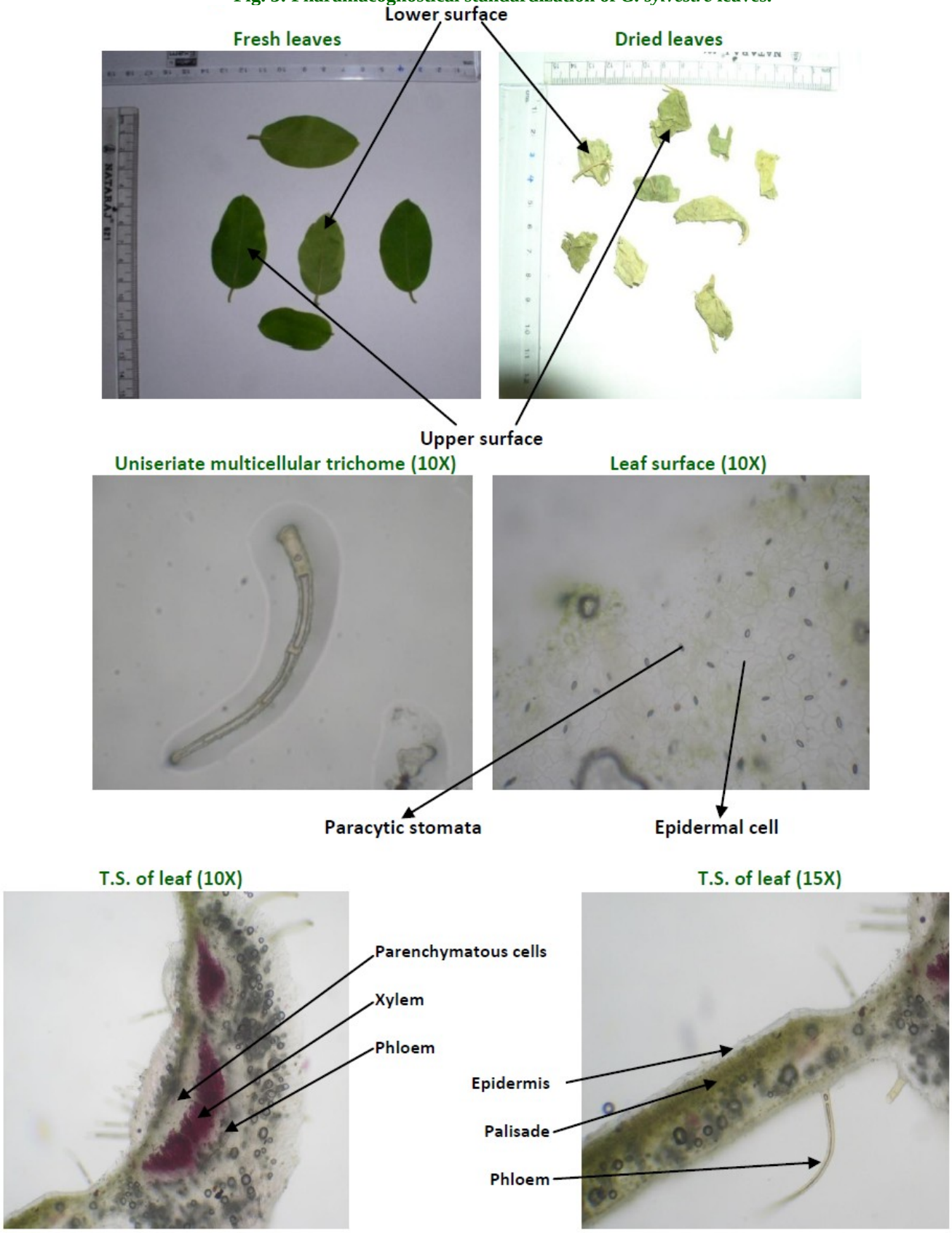


Table 5. Estimation of microbial load and contamination in *G. sylvestre* samples.

Parameters analyzed	Results			WHO and API limits
	Sample 1 - GB1	Sample 2 - GB2	Sample 3 - GB3	
Total bacterial count	$22X10^2$	14X10	12X10	10^5 cfu/g
Total fungal count	$43X10^2$	$79X10^2$	$35X10^2$	10^3cfu/g
Salmonella spp.	Absent	Absent	Absent	Nil
Staphylococcus aureus	Absent	Absent	Absent	Nil
Escherichia coli	Absent	Absent	Absent	Nil
The drug samples represented were procured from the regions: GB1 = Delhi Vendor; GB2 = Hyderabad Vendor; GB3 = CRIUM Herbal garden, Hyderabad.				

Table 6. Estimation of heavy metals contamination in *G. sylvestre* samples.

Parameters analyzed	Results			WHO and API limits
	Sample1 - GB1	Sample2 - GB2	Sample3 - GB3	
Arsenic	Not detected	Not detected	Not detected	3.0 ppm
Cadmium	Not detected	Not detected	Not detected	0.3 ppm
Lead	Not detected	Not detected	Not detected	10.0 ppm
Mercury	Not detected	Not detected	Not detected	1.0 ppm
The drug samples represented were procured from the regions: GB1 = Delhi Vendor; GB2 = Hyderabad Vendor; GB3 = CRIUM Herbal garden, Hyderabad.				

Table 7. Estimation of aflatoxins contamination in *G. sylvestre* samples.

Parameters analyzed	Result			WHO and API limits
	Sample1, - GB1	Sample2, - GB2	Sample3, - GB3	
B1	Not detected	Not detected	Not detected	0.5 ppm
B2	Not detected	Not detected	Not detected	0.1 ppm
G1	Not detected	Not detected	Not detected	0.5 ppm
G2	Not detected	Not detected	Not detected	0.1 ppm
The drug samples represented were procured from the regions: GB1 = Delhi Vendor; GB2 = Hyderabad Vendor; GB3 = CRIUM Herbal garden, Hyderabad.				

Conclusion

The present pharmacognostical and physicochemical data have shown that the collected drug samples of *G. sylvestre*, GB1, GB2 and GB3 from Delhi vendor, Hyderabad vendor and from CRIUM Herbal garden, Hyderabad are free from adulterants. The results on quality control and quality assurance revealed the samples can be used for the preparation of classic and patent, coded branded, Ayurvedic and Unani formulations. The results also revealed that the drug samples taken for study are free from toxic substances like toxic microbes, heavy metals and aflatoxins. The study of different samples of drug can provide referential support to botanical identification, reinvestigation and pharmacovigilance of the drug.

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References

Anonymous, 2006. The Ayuervedic Pharmacopeia of India. Part-I (V). The Controller of Publications Civil Lines, Delhi. pp.128-130.

Anonymous, 2007. The Unani Pharmacopeia of India. Part-I (II). Ministry of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy (AYUSH), Government of India, New Delhi. pp.45-46.

AOAC, 2000. Official Methods of Analysis of Association of Official Analytical Chemists (AOAC).17th Edn. AOAC International.

Britto, J. De A., Rekha, G.S., Simanek, V., Kosina, P., 2010. Molecular characterization of *Gymnema sylevstre* (Retz.) R.Br. in Tirunelveli hills in the Western Ghats. J. Adv. Plant Sci. 23(1), 35-37.

Chakravarthi, D.C., 1981. Isolation of gymnemagenin, the sapogenin from *Gymnema sylvestre* R.Br. (Asclepiadaceae). J. Inst. Chemist. 53, 155-158.

Chopra, R.N., Nayar, S., Chopra, I.C., 1966. In: Glossary of Indian Medicine. CSIR Publication, New Delhi. 128p.

Deokule, S.S., Pokhakar, A.A., 2010. Pharmacognostic study of leaf of *Gymnema sylevstre* (Retz.) R.Br. Ex. Schulters. J. Econ. Taxon. Bot. 34(1), 18-24.

Johansen, D.A., 1940. Plant Microtechnique. McGraw Hill Book Company Inc., New York and London. pp.181-186.

Kirtikar, K.R., Basu, B.D., 1984. Indian Medicinal Plants. Vol. 3. Bishen Sigh Mahendra Pal Singh, Dehradun. 1625p.

Liu, X., Ye, W., Yu, B., Zhao, S., Wu, H., Che, C., 2004. Two new flavonol glycosides from *Gymnema sylvestre* and *Euphorbia ebracteolata*. J. Carb. Res. 39(4), 891-895.

Rahaman, M.M., Habib, M.R., Hasan, M.A., Al Amin, M., Saha, A., Mannan, A., 2014. Comparative assessment on *in vitro* antioxidant activities of ethanol extracts of *Averrhoa bilimbi*, *Gymnema sylvestre* and *Capsicum frutescens*. J. Pharm. Res. 6(1), 36-41.

Romaiyan, A., Liu B., Asar-Anane, H., Maity, C.R., Chatterjee, S.K., Koley, N., Biswas, T., Chatterji, A.K., Huang, G.C., Amiel, S.A., Persaud, S.J., Jones, P.M., 2010. A noval *Gymnema sylvestre* extract stimulate insulin secretion from human islets *in vivo* and *in vitro*. J. Physiol. Res. 24(9), 1370-1376.

Sagar, P.K., 2013. Emerging Trends of Selective Medicinal Plants used in preparation of traditional Ayurvedic and Unani, patent formulated medicines, having effective anti-diabetic, hyperglycaemic medicinal values - A review. Int. J. Nat. Prod. Sci. 3(4), 1-19.

Sagar, P.K., Kazmi Munawwar Husain, Rasheed, M.A., 2015. HPTLC. Finger printing and standardization comparative screening, assessment of Anti-diabetic Gurmarbuti leaves (*Gymnema sylvestre* R.Br.) samples. J. Med. Chem. Drug Disc. 3, 20-36.

Sahu, N.P., Mahato, S.B., Sarkar, S.K., Poddar, G., 1996. Triterpenoid saponins from *Gymnema sylvestre*. J. Physiol. Chem. 41(4), 1181-1185.

Sairam, R.P., Rama Gopal, G., Lakshmi Sita, G., 1998. In vitro multiplication of *Gymnema sylvestre* R.Br.-An important medicinal plant. Curr. Sci. 75(8), 843-845.

Vimalanathan, S., Ignacimuthu, S., Hudson, J.B., 2009. Medicinal plants of Tamil Nadu (Southern India) are a rich source of antiviral activities. J. Pharm. Biol. 47(5), 422-429.

Wagner, H., Bladt, S., Zgainski, E.M., 1984. Plant Drug Analysis, A Thin Layer Chromatography Atlas (2nd Edn.). Springer-Verlag, Germany.

WHO, 1998. Quality Control Methods for Medicinal Plant Materials. World Health Organization, Geneva. pp.25-28.

Ye, W., Liu, X., Zhang, Q., Che, C.T., Zhao, S., 2001. Antisweet saponins from *Gymnema sylvestre*. J. Nat. Prod. 64(2), 232-235.

Ye, W.C., Zhang, Q.W., Liu, X., Che, C.T., Zhao, S.X., 2000. Oleanane saponins from *Gymnema sylvestre*. J. Physiol. Chem. 53(8), 893-899.

Yoshikawa, K., Arihara, S., Matsuura, K., Miyaset, T., 1992. Dammarane saponins from *Gymnema sylvestre*. J. Physiol. Chem. 31, 237-241.