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# Pharmacognostic standardisation and high performance thin layer chromatography fingerprint profile of *Gymnema sylvestre* R. Br. leaves

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**Abstract**
**Introduction**: *Gymnema sylvestre* R. Br. (Family: Asclepiadaceae) commonly called as Madhunashini (Sugar Killer) in Sanskrit is a high valued medicinal plant due to its principal phytoconstituent Gymnemic acid mainly found in its leaves.
**Methods**: *Gymnema sylvestre* R. Br. leaf, its powder and leaf extracts were studied using various standardization parameters like macroscopical, microscopical, physicochemical, phytochemical and fluorescence analysis. Further, HPTLC fingerprint profile was developed for methanolic leaf extract.
**Results:** Macroscopically, the leaves were simple, petiolate, broadly ovate in shape, acute apex and rounded base with reticulate venation. Microscopically, the leaf showed the presence of fan shaped vascular bundle, epidermal cells covered trichomes, xylem lying towards upper and phloem towards lower epidermis. Powder microscopy of leaves revealed the presence of vessels, calcium oxalate crystals, fibers, etc. The study also included quantitative leaf microscopy, histochemical and fluorescence analysis. Physicochemical analysis revealed that the water-soluble extractive value of leaf was three times higher than alcohol soluble extractive value. Preliminary phytochemical screening showed the presence of various primary and secondary metabolites *viz*. Aleurone grains, Alkaloids, Flavonoids, etc. A developed HPTLC fingerprint profile of methanolic leaf extract is unique for *Gymnema sylvestre* R. Br. and can be used as an identifying marker.
**Conclusion**: The data generated from the present pharmacognostical studies are significant for establishing correct identification, standardization and authentication of genuine plant material.

**Keywords:** *Gymnema sylvestre* R. Br.; pharmacognostic evaluation; phytochemical; physicochemical; HPTLC fingerprint profile

## 1. Introduction
The Indian traditional system of medicine namely Ayurveda and Siddha are primarily plant based system and use of plants as medicine is dated back to early man (Desai and Chanda, 2014 and Phillipson, 2001) [4, 20] . They constitute an effective source of traditional and modern medicine and play an important role in health care program. Therefore, it becomes extremely important to make an effort towards standardization of plant material as medicine (Nisha Raj Radhamany, 2012) [18]. The process of standardization can be attained by stepwise pharmacognostic studies (Ozarkar, 2005) [19]. Pharmacognosy is an essential measure of authentication in which most of the attention is paid to quality indices including macro- and microscopic examination, fluorescence and histochemical analysis, determination of ash values, moisture content, extractive values, qualitative and quantitative chemical evaluation and chromatographic examination (WHO, 1996 a and b) [24, 23].
*Gymnema sylvestre* R. Br. is a high valued multipurpose medicinal plant belonging to Asclepiadaceae family. It is a slow growing herb with a huge demand in the pharmaceutical industries. Traditionally, the herb is well known as 'Gudmar' in Hindi and 'Madhunashini' in Sanskrit due to its sugar masking property (Kumar, 2016 and Anonymous, 2016) [14, 2]. The species is native to tropical and subtropical Asia, Oceania, South Africa and Australia (Rapini *et al*., 2003) [21].
The medicinal properties of this climber are mainly due to Gymnemic acids present in its leaves (Nikalje *et al*., 2012) [17]. Hence, the leaves are widely used in almost all the Indian system of medicines as an effective natural remedy for diabetes, arthritis, anemia, osteoporosis, hypercholesterolemia, cardiopathy, asthma, constipation, microbial infections and indigestion. Moreover, it possesses antimicrobial, diuretic, stomachic, anti-hypercholesteremic, hepatoprotective and antisaccharine properties (Komalavalli and Rao, 2000 and Nadkarni, 1996 b) [13, 16].
Thus, the main objective of the present study was to establish botanical and chemical standards like pharmacognostic characterization, physicochemical analysis, preliminary phytochemical testing and HPTLC fingerprint profiling of leaves that would help to prepare a monograph for the proper identification of the plant.

## 2. Materials and methods
### 2.1. Plant material
*Gymnema sylvestre* R. Br. Plants were collected from Alibaug, Raigad district, Maharashtra, India. The plant was authenticated from Blatter Herbarium, St. Xavier's College, Mumbai. The fresh mature leaves were used for macroscopic and microscopic studies and remaining leaves were shed dried, ground into powder and stored in airtight container for further studies.

### 2.2. Chemicals and Reagents
All the chemicals and reagents used were of analytical grade, purchased from SDFCL, Mumbai (India), E. Merck Limited India and Hi-Media Laboratories, Mumbai, India.

### 2.3. Pharmacognostic evaluation
#### 2.3.1. Macroscopic characteristics
Macroscopic characteristics of leaf and leaf powder were determined according to standard methods. Leaves were macroscopically examined for color, odour, texture, taste, size and shape (Kokate *et al*., 2008) [12].

#### 2.3.2. Microscopy
Microscopic studies were carried out by preparing thin free hand section of leaves of *Gymnema sylvestre* R. Br. The sections were stained with safranin and powdered leaf was stained with pholoroglucinol- hydrochloric (1:1) solution. The sections were cleared with chloral hydrate solution and stained with Phloroglucinol- hydrochloric acid (1:1). Powder of the dried leaves used for the observation was separately treated with pholoroglucinol-hydrochloric acid (1:1) solution and diluted safranin. Sections and powder were examined under compound microscope.
Leaves were also studied for their quantitative microscopy, which includes Stomatal Number, Stomatal Index, Pallisade Ratio, Vein Islet Number and Vein Termination Number (Kokate *et al*., 2008 and Khandelwal, 2016) [12,10]. Photomicrographs were obtained under 10 X and 45 X by observing free-hand sections of leaf under compound binocular microscope.

#### 2.3.3. Fluorescence studies
The fluorescence analysis of powdered leaf and the leaf extracts in different solvents were placed on grease free slide. It was treated with 1-2 drops of freshly prepared reagents and examined under short ultra-violet lamps (254 nm), long (365 nm) and visible light wavelengths. Finally, the change in color of the leaf powder and leaf extract was noted down (Chase & Pratt, 1949; Kokashi *et al.*,1958) [3, 11].

#### 2.3.4. Histochemical Analysis
Leaves of *Gymnema sylvestre* R. Br. were washed thoroughly, blotted dry, and then used for histochemical study. Transverse section of leaf was placed on grease free clean microscopic slide and were treated with different reagents like Iodine solution, Ferric chloride (10 %), Conc. HCL, Ruthenium red, Sudan red, Alcoholic picric acid, Conc. $H_2SO_4$ and Dragendorff's reagent. These sections were then observed under 10X and 45X magnification.

#### 2.3.5. Physico-chemical evaluations
Physicochemical parameters like total ash, water-soluble ash, acid-insoluble ash, moisture content, alcohol and water-soluble extractive values, swelling index and foaming index were determined according to the well-established official method and procedure (Anonymous, 2016; WHO, 1992) [2, 25]. The tests were performed in triplicates and results were expressed as mean ± S.D.

#### 2.3.6. Preliminary phytochemical analysis
Phytochemical analysis was performed as per standard method (Khandelwal, 2016) [10]. 1 gm of powdered leaf was soaked overnight in 10 ml of solvents having different polarity *viz*., Water, Chloroform, Methanol and n-Hexane, filtered and subjected to qualitative tests for the identification of various phytoconstituents.

#### 2.3.7. HPTLC fingerprint profile
A qualitative densitometric HPTLC analysis was performed with methanolic leaf extract using winCATS software for the development of characteristic fingerprint profile. 10 µl of extract were applied in triplicate on precoated TLC aluminium plate (silica gel 60 $F_{254}$ - Merck) with the help of CAMAG Linomat 5 sample applicator equipped with 100 µL syringe (Hamilton, Bonaduz, Switzerland). The band length was kept as 8 mm. After application, the vertical development of plate was carried out in a twin trough chamber (CAMAG, Switzerland) pre-saturated using Toluene: Chloroform: Ethyl alcohol as mobile phase (10 ml) in the ratio of 4:4:1 (v/v/v) for 20 minutes at room temperature. The TLC plates were developed up to 8 cm and then dried at room temperature. The plate was further derivatized by dipping in Anisaldehyde sulphuric acid reagent and dried at room temperature. After drying, the plate was heated on HPTLC Plate heater at 110 ºC for 10 sec. and photo documented using photo-documentation chamber (CAMAG REPROSTAR 3). Densitometric scanning was then performed at visible light, 366 nm and 254 nm using CAMAG TLC scanner 4 with winCATS software version 1.4.6. The slit dimension used was 6.0 × 0.45 mm with scanning speed of 20 mm/sec throughout the analysis.

## 3. Results
### 3.1. Macroscopic analysis
Leaves are opposite, elliptic in shape and cordate at base (Fig. 1A). In addition, they are simple, petiolate with entire margin, acute apex and rounded base. The venation is reticulate, pubescent on both surfaces, however, the dorsal one is highly pubescent; about 1.5- 7.2 cm long and 1 to 4 cm broad, yellowish brown on adaxial surface and dark green on abaxial surface (Fig. 1B).

### 3.2. Microscopic analysis
#### 3.2.1. T.S of leaf
Transverse section of leaf shows a typical dorsiventral structure. The epidermal cells of the lamina are single layered, having rectangular shape with outer convex wall and thin cuticle. Epidermis of both the surfaces are externally covered by unicellular and uniseriate multicellular (2-5 celled) trichomes. Trichomes are slightly curved at the bulbous base. Single layer of palisade cells are present below the epidermis, followed by 2-3 layered spongy parenchyma. Spongy parenchyma shows the presence of starch grains and different types of calcium oxalate crystals. Palisade tissue is single layered, cylindrical, compact and occupies one-third thickness of lamina. The spongy parenchyma cells are lobed, four to five layered and loosely arranged. Spongy parenchyma shows the presence of starch grains and different types of calcium oxalate crystals *viz*. prism, rosette and needle shape.
The midrib is broadly hemispherical on the abaxial side with short lump on the adaxial side. Multicellular unbranched trichomes consist of single rows of cells on both sides of midrib. Also, there is a fan shaped vascular bundle in the centre of the midrib. Each vascular bundle is compact and collateral, surrounded by parenchymatous cells. The xylem is characterized by the presence of small vessels, tracheids

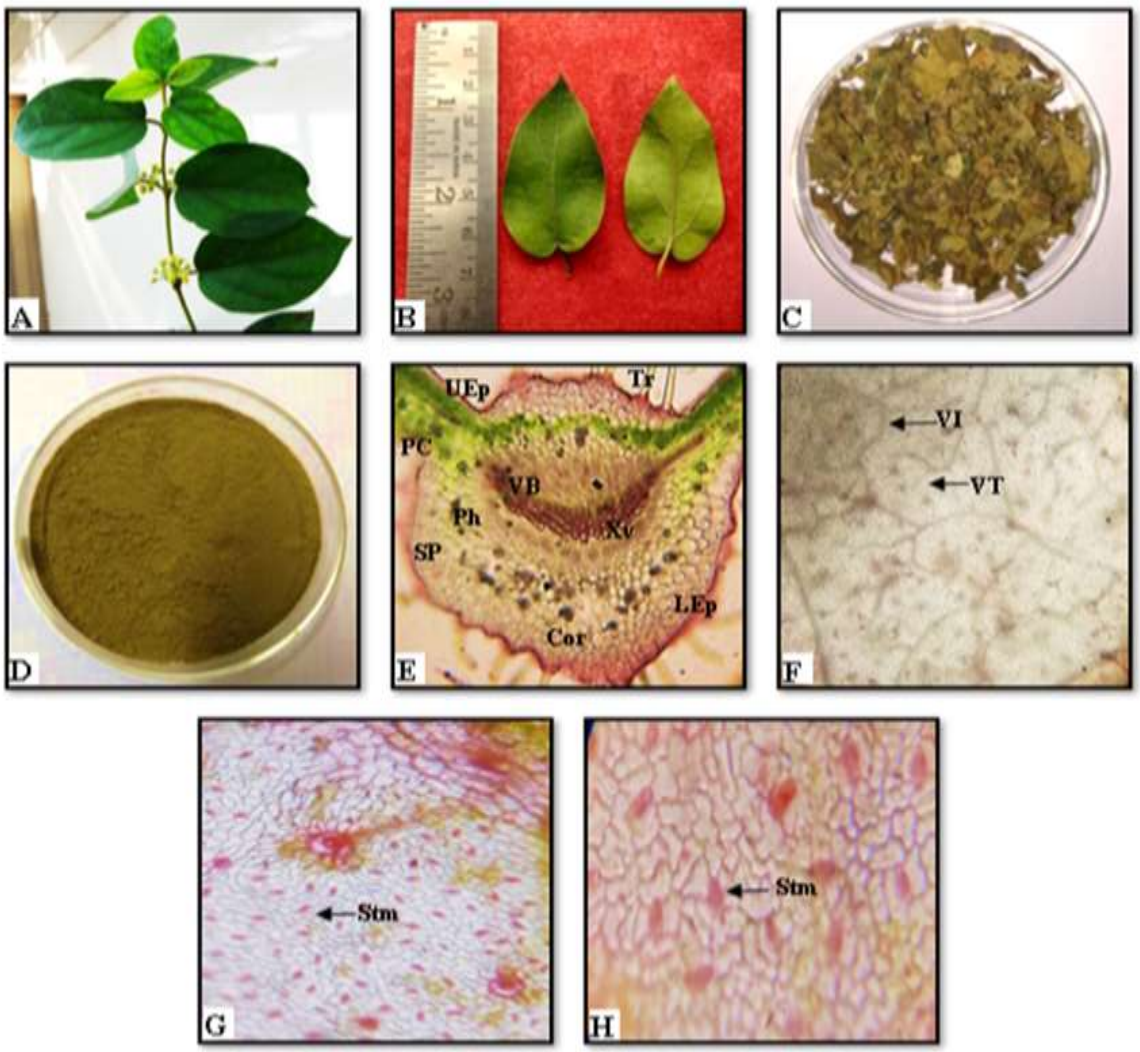
and fibres and lies towards upper epidermis and phloem lies towards lower epidermis beneath the xylem (Fig. 1E).

### 3.2.2. Organoleptic characteristics of leaf powder

Organoleptic evaluation can be carried out by visual inspection. This evaluation provides the simplest and quickest means to establish the identity and purity and thereby ensure quality of a particular drug (WHO, 1992; Mukherjee, 2008) [25, 15]. Organoleptic characters of powder are depicted in Table 1.

### 3.2.3. Quantitative determination

Leaf constants *viz*. Vein islet number, Vein termination number (Plate 1 F), Palisade ratio, Stomatal number (Fig. 1 G and H) and Stomatal index were measured and the values for the same are tabulated in Table 2.



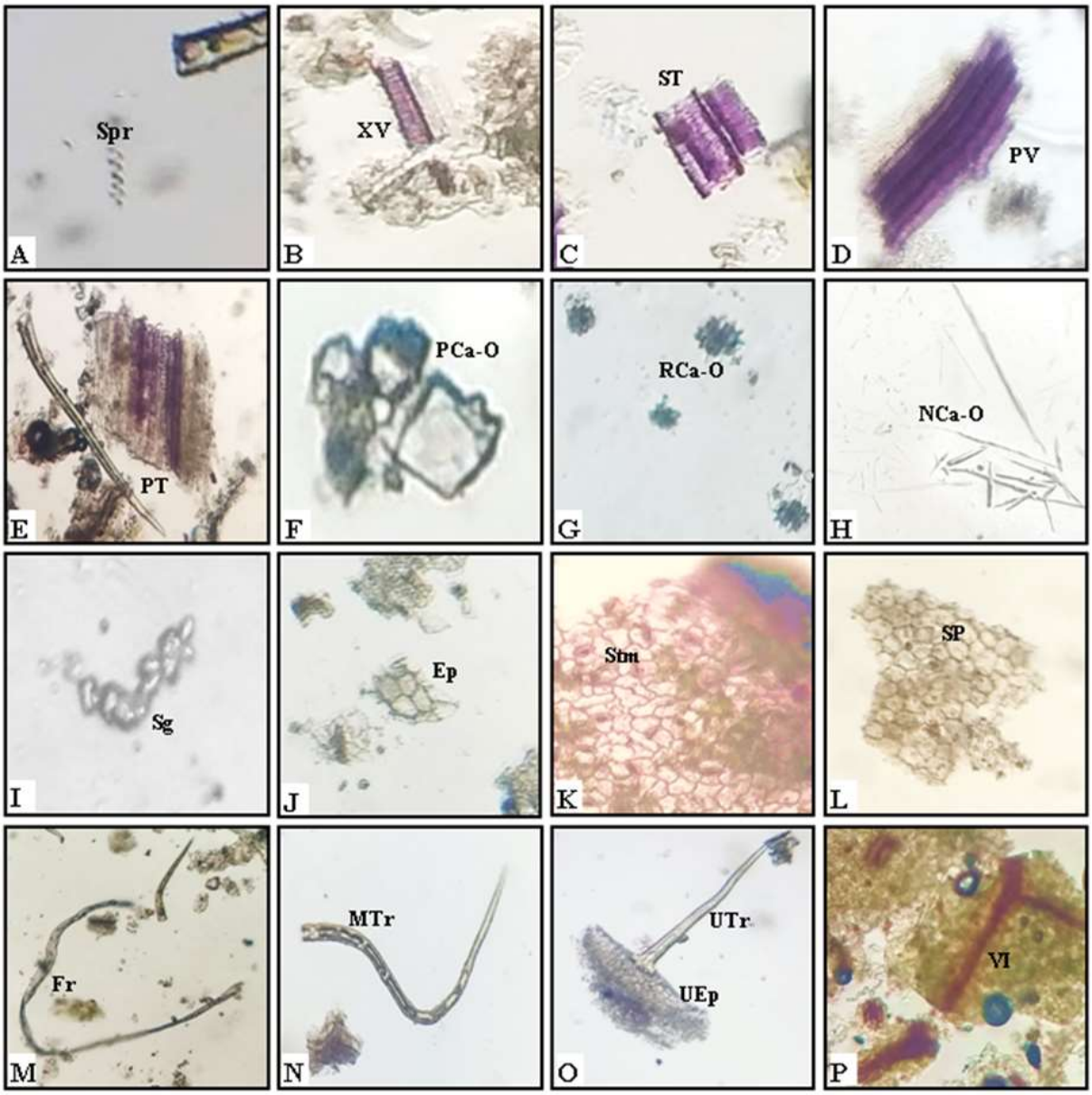

**Keywords:** A – Flowering plant, B – Leaf showing length, C – Dried leaves, D – Leaf Powder, E – T. S. of leaf (UEp: Upper epidermis; LEp: Lower epidermis; PC: Palisade cell; Tr: Trichome; Cor: Cortex; VB: Vascular Bundle; Ph: Phloem; Xy: Xylem; SP: Spongy parenchyma), F – Leaf in surface view showing Vein islet (VI) and Vein termination (VT), G – Stomata (Stm) under 10X; H – Stomata (stm) under 45X.

**Fig 1:** Macroscopy and microscopy of *Gymnema sylvestre* R. Br. leaf

### 3.2.4. Powder microscopy of leaf powder

The powdered leaf material of *Gymnema sylvestre* R. Br. was yellowish green to brown in color. The powder characteristics are shown in Plate 3.2.

Powder microscopy of leaves revealed the presence of vessels (Fig. 2 A, B, C, D, E), different types of calcium oxalate crystals like prism, rosette and needle shape (Fig. 2 F, G, H), starch grains, group of epithelial cells, stomata, spongy parenchymatous cells, fibers (Fig. 2 I, J, K, L, M), multicellular, unicellular trichome with upper epidermal cells and vein islet (Fig. 2 N, O, P).




**Keywords:** A – Spiral vessel (Spr), B – Xylem vessel (XV), C – Vessels showing spiral thickening (ST), D – Pitted vessels (PV), E – Spiral Vessels showing pitted thickening (PT), F – Prism shape of Calcium oxalate crystal (PCa-o), G– Rosette shape of Calcium oxalate crystals (RCa-o), H – Needle shape of Calcium oxalate crystal (NCa-o), I – Starch grains (Sg), J– Group of Epithelial cells (Ep), K – stomata (stm), L – Spongy parenchymatous (SP) cells, M– Fibres, N– Multicellular Trichome (MTr), O – Unicellular Trichome (UTr) with upper epidermal cells (UEp) , P – Fragment of leaf showing vein islet

**Fig 2:** Powder microscopy of *Gymnema sylvestre* R. Br. leaf

**Table 1:** Organoleptic evaluation of *Gymnema sylvestre* R. Br. Leaf

| Parameters | Observation |
|---|---|
| Color | Upper surface dark green and lower surface light green |
| Odour | Unpleasant |
| Texture | Papery |
| Taste | Bitter |

**Table 2:** Quantitative microscopy of *Gymnema sylvestre* R. Br. Leaf

| Parameters | Value (in $mm^2$ area) |
|---|---|
| Stomatal number | 11.20 ± 0.59 |
| Stomatal index | 21.68 ± 0.93 |
| Vein – islet number | 8.74 ± 0.75 |
| Veinlet termination number | 13.08 ± 0.68 |
| Palisade ratio (per epidermal cell) | 11.40 ± 0.48 |

### 3.3. Fluorescence study

Fluorescence analysis of *Gymnema sylvestre* R. Br. leaf powder and leaf extract in different solvents *viz*. Water, Methanol, n-Hexane and Chloroform showed their characteristic fluorescent color. Change in color was examined under long UV (366nm), UV (254nm) and visible light. The results are presented in table 3 and 4.

**Table 3:** Fluorescence analysis of *Gymnema sylvestre* R. Br. leaf powder

| No. | Fluorescence tests | Observation in/under | | |
|---|---|---|---|---|
| | | Day light | UV 254 nm | UV 366 nm |
| 1. | Powder as such | Black | Light Green | Green |
| 2. | Powder + 1N HCl | Black | Brown | Green |
| 3. | Powder + 1N NaOH in water | Black | Brown | Brown |
| 4. | Powder + $HNO_3$ (1:1) | Black | Yellow Orange | Brown Orange |
| 5. | Powder + $H_2SO_4$ (1:1) | Black | Fluorescent Green | Yellow Orange |
| 6. | Powder + 1 % Picric acid | Black | Black | Orange |
| 7. | Powder + 5% Iodine | Black | Brown | Yellow Orange |
| 8. | Powder + 5% FeCl3 | Black | Brown | Brown |
| 9. | Powder + 25% $NH_3$ + $HNO_3$ | Light Brown | Light Green | Pale Green |
| 10. | Powder + Conc. $HNO_3$ | Black | Orange | Green |
| 11. | Powder + 50% KOH | Brown | Dark Brown | Dark Green |
| 12. | Powder + Methanol | Dark Green | Dark Green | Black |
| 13. | Powder + Ethanol | Dark Red | Dark Green | Black |
| 14. | Powder + Toluene | Black | Fluorescent Green | Green |
| 15. | Powder + Glacial acetic acid | Yellow | Fluorescent Green | Fluorescent Green |

**Table 4:** Fluorescence analysis of various extracts of *G. sylvestre* R. Br. Leaf

| No. | Solvents | In ordinary day light | UV - 254 nm | UV - 366 nm |
|---|---|---|---|---|
| 1. | Aqueous | Black | Brown | Green |
| 2. | Methanol | Black | Green | Yellow Orange |
| 3. | n-Hexane | Black | Green | Dark Green |
| 4. | Chloroform | Dark green | Green | Brown |
| 5. | Petroleum Ether | Black | Green | Dark Green |
| 6. | Alcohol | Black | Green | Orange |

### 3.4. Histochemical analysis

During the present study, free hand sections of *Gymnema sylvestre* R. Br. leaf were taken and treated with the respective reagents to confirm the presence of different ergastic substances like calcium oxalate crystals, tannins, etc. The results are shown in Table 5.

**Table 5:** Histochemical Test for *Gymnema sylvestre* R. Br. leaf sections

| No. | Reagents | Test for | Color observation | Leaf section |
|---|---|---|---|---|
| 1. | Iodine | Starch | Blue | - |
| 2. | Ferric chloride (10 %) | Tannin | Black | + |
| 3. | Dil. HCl + Phloroglucinol | Lignin | Pink | + |
| | Conc. HCl | Ca- oxalate crystals | Dark black | + |
| | Ruthenium red | Mucilage | Pink | + |
| 4. | Sudan red | Oil globules | Red | - |
| 5. | Alcoholic Picric acid | Aleurone grains | Bluish Green | - |
| 6. | Conc. $H_2SO_4$ | Stone cells | Green | - |
| 7. | Dragendorff's reagent | Alkaloids | Light orange | + |

Keywords: '+' – Present; '-' – Not detected

### 3.6. Physicochemical Evaluation

Physicochemical analysis of leaf powder *viz.* the Moisture content, Swelling index (ml), Foaming index, Ash values and Extractive values are presented in Table 6.

**Table 6:** Physicochemical parameters for *G. sylvestre* R. Br. Leaf

| No. | Physicochemical Parameters | Result (Mean ± S.D.) |
|---|---|---|
| 1. | Moisture Content (% w/w) | 5.73 ± 0.65 |
| 2. | Swelling Index (ml) | 8.00 ± 0.81 |
| 3. | Foaming Index | 111.10 ± 0.18 |
| 4. | Ash values | |
| | a. Total ash value (% w/w) | 11.83 ± 0.21 |
| | b. Acid insoluble ash value (% w/w) | 3.60 ± 0.16 |
| | c. Water soluble ash value (% w/w) | 7.46 ± 0.34 |
| 5. | Extractive values | |
| | a. Alcohol Soluble (% w/w) | 11.12 ± 0.49 |
| | b. Water Soluble (% w/w) | 46.76 ± 0.48 |

### 3.7. Preliminary phytochemical Evaluation

Preliminary phytochemical screening of leaves in four different solvents (n-Hexane, Chloroform, Methanol and Water) showed the presence of primary metabolites *viz.* amino acids, carbohydrates, fats and fixed oils, proteins and starch and secondary metabolites *viz.* tannins, alkaloids, steroids triterpenoids, aleurone grains, saponins, acid compounds, glycosides, mucilage, flavonoid, resins, essential oils and anthraquinones (Table 7).

**Table 7:** Preliminary phytochemical screening of *G. sylvestre* R. Br. leaf extracts

| No. | Phytoconstituents | AE | CE | ME | n-HE |
|---|---|---|---|---|---|
| 1. | Acid compounds | + | ND | ND | + |
| 2. | Aleurone grains | + | + | + | + |
| 3. | Amino acids | + | ND | + | + |
| 4. | Carbohydrates | + | + | + | ND |
| 5. | Fats and Fixed oils | ND | + | ND | + |
| 6. | Protein | + | + | ND | ND |
| 7. | Starch | + | + | + | ND |
| 8. | Alkaloids | + | + | + | ND |
| 9. | Anthraquinones | + | ND | + | ND |
| 10. | Essential oils | + | ND | ND | ND |
| 11. | Flavonoid | + | ND | + | ND |
| 12. | Glycosides | + | ND | + | + |
| 13. | Mucilage | + | + | + | + |
| 14 | Resins | + | ND | + | ND |
| 15 | Saponins | + | + | ND | ND |
| 16. | Steroids | ND | + | ND | ND |
| 17. | Tannins | + | ND | ND | + |
| 18 | Triterpenoids | + | + | + | + |

**Keywords:** AE - Aqueous Extract; CE - Chloroform Extract; ME - Methanolic Extract; n-HE- n-Hexane Extract; ND - Not detected and + - Detected

### 3.8. HPTLC Fingerprint profile

HPTLC fingerprint profile of methanolic leaf extract showed characteristic band pattern before and after derivatization with Anisaldehyde sulphuric acid. Rf values under different wavelengths before and after derivatization are tabulated in table 8 (Fig. 3).

**Table 8:** HPTLC Fingerprint Profile (Rf values) of Methanolic extract of *G. sylvestre* R. Br. Leaf

| No. | Before Derivatization | After Derivatization | |
|---|---|---|---|
| | 254nm | 366 nm | 540 nm |
| 1. | 0.02 | 0.05 | 0.01 |
| 2. | 0.22 | 0.10 | 0.02 |
| 3. | - | 0.15 | 0.10 |
| 4. | - | 0.19 | 0.19 |
| 5. | - | 0.21 | 0.21 |
| 6. | - | 0.28 | 0.24 |
| 7. | - | 0.32 | 0.28 |
| 8. | - | 0.36 | 0.32 |
| 9. | - | 0.43 | 0.35 |
| 10. | - | 0.48 | 0.39 |
| 11. | - | 0.54 | 0.43 |
| 12. | - | 0.64 | 0.54 |
| 13. | - | 0.79 | 0.85 |
| 14. | - | 0.85 | 0.94 |
| 15. | - | 0.92 | - |

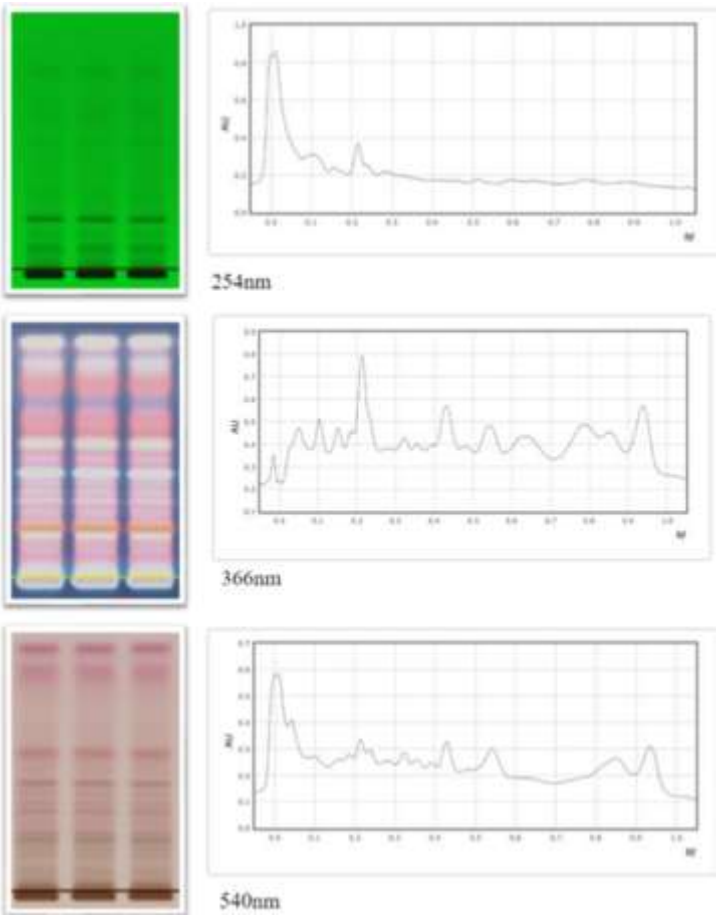



**Fig 3**: High performance thin layer chromatography finger print profile of *G. sylvestre* R. Br. leaf extract

## 4. Discussion

Pharmacognostic standardization including physicochemical and phytochemical evaluation have become of immense concern as it signifies the identification, authentication and detection of adulteration of herbal drugs (Kokashi *et al.*, 1958) [11]. The present study reports the pharmacognostic standardisation of *G. sylvestre* R. Br. leaf. Macro and microscopic characterization which constitutes the major part of pharmacognosy are found to be simple, reliable and inexpensive technique to establish the identity and purity of source materials (Edwin *et al*., 2008 and Anonymous, 1998) [5, 1].

Physicochemical analysis through the determination of Ash values and extractive values are useful for judging the purity of a plant material (Kala *et al*., 2011) [8]. Extractive values also help to determine the solubility of chemical compounds in a specific solvent (Sridharan and Chinna, 2016) [22]. Ash values were less in the proposed study, which indicates that foreign inorganic matter is present in a smaller amount in the *G. sylvestre* R. Br. leaf powder. Water and methanol extractive values were determined to find out the amount of water and methanol soluble components. Moisture content of crude drug is directly related to its stability when there are chances of microbial growth. The moisture content of the drug was not too high, thus it could discourage bacterial or fungal growth.

Fluorescence phenomenon exhibited by various chemical constituents showed different color in the visible range in day light and the UV light (which do not visibly fluorescence in day light). Hence, the results obtained from the present fluorescent studies will help in qualitative evaluation of crude drug (Desai and Chanda, 2014) [4].

Histochemical analysis is one of the valuable standardization tool for quality control of crude drugs to locate the presence of cell contents in the histological zones of the plant organs. The study revealed the occurrence of ergastic content, *viz*., tannin, lignin, Ca- oxalate crystals, mucilage and alkaloids in *G. sylvestre* R. Br. leaf sections.

Preliminary phytochemical analyses gives an indication of the presence of particular pharmacologically active metabolites in the plant. A variety of constituents *viz*. primary and secondary metabolites have been isolated from *G. sylvestre* R. Br. leaf extract (Table 7) that have been claimed to be pharmacologically active. This reveals that the plant part is medicinally important and can be explored further for its phytochemical and biological assays.

The evaluation and quality control of herbal medicines is moving a step ahead towards an integrative and comprehensive direction, in order to tackle the complex herbal drugs. Therefore, High-instrumental techniques are used for fingerprint analysis of the herbs and herbal drugs, HPTLC fingerprint profile as an identification and quality evaluation technique for medicinal plants are recommended by WHO since 1991. The unique band pattern obtained from present HPTLC fingerprint profile of leaf extract will serve as useful data for identification, quality control and detection of any type of adulteration, especially of similar species of this drug.

Also, a thorough literature survey reveals that numerous reports have been published on pharmacognostic standardisation and HPTLC fingerprint profile of leaf from different plants *viz*. *Terminalia catappa* leaf (Kadam *et al*., 2011) [7], *Psidium guajava* L. leaf (Kaneria and Chanda 2011) [9], *Bauhinia variegata* Linn. (Gunalan *et al*., 2012) [6], etc. Thus, the present work was carried out to report various necessary pharmacognostical standards of *Gymnema sylvestre* R. Br. leaves.

In this way, Pharmacognostic study gives the scientific information regarding the purity and quality of crude drug and is therefore considered as the preliminary step in the standardization process. Also, further studies are needed to identify, isolate, characterize and elucidate the structure of pharmacologically active compounds to establish it as a safe and effective drug.

## 5. Conclusion

The present study on Pharmacognostic standardization is an attempt to establish the diagnostic features of *Gymnema sylvestre* R. Br. The various parameters studied serve as a basis for proper identification, collection and investigation of the traditionally important medicinal plant *Gymnema sylvestre* R. Br. The results obtained can be employed as suitable quality control measures to ensure the purity, quality, safety and efficacy of *Gymnema sylvestre* R. Br. Further, this study will also reduce the chances of adulteration of plant material when it is available in powdered form. An efficient HPTLC fingerprint profile was developed with simple sample preparation protocol for detection of various secondary metabolite from the methanolic leaf extract of *Gymnema sylvestre* R. Br. Thus in this way, the detailed study of *Gymnema sylvestre* R. Br. leaf will help in its standardization process.

## 6. Acknowledgement

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