Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 664-668
Received: 10-03-2019
Accepted: 12-04-2019

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Chemo-profile development of *Gymnema sylvestre* R. Br. leaves using High performance thin layer chromatography (HPTLC) technique

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Abstract
Compounds derived from natural sources have been gaining importance since past few decades because of the vast chemical diversity they offer. This has led to phenomenal increase in the demand of herbal medicines which necessitates ensuring the quality, safety and efficacy of herbal drugs. One such reputed plant in the Ayurvedic system of medicine is *Gymnema sylvestre* R. Br. (Family: Asclepiadaceae), commonly called as "gurmar" for its distinct property as sugar destroyer. The plant exhibits a broad range of secondary metabolites which act as an effective natural remedy for diabetes, arthritis, diuretic anemia, osteoporosis, hypercholesterolemia and many other diseases. One of the important modern analytical technique for the qualitative, semi quantitative and quantitative phytochemical analysis of the secondary metabolites is High-performance thin-layer chromatography (HPTLC). In the present study, a pioneering attempt has been made to focus on HPTLC chemoprofiling of *Gymnema sylvestre* R. Br. leaf for presence of thirteen different secondary metabolites. Results of this phytochemical screening revealed the presence of all thirteen secondary metabolites in *Gymnema sylvestre* R. Br. leaf.

Keywords: *Gymnema sylvestre* R. Br., HPTLC, Secondary metabolites, Chemo-profiling, Leaf

Introduction
India is one of the 12 mega biodiversity centers having 45,000 plant species, 16 different agro climatic zones, 10 vegetative zones and 15 biotic provinces [1]. The country has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. In addition, it has the unique distinction of having six recognized systems of medicines in this category, *viz.* Ayurveda, Siddha, Unani, Yoga, Naturopathy and Homoeopathy [2].

Among the remedies used, plant drugs constitute an important part. A number of scientific investigations have highlighted the importance and the contribution of medicinal plants towards the development of new drugs [1]. Standardization and evaluation of these drugs is a very crucial parameter and is mostly based on phytochemical, pharmacological and allied approaches including various instrumental techniques like chromatography, microscopy and many other [3]. This will help to develop the drugs with fewer side effects, lower costs and better compatibility. The nature of phytoconstituents determine the pharmacological action of the plant material. Hence, the plants species may be considered as a biosynthetic laboratory for a magnitude of primary metabolites (carbohydrates, proteins and fats) as well as secondary metabolites (alkaloids, terpenoids, saponins, flavonoids, glycosides etc.) which exert a definite physiological effect [3].

Secondary metabolites represent a unique sources for pharmaceuticals, food additives, flavors and others industrial values. Though they are not essential for growth, energy conservation or for primary metabolism, plant require them to interact with its environment and other organisms [4]. Also, these phytocompounds are extremely useful to mankind as they are responsible for the desired therapeutic properties. To obtain these pharmacological effects from the plant materials, they may either be used in their crude form or the desired components can be extracted with suitable solvent and the resulting principle compound can then be utilized as therapeutic agent [5, 3].

Gymnema sylvestre R. Br. (Asclepiadaceae), popularly known as "Gudmaar" in Hindi and "Madhunasini" in Sanskrit for its distinctive property as sugar destroyer, is a reputed plant in the Ayurvedic system of medicines [6, 7]. It is a slow growing, perennial, woody climber, found in India and the southwestern region of China [8]. The plant is considered to be a good source of a large number of bioactive substances. The leaves of *Gymnema sylvestre* R. Br. have a growing demand in the

pharmaceutical trade since it is used in many of the crude as well as purified drugs in both traditional and modern systems of medicines [9].

Gymnema sylvestre R. Br. leaves have been widely used in Ayurvedic traditional system of medicine since the time immemorial. The leaves are bitter, acrid, thermogenic, antiinflammatory, anodyne, digestive and liver tonic [10, 11]. It is also used in the treatment of asthma, eye complaints, inflammations and snake bite. In addition, it possesses antimicrobial, antihypercholesterolemic [12], hepatoprotective [13] and sweet suppressing [14] and anti-arthritic activity [10].

Analytical separation techniques, for example, high-performance liquid chromatography (HPLC), high-performance thin layer chromatography (HPTLC), gas chromatography (GC) and mass spectrometry (MS) were among the most popular methods of choice used for quality control of raw materials and finished herbal products. Among these chromatographic methods, HPTLC has become the most potent tool for identification, authentication and for qualitative, semi-quantitative and quantitative analysis of herbal drugs [15, 16].

Also, HPTLC is one of the most versatile technique, known for its uniformity, purity profile, assay values, precision, simplicity and reliability of results. It can handle several samples of even divergent nature and composition. It gives better resolution and assessment of active components with minimum troubleshooting in a lesser time [17].

Thus, the focus area of the present research is to explore detailed phytochemical profile of *Gymnema sylvestre* R. Br. leaves using HPTLC technique which may lead to confirm the presence of phytochemicals responsible for its pharmacological activities.

2. Materials and Methods

2.1 Plant Material

Plants of *Gymnema sylvestre* R. Br. were collected from Alibaug and authenticated from Blatter Herbarium, St. Xavier's college, Mumbai. After authentication, the fresh leaves were collected, air dried, ground into fine powder and stored in airtight container at room temperature for further studies.

2.2 Chemicals and Reagents

All the chemicals and reagents used were of analytical grade, purchased from Sigma-Aldrich Chemical Co. The precoated TLC silica gel $60F_{254}$ plates were obtained from E. Merck (India).

2.3 Sample Preparation

Proper sample preparation is an important prerequisite for the successful HPTLC separation. Samples of *Gymnema sylvestre* R. Br. leaves were prepared according to standard method described by Wagner and Bladt (1984) [18]. All the samples were filtered through Whatman filter paper before HPTLC analysis.

2.4 High Performance Thin Layer Chromatography (HPTLC) Analysis

HPTLC chemo-profiling of *Gymnema sylvestre* R.Br. leaves was carried out for secondary metabolites *viz*. Alkaloid Drugs, Anthracene Derivatives, Arbutin Derivatives, Bitter Drugs, Cardiac Glycosides, Coumarin Drugs, Essential Oils, Flavonoids, Lignans, Pungent -Tasting Principles, Saponins, Triterpenes and Valepotriates as per the standard methodology described by Wagner and Bladt [20].

Analysis work was carried out on HPTLC equipment, CAMAG made (Muttenz, Switzerland) which consist of Linomat-V sample applicator fitted with a 100 µL syringe (Hamilton, Switzerland), CAMAG TLC visualizer, CAMAG TLC Scanner 3 and WinCATS software. Analysis was performed by using TLC precoated silica gel 60 F_{254} aluminium plates with 200 µm thickness (E. Merck, Mumbai, India). 10 µl of samples were applied to the plate using the Linomat-V sample applicator fitted with a 100 µL syringe. After the application, plates were developed in CAMAG twin-trough glass chamber presaturated for 20 mins at room temperature, with respective mobile phase. The TLC plates were developed up to the distance of 8 cm. After development, plates were dried at room temperature, derivatized with freshly prepared derivatizing reagents for 30 seconds and dried at room temperature. After drying plates were heated on HPTLC Plate heater at 110ºC for 10 mins. The plate was kept in CAMAG TLC visualizer and the images were captured. Densitometric scanning was then performed at 254nm, 366 nm and visible light using CAMAG TLC Scanner 3 with winCATS software version 1.4.6. [19, 20].

Table 1: Mobile phases and Derivatizing agents for respective secondary metabolites

Sr. No.	Phyto-constituents	Mobile phases	Derivatizing agents	Inference
1.	Alkaloid Drugs	Toluene: Ethyl Acetate: Methanol: Ammonia (30:30:15:1)	Dragendorff reagent	Brown zones appear immediately after derivatization.
2.	Anthracene Derivatives	Ethyl acetate: Methanol: Water (81:11:8)	Potassium hydroxide reagent	Yellow or red-brown fluorescence at 366nm.
3.	Arbutin Derivatives	Ethyl acetate: Methanol: Water (100:13.5:10)	Gibb's reagent	Blue violet bands at visible light.
4.	Bitter Drugs	Ethyl acetate: Methanol: Water (77:15:8)	Anisaldehyde – Sulphuric acid reagent	Red-violet, brown, blue green, blue, grey bands under visible light.
5.	Cardiac Glycosides	Ethyl acetate: Methanol: Water (81:11:8)	Sulphuric acid reagent	Yellow, brown and blue zones under visible light.
6.	Coumarin Drugs	Toluene: Ether (1:1saturated with 10% acetic acid) lower phase	Potassium hydroxide reagent	Blue, blue green and yellow fluorescence under 366 nm
7.	Essential Oils	Toluene: Ethyl acetate (93:7)	Anisaldehyde Sulphuric acid reagent	Blue, red, green and brown coloration in visible light.
8.	Flavonoids	Ethyl acetate :Formic acid: glacial acetic acid: water (10:0.5:0.5:1.3)	Sulphuric acid reagent	Blue, green and red fluorescence under UV-366 nm
9.	Lignans	Toluene: Ethyl acetate (70:30)	Sulphuric acid reagent	Blue fluorescence at 366 nm

10.	Pungent -Tasting Principles	Toluene: Ethyl acetate (70:30)	Vanillin – Sulphuric acid reagent	Lemon yellow and blue to violet bands in visible light.
11.	Saponins	Chloroform: Glacial acetic acid: Methanol: Water (64:32:12:8)	Vanillin – Sulphuric acid reagent	Blue, blue-violet and yellow brown zones in visible light.
12.	Triterpenes	Ethyl acetate: Glacial acetic acid: Formic acid: Water (90:3:3:2)	Anisaldehyde Sulphuric acid reagent	Blue violet dumb bell shape quenching under 366 nm.
13.	Valepotriates	Toluene: Ethyl acetate (75:25)	Anisaldehyde Sulphuric acid reagent	Violet and blue zone in visible light

3. Results and Discussion

Since long, medicinal plants have always been closely associated with our daily life, either as food products or for their medicinal value. Each medicinal plant that has been used in the traditional system of medicine is scientifically tested in order to bring forth its active principle that can be effectively used as a phytomedicine. Such phytomedicine have played a vital role in the past and will continue to do so in the future.

In this vast resource of phytoproducts there are various plants that are claimed to be effective against various ailments. *Gymnema sylvestre* R. Br. is one such plant that has been used for a long time in the Indian systems of medicine and is said to possess various pharmacological properties.

In the present study, *Gymnema sylvestre* R. Br. leaf extracts are used to evaluate the presence of thirteen major class of secondary metabolites. The results confirmed the presence of all thirteen secondary metabolites like Alkaloid Drugs, Anthracene Derivatives, Arbutin Derivatives, Bitter Drugs, Cardiac Glycosides, Coumarin Drugs, Essential Oils, Flavonoids, Lignans, Pungent-Tasting Principles, Saponins, Triterpenes and Valepotriates. Also, it was observed that the developed mobile phase and the particular derivatizing agents used gave well-resolved bands for secondary metabolites present in the leaf extract of *Gymnema sylvestre* R. Br.

The chemoprofile of *Gymnema sylvestre* R. Br. leaf extract provided a set of peaks with R_f values and their corresponding area percentage which indicates the presence of specific chemical compounds. The R_f values of respective compounds in all the chromatograms are given in table 3. The developed chromatograms for respective secondary phytocompounds of leaves are presented in figure 1.

Table 2: Detection of secondary metabolites in *Gymnema sylvestre* R. Br. leaves by HPTLC

Sr. No.	Phytoconstituents	Present/Absent
1.	Alkaloid Drugs	D
2.	Anthracene Derivatives	D
3.	Arbutin Derivatives	D
4.	Bitter Drugs	D
5.	Cardiac Glycosides	D
6.	Coumarin Drugs	D
7.	Essential Oils	D
8.	Flavonoids	D
9.	Lignans	D
10.	Pungent –Tasting Principles	D
11.	Saponins	D
12.	Triterpenes	D
13.	Valepotriates	D

Keywords: '**ND**'–Not detected and **'D'-**Detected

Table 3: Retention factor (R_f) of secondary metabolites in *Gymnema sylvestre* R. Br. leaves by HPTLC

Sr. No.	Phytoconstituents	R_f values
1.	Alkaloid Drugs	0.35, 0.52, 0.55, 0.80
2.	Anthracene Derivatives	0.27, 0.33, 0.49, 0.59, 0.65, 0.83, 0.88
3.	Arbutin Derivatives	0.32, 0.76, 0.82
4.	Bitter Drugs	0.28, 0.35, 0.41, 0.63, 0.86
5.	Cardiac Glycosides	0.27,0.32, 0.37, 0.41, 0.49, 0.52, 0.61, 0.68, 0.75, 0.81
6.	Coumarin Drugs	0.20, 0.36, 0.50, 0.73, 0.89
7.	Essential Oils	0.34, 0.47, 0.66, 0.73, 0.79
8.	Flavonoids	0.17, 0.19, 0.25, 0.38,0.51,0.77
9.	Lignans	0.30, 0.41, 0.51, 0.56, 0.67, 0.85
10.	Pungent -Tasting Principles	0.13, 0.16, 0.21, 0.27, 0.41, 0.67, 0.82
11.	Saponins	0.15, 0.20, 0.36, 0.39, 0.55, 0.76, 0.83, 0.85
12.	Triterpenes	0.19, 0.27, 0.38, 0.51, 0.63, 0.68,0.74
13.	Valepotriates	0.14, 0.20, 0.27, 0.34, 0.37, 0.43, 0.57, 0.67, 0.74, 0.85

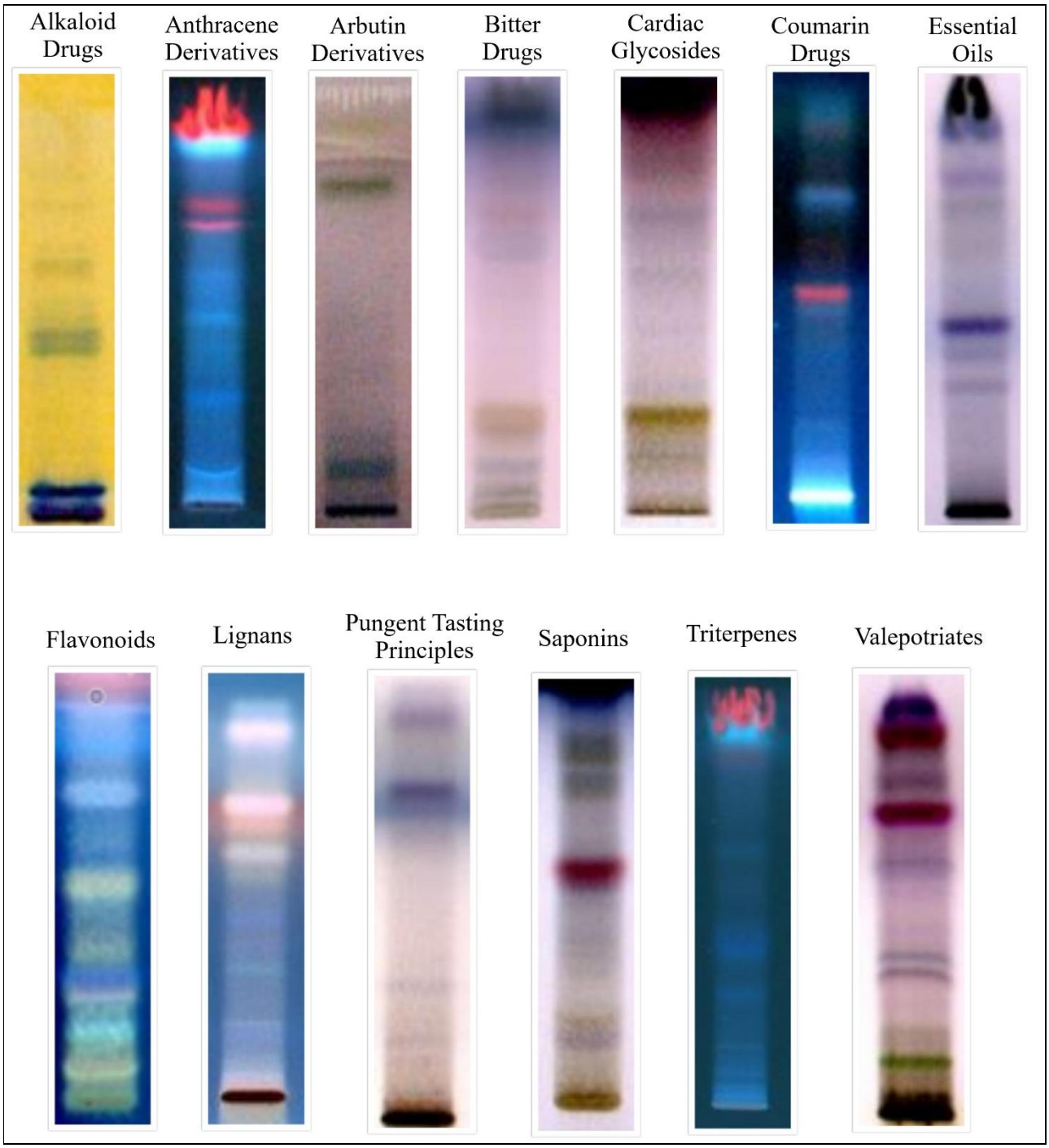

Fig 1: HPTLC chromatograms of secondary metabolites present in *Gymnema sylvestre* R. Br. leaf

Acknowledgement
The author and coauthors are grateful to Management, B.K.Birla College of Arts Science and Commerce (Autonomous), Kalyan, Maharashtra (India) for providing the essential facilities to carry out this research work. We would also like to thank Anchrom Laboratories for conducting training program on HPTLC technique.

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