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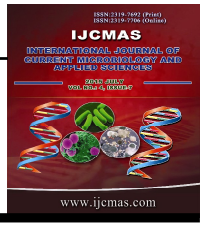

Original Research Article

TLC, FTIR and GCMS analysis of leaves of *Gymnema sylvestre* R.Br from Kolli Hills, Tamil Nadu, India

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Keywords

FTIR,
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A B S T R A C T

This study was aimed at analysis of potential bioactive constituents of leaves of *Gymnema sylvestre* R.Br. Methanol extracts of the leaves were subjected to thin layer chromatography (TLC), Fourier transform infrared spectroscopy (FTIR) and Gas chromatography- mass spectroscopic (GCMS) analysis. TLC analysis with methanol: Chloroform solvent revealed 6 fractions with R_f values of 0.23, 0.35, 0.45, 0.59, 0.69 and 0.85. FTIR spectroscopic investigation gave characteristic peak values with various functional compounds such as alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids and aromatics. GC-MS analysis gave a spectrum of compounds with 10 major peaks corresponding to 2-(amino oxy) propanoic acid, 3-heptanone-4- methyl-, Undecanoic acid, (2 propyl-1,3-dioxolan-2-yl)acetic acid, 2,2-dimethyl propyl sulfinyl sulfonyl)-2,2 dimethyl propane, Octatriene,1-3,5, squalene, Genistin, Diphenyl acetic acid-4-((pyrrolidincarbonyl)diphenyl methyl), -3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane and Ethanone 1,1,6 hydroxy-2,5 benzofuran diyl bis most of which are bioactive compounds which may act as good antimicrobial, antiviral, antioxidant and anti-inflammatory agents.

Introduction

Microbial infections continue to scourge mankind for several million years. Allopathy is the mainstay of therapeutics for several years. Owing to development of drug resistance, various side effects, expensive medications and emergence of mutants, search for alternatives led to the use of medicinal plants for the betterment of the disease (Rates, 2001). There is also an upsurge in use of herbal preparations and active ingredients isolated from medicinal plants in health care. More than 40% of modern drugs are derived from natural sources using either the natural substance or a synthesized version (Jassim et al., 2003). A review on natural products by Butler et al., (2014) states that the short term prospect for new NP (natural products) and NP-derived drug approvals is bright, with 31 compounds in phase III or in registration, which should ensure a steady stream of approvals for at least the next five years.

A plant extract contains low concentration of active compounds and a large number of promising compounds, requiring the use of sensitive bioassays suitable for the wide chemical variety and small amounts of tested samples. Analysis of these compounds have become easier and more cost effective due to the development of Thin layer (TLC) and hyphenated chromatographic techniques such as GC-MS, LC-MS and various spectroscopic methods such as FTIR and UV-Vis Spectophotometry (Sahaya et al., 2012).

Thin layer chromatography is simple, quick and inexpensive process that can be used for analysis of mixtures. (Fried and Sharma, 1994) FTIR is one of the widely used methods to identify the chemical constituents and has been used as requisite method to medicines in pharmacopeia of many countries (Liu et al., 2006). GC-MS analysis is a breakthrough in analysis of phyto constituents and structure elucidation of these compounds as they have a sensitivity of detecting compounds as low as 1ng (Liebler et al., 1996).

Gymnema sylvestre R.Br. (Asclepiadaceae) is a slow growing perennial, medicinal woody climber native to central, western and southern India and several tropical countries. It is known as Sirukurinja in Tamil, and is widely used in herbal medicine owing to its potent antidiabetic property (Thakur et al., 2012). Leaves are opposite, usually elliptic or ovate (1.25– 2.0 inch. 0.5–1.25 inch). Flowers are small, yellow, in umbellate cymes. Follicles are terete, lanceolate up to 3 inches in length (Kanetkar et al., 2007). It possesses antimicrobial (Satdive et al., 2007), anti hypercholesterolemic and hepatoprotective activity in addition to potent anti-inflammatory activity, anti cancer activity (Arunachalam et al., 2015) and antiviral activity (Sinsheimer et al., 1968, Subashini and Rajendran, 2015)

Gymnema sylvestre R.Br. leaves contain several phytoconstituents such as saponins, gymnemic acids, gymnemasides (Khramov et al., 2008), flavones, anthraquinones, hentri-acontane, pentatriacontane, triterpenoid saponins (Zhu et al., 2008) α and β-chlorophylls, phytin, resins, quercitol, tartaric acid, formic acid, butyric acid, lupeol, β-amyrin related glycosides and stigmasterol. This study is mainly aimed at metabolic profiling of methanolic extracts of leaves of *Gymnema sylvestre* R.Br.

Materials and Methods

Plant collection and extraction

The leaves of *Gymnema sylvestre R.Br.* were collected from Kolli hills adjoining downstream areas of Namakkal district of Tamil Nadu, India and authenticated (PARC/2011/943) at Plant Anatomy Research Centre, National Institute of Herbal Science, Chennai, India. The plant samples were washed, shade dried, powdered and extracted in methanol and filtered. The extract was then concentrated to dryness under reduced pressure using rotary vacuum evaporator, stored at 4ºC until further analysis.

Thin layer Chromatographic analysis

TLC was performed on a pre-coated silica gel TLC plates grade F254 (E-Merck, Darmstadt, Germany) to determine the number of compounds present in the plant crude extract. A total of 5 µl (10 mg/ml) of sample was spotted at 1 cm from the bottom of silica gel plates using capillary tubes. Different solvents at various combinations and concentrations were used for metabolites profiling. Development of the

chromatogram was done in closed tanks, in which the atmosphere has been saturated with eluent vapour by wetting a filter paper lining. The chromatogram was visualized under UV light (365 nm and 254 nm), white light and iodine vapour and sulpho-vanillin reagent spray. The R_f values of the compounds were calculated using the following formula.

$$Rf = \frac{\text{distance travelled by the compound}}{\text{distance travelled by the solvent front}}$$

FTIR analysis

Dried powder of methanolic extract was used after performing KBr pelleting. The sample was loaded onto FTIR spectroscope (Shimadzu, IR Affinity Japan) in a scan range of 400 -4000 cm^{-1}.

GC-MS analysis

The extract was subjected to GC MS analysis to identify the various bioactive compounds present. The sample was analyzed in Perkin Elmer- Clarus-600 instrument using software Turbomass 5.2 version. Capillary standard non-polar column (30 m X 0.25 mm, 0.25 mm film thickness) was used. The volume of injected specimen was 1 µ l of methanol/ ethanol extract, injector temp. 220°C with a split ratio of 25:1 Carrier gas Helium, Solvent Delay=3.00 min, source Temp=180°C, oven temperature program initial temp 60°C for 5 min, ramp 7°C/min to 300°C, hold 15 min, Scan: 50 to 600Da, ionization energy 70 eV, in the electronic ionization mode. The identification of compounds was done using computer matching of mass spectra with those of standards (Mainlib, Replib and NIST library). The name, molecular weight and the structure of the components of the test materials were ascertained.

Results and Discussion

Thin layer chromatographic analysis revealed the presence of diverse potent biomolecules in the plant extract. Solvent system of Chloroform: Methanol (9:1) gave a better separation of compounds (Fig.1 and Table1). Methanolic extract revealed 6 spots with R_f values of 0.23, 0.35, 0.45, 0.59, 0.69 and 0.85.

FTIR analysis was used to identify the functional group of active components based on peak values in the region of infrared radiation. FTIR spectrum was analyzed (Coats et al., 2000) and the results of FTIR spectrum profile was illustrated in the Fig.2 and tabulated in Table 2.

The study on the active principles of leaves of *Gymnema sylvestre* by GCMS analysis showed the presence of 10 major peaks in methanolic extract corresponding to compounds 2-(amino oxy) propanoic acid, 3-heptanone-4- methyl-, Undecanoic acid, (2 propyl-1,3-dioxolan-2-yl)acetic acid, 2,2-dimethyl propyl sulfinyl sulfonyl)-2,2 dimethyl propane, Octatriene,1-3,5, squalene, Genistin, Diphenyl acetic acid-4-((pyrrolidincarbonyl)diphenyl methyl), -3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane, Ethanone 1,1,6 hydroxy-2,5 benzofuran diyl) bis. The results of GCMS analysis was illustrated in Fig. 3 and Table 3.

TLC analysis provide an idea about polarity of various chemical constituents in such a way that compounds showing high R_f value in less polar system have low polarity and vice versa. TLC analysis of gymnemic acid from *Gymnema sylvestre* by Balamurali Krishna et al., (2012) revealed R_f value of 0.66- 0.82 by using Chloroform: Methanol (6:5)

Table.1 R_f values of methanolic extract of leaves of *Gymnema sylvestre R.Br*

Compound Position	R_f values
01	0.23
02	0.35
03	0.45
04	0.59
05	0.69
06	0.85

Table.2 FTIR analysis of methanolic extract of leaves of *Gymnema sylvestre R.Br*

Peak values Frequency, cm-1	Bond	Functional groups
3360,3367	O-H stretch	Alcohols/ Phenols
2862,2926	C-H stretch	Alkanes
2109	-C=C- Stretch	alkynes
1711	C=O stretch	Ketones
1611,1645	N-H bend	Primary amines
1512.26	N-O asymmetric stretch	Nitro compounds
1448,1420	-C-C- stretch	Aromatics
533,545,598,646	C-Br stretch	Alkyl halides
824,876	C-H oop	Aromatics

Figure.1 TLC Plate of methanolic extract of leaves of *Gymnema sylvestre R.Br.* developed using chloroform and methanol (9:1)

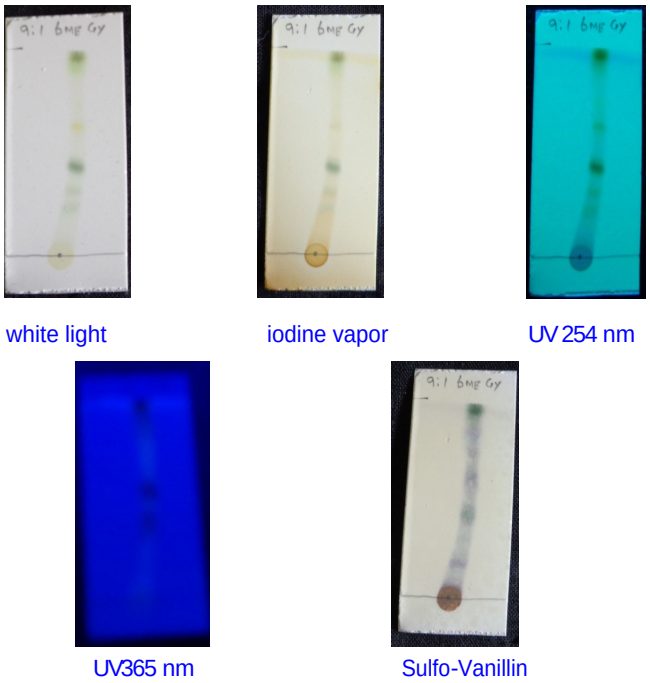


Table.3 List of compounds identified at various retention times from methanolic extract of leaves of Gymnema sylvestre R.Br.by GCMS

Pk	Retention Time	Compound	Structure
1	22.10	2-(amino oxy) propanoic acid	$C_3H_7NO_3$
2	24.73	3-heptanone-4- methyl-	$C_8H_{16}O$
3	26.50	Undecanoic acid	$C_{11}H_{22}O_2$
4	27.02	(2propyl-1,3-dioxolan-2-yl)acetic acid	$C_8H_{14}O_4$
5	28.138	2,2-dimethyl propyl sulfinyl sulfonyl)-2,2 dimethyl propane	$C_{10}H_{22}O_3S_2$
6	29.529	Octatriene,1-3,5	C_8H_{12}
7	36.342	Squalene	$C_{30}H_{50}$
8	39.493	Enalapril	$C_{20}H_{28}N2_{O5}$
9	40.26	1-propanone-2-	$C_{14}H_{24}O$
10	40.49	Genistin	$C_{21}H_{20}O_{10}$
11	41.154	Ethane,1,1,1- trinitro-	$C_2H_3N_3O_6$
12	42.15	Diphenyl acetic acid-4-((pyrrolidincarbonyl)diphenyl methyl)	$C_{32}H_{29}NO_3$
13	42.464	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	$C_{15}H_{26}O$
14	43.895	Cyclohexane methanol, proponoate	$C_{10}H_{18}O_2$
15	44.715	2,5-octadiyne4,4	$C_{12}H_{18}$
16	45.71	Ethanone 1,1,6 hydroxy-2,5 benzofuran diyl bis	$C_{12}H_{10}O_4$

Figure.2 FTIR Spectrum of Methanolic Extract of leaves of *Gymnema sylvestre* R.Br.

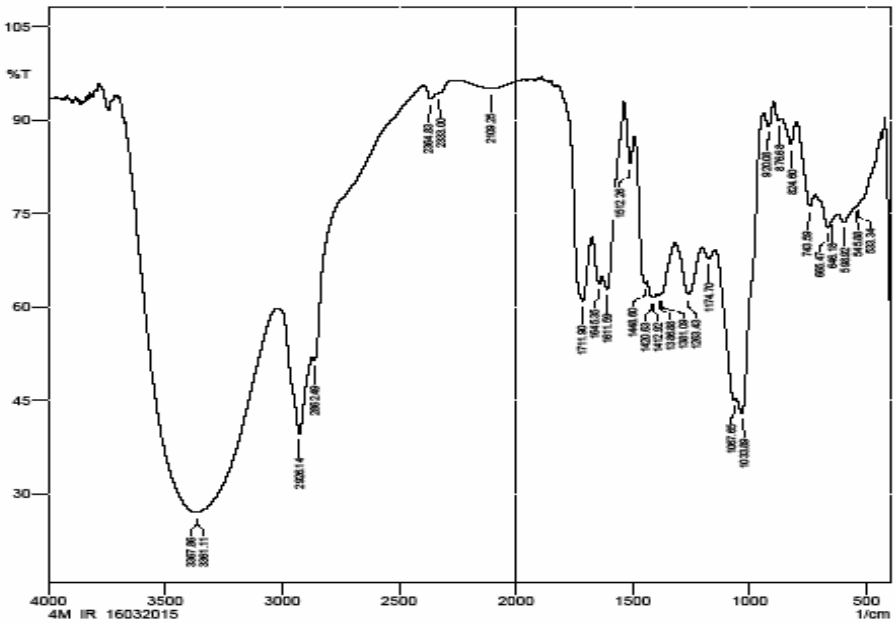
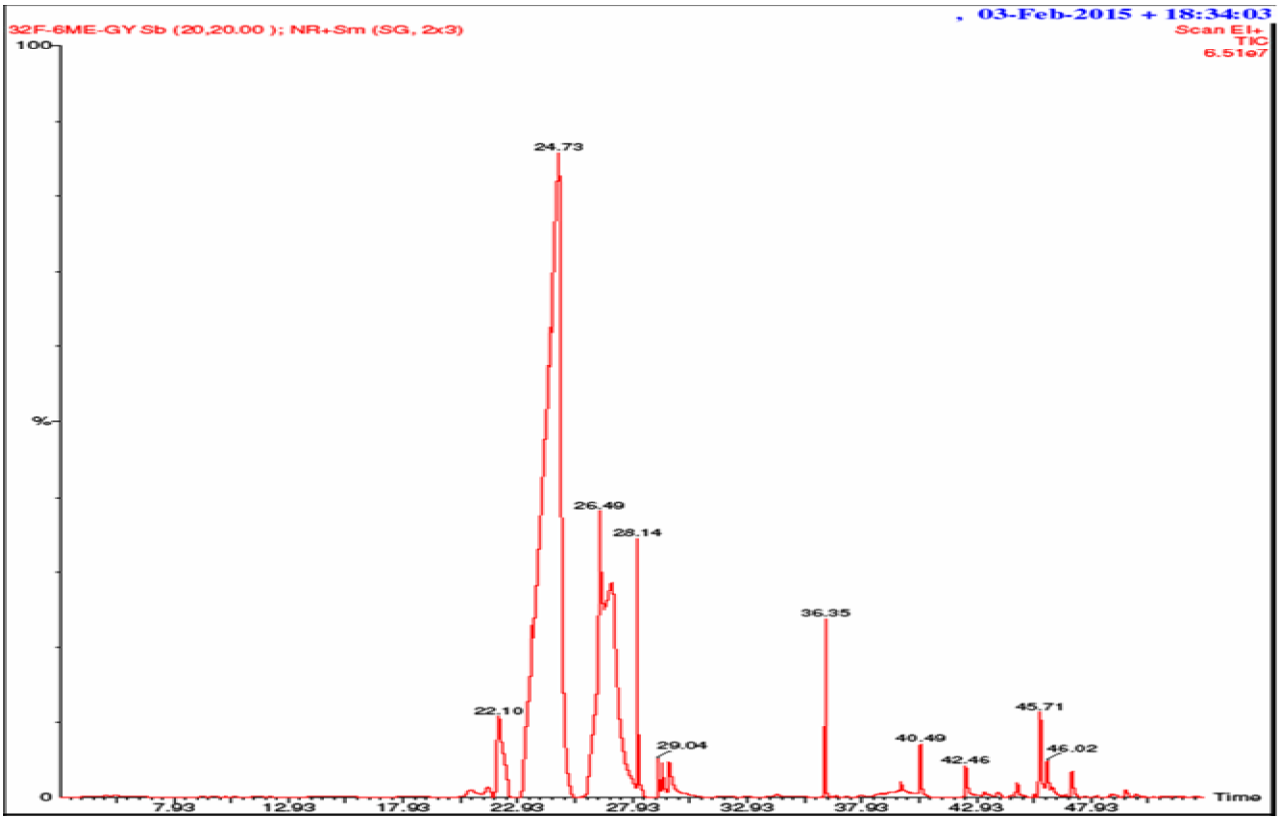


Figure.3 Mass Chromatogram of Methanolic extract of leaves of *Gymnema sylvestre* R.Br.



FTIR analysis confirmed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides aldehydes, carboxy acids aromatics, aromatic amines. A study on aqueous extracts of *Gymnema sylvestre* by Sangeetha et al., (2014) revealed the presence of excess of aliphatic and aromatic amines and reported that the plant possessed highest antioxidant activity. FTIR analysis of four medicinal plants by Ashokkumar and Ramaswamy (2014) with various solvents like petroleum ether, chloroform, methanol and ethyl acetate revealed that potential OH groups in methanolic extract which demonstrated high antimicrobial activity.

A total of 7 compounds had been identified from the methanolic extract of *G.sylvestre* by Parimala Devi (2010) where terpenes, saturated and unsaturated fatty acids such as 9-Octadecenoic acid (Z)-, methyl ester were predominantly found. Similar study on essential oil of *Gymnema sylvestre* indicated the major compounds to be palmitic acid, hydroquinone, phytol, pentadecanoic acid , 4-vinyl guaiacol and eugenol (Naick et al., 2011). Gas chromatography-mass spectrometry analysis of different organic crude extracts from the local medicinal plant of *Thymus vulgaris* done by Hashmi et al., (2013) revealed several chemical constituents of medicinal value.

The results of the present study indicate that the methanolic extract of leaves of *Gymnema sylvestre* R.Br. have isoflavanoids, carboxylic acids, fatty acids, sesqueterpenes, steroids, phenolics which are potent phytochemicals with many biological activities.

In conclusion, the presence of various bioactive compounds in the leaves of *Gymnema sylvestre R.Br* necessarily indicates its potential in treating various infectious diseases. Further research is necessary to identify and purify the compounds responsible for antimicrobial and antiviral activity. An in silico approach to the drug receptor binding (molecular docking) analysis is an essential key which can throw more light for better understanding and can open up new molecules with essential biological activity.

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