PUBELESE PUBLISHER

JOAASR Journal of Advanced Applied Scientific Research

IDENTIFICATION OF BIOACTIVE COMPOUNDS FROM THE METHANOLIC LEAF EXTRACT OF *GYMENEMA SYLVESTRE*

P. Vijaya Kumar and A. Jafar Ahamed*

P G and Research Department of Chemistry,
Jamal Mohamed College (Autonomous), Tiruchirappalli - 620020, Tamil Nadu, India
E-mail: agjafar@yahoo.co.in

Abstract

This study was aimed to analysis of potential bioactive phytoconstituents from the methanolic leaf extract of *Gymnema sylvestre*. Qualitative phytochemical screening of this plant confirms the presence of various secondary metabolites like steroids, alkaloids, saponins, tannins, ester, phenol and flavonoids. Gas chromatography mass spectroscopy analysis (GC-MS) also confirmed the presence of various phytochemical compounds with different chemical structure.

Keywords: *Gymnema sylvestre*, alkaloids, steroids, GC-MS analysis

1. Introduction

The plant is a biosynthetic laboratory not only for chemical compounds such as proteins, carbohydrates and lipids that are utilized as food by man, but also for a multitude of compounds like alkaloids, glycosides, tannins, volatile oils, etc., that exert a physiological and therapeutic effect. The compounds that are responsible for the therapeutic effects are mostly secondary metabolites. A systematic phytochemical study therefore, involves through screening of primary as well as secondary metabolites derived as a result of plant metabolism [1].

Secondary metabolites were once thought to be waste compounds [2]. The significant is in planta function of many secondary metabolites is gradually expanding. Many of these secondary metabolites are used in the veterinary, agriculture, human therapy, scientific research

and countless other areas [3]. Semiochemicals are relied on as means of defense against pathogens and predators, as attractants to lure mobile creatures for fertilization and dissemination and also for aerial allelopathy (interplant communication). Though, volatile organic compounds and pigments are revealed to be attractive to insects that help with fertilization or warning colors to defend against predators whilst other plant pigments can provide protection against environmental damage such as free radicals and UV radiation [4, 5]. Some secondary products perform signaling functions as plant hormones and pheromones.

Phytochemists play a vital role in the chemical investigation of these plants. So the phytochemists need more basic knowledge of several aspects of taxonomy. This phytochemical study may be directed towards characterizing the chemical composition of plant extracts or complex essential oils. Phytochemical screening can assist taxonomic classification whilst bioassay guided studies can target and identify biologically active compounds in complex plant extracts [6]. Nearly 75000 species of Indian medicinal plants are widely used by all sections of the population [7]. Among them, *Gymnema sylvestre* is a medicinal plant, belongs to the family Asclepiadaceae. It is commonly known as gurmar and it has been used as a natural treatment for diabetes for nearly two millennia [6]. *Gymnema sylvestre* has its native to the tropical forest of central and southern India, it has wider distribution and it grows in the plains from the coast, in scrub jungles and in thickets at an altitude ranging from 300-700 metre. The genus *Gymnema* comprises nearly 40 species distributed from western Africa to Australia. In India, it is found in Uttar Pradesh, Madhya Pradesh, Maharashtra, Punjab, Haryana, Tamil Nadu, Kerala, Bihar and Bengal [8].

The active constituent of the plant is a group of oleanane type of triterpenoid saponins termed as Gymnemic acid and the amount and type of phytochemical compounds vary from

plant to plant and place to place with respect to nature of the soil [9]. In Gymnema species a number of phytochemical constituents such as alkaloids, flavonoids, saponins, tanins, terpenoids and steroids have been reported, which have anti-inflammatory effects. Saponins possess hypo-cholesterolemic and antidiabetic properties [10].The terpenoids have also been shown to decrease blood sugar level in animal studies [11]. The steroids and saponins are responsible for central nervous system activities [12].

Hence, the present investigation was aimed at to identify the functional groups present in crude powder and phyto components present in methanol extract of *Gymnema sylvestre* leaf with the aid of TLC, FT-IR and GC-MS analytical techniques.

2. Materials and methods

2.1. Collection and authentication of Plant of *Gymnema sylvestre*

Healthy, disease free leaves of *Gymnema sylvestre* were collected during the month of December 2013 in Veeramalai forest, Manapparai Taluk, Tiruchirappalli district, Tamil Nadu, India. Plants with complete herbarium were identified and authenticated by Dr. S. Soosairaj, Assistant Professor, Post Graduate and Research Department of Botany (Rapinat Herbarium, A centre for plant Taxonomic Research), St. Joseph's College (Autonomous), Tiruchirappalli, India. It is compared with the flora of Tamil Nadu Carnatic by Mathew (1983). A voucher specimen no. SJCBOT2184/2014 has been deposited in the herbarium.

2.2. Soxhlet extraction

It was carried out to obtain extracts for the phytochemical screening. *Gymnema sylvestre* plant leaves were first washed, shade dried, and ground in a mechanical grinder to obtain the powder of the crude drug which was later sieved through mesh size 80 µm to get the powder of uniform size. Around 40 g of the powder was packed in a thimble of filter paper prepared

manually. The thimble was then inserted into the Soxhlet apparatus and extraction was done by using 200 ml methanol as a solvent. Yet, temperature was maintained at 85 °C and extraction was continued for 3 hours. Then the methanol extract was collected and the powder from the thimble was discarded. Then the obtained methanolic extract was dried and used for the phytochemical screening.

2.3. Chromatographic studies

Chromatography is a separation process which depends on the differential distribution of the components of a mixture between a mobile bulk phase and an essentially thin film stationary phase. This technique is used as an analytical tool to establish the complexity of mixtures and the purity of samples, and as preparative tools for the separation of mixtures into individual components.

2.3.1. Thin layer chromatographic studies (TLC)

TLC is based on adsorption chromatography in which separation depends on the selective adsorption of the components of a mixture on the surface of solid. The stationary phase was in the form of a thin layer adhering to a suitable form of backing material over which the mobile phase was allowed to ascend by capillary action. Traditionally, analytical TLC has found application in the detection and monitoring of compound through a separation process. Silica gel coated TLC plates were used to determine the number of compounds present in the plant crude extract. A total of 5µl (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.

2.3.2. 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}.

2.3.3. 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 µl of methanol 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.

3. Results and discussion:

The extracts of leaf of *Gymnema sylvestre* was subjected to TLC. Tolune, chloroform, ethylacetate, ethylacetate + methanol and methanol were used as solvent systems which showed good resolution. From this analysis 6 bands were visualized. Bluish-green colour bands were observed in methanol solvent system indicating the presence of terpene-rich compounds was TLC chromatogram can be used as a reference standard for the separation and isolation of compound from plant. (Fig. 1)

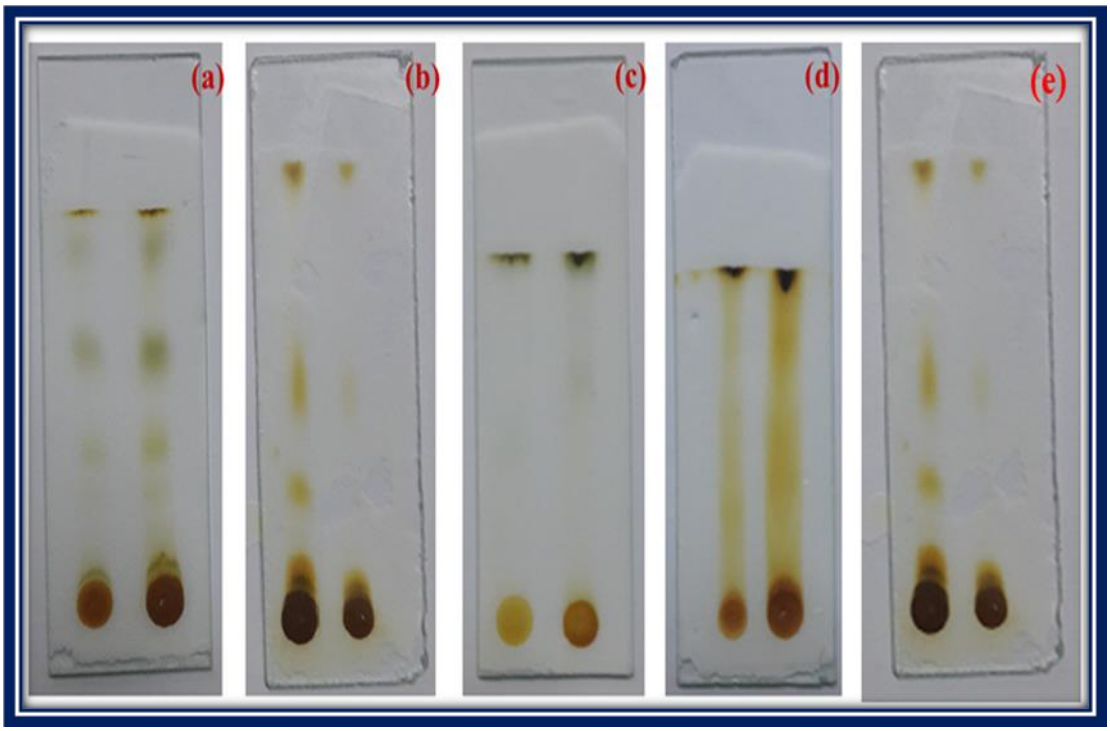


Figure 1 TLC Plate of methanolic extract of leaves of *Gymnema sylvestre* developed using. (a) Tolune, (b) Chloroform, (c) Ethylacetate, (d) Ethylacetate + methanol (12:3) and (e) methanol

The FTIR spectrum was used to identify the functional group of the active components. Fig. 2 represents the FTIR spectrum of *Gymnema sylvestre* plant extract. The band appears at 3402 cm^{-1} is due to the presence of Alcohols/Phenols groups. The band appears at 2844, 2947 cm^{-1} is due to the presence of alkane groups present in the leaf extract. The band appears at 1645 cm^{-1} is due to the presence N-H bending frequency. The stretching frequency appears at the 671 cm^{-1} id due the presence of alkyl halide groups. So the FTIR analysis confirmed the presence of alcohol, phenols, alkane, alkynes, alkyl halides, carboxylic acids and aromatic amines in the *G. sylvestre* leaf extract [13].

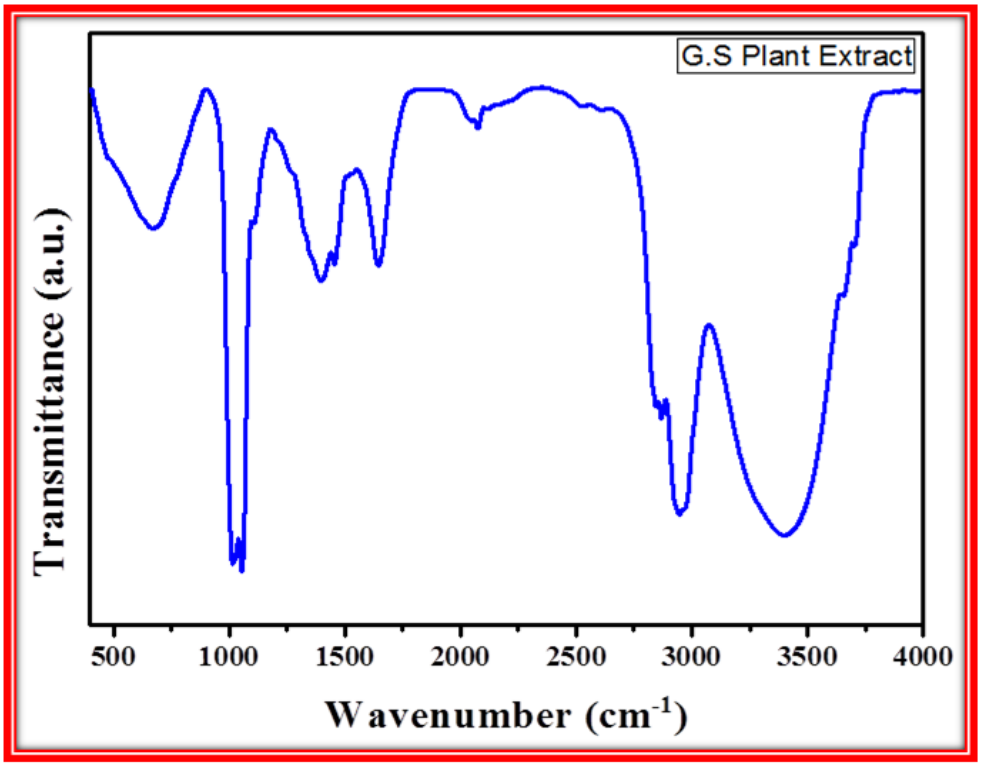


Figure 2 FT-IR Spectrum of *Gymnema sylvestre leaf extract*

GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain and branched chain hydrocarbons, alcohols acids, esters etc. Fig.1 shows that the GC-MS analysis of the *G. sylvestre* leaf extract. It was identified that 12 major phytoconstituents and totally 41 chemical compounds from the leaf extract of *G. sylvestre*. The major chemical compounds and their molecular weight were presented in the table 1.

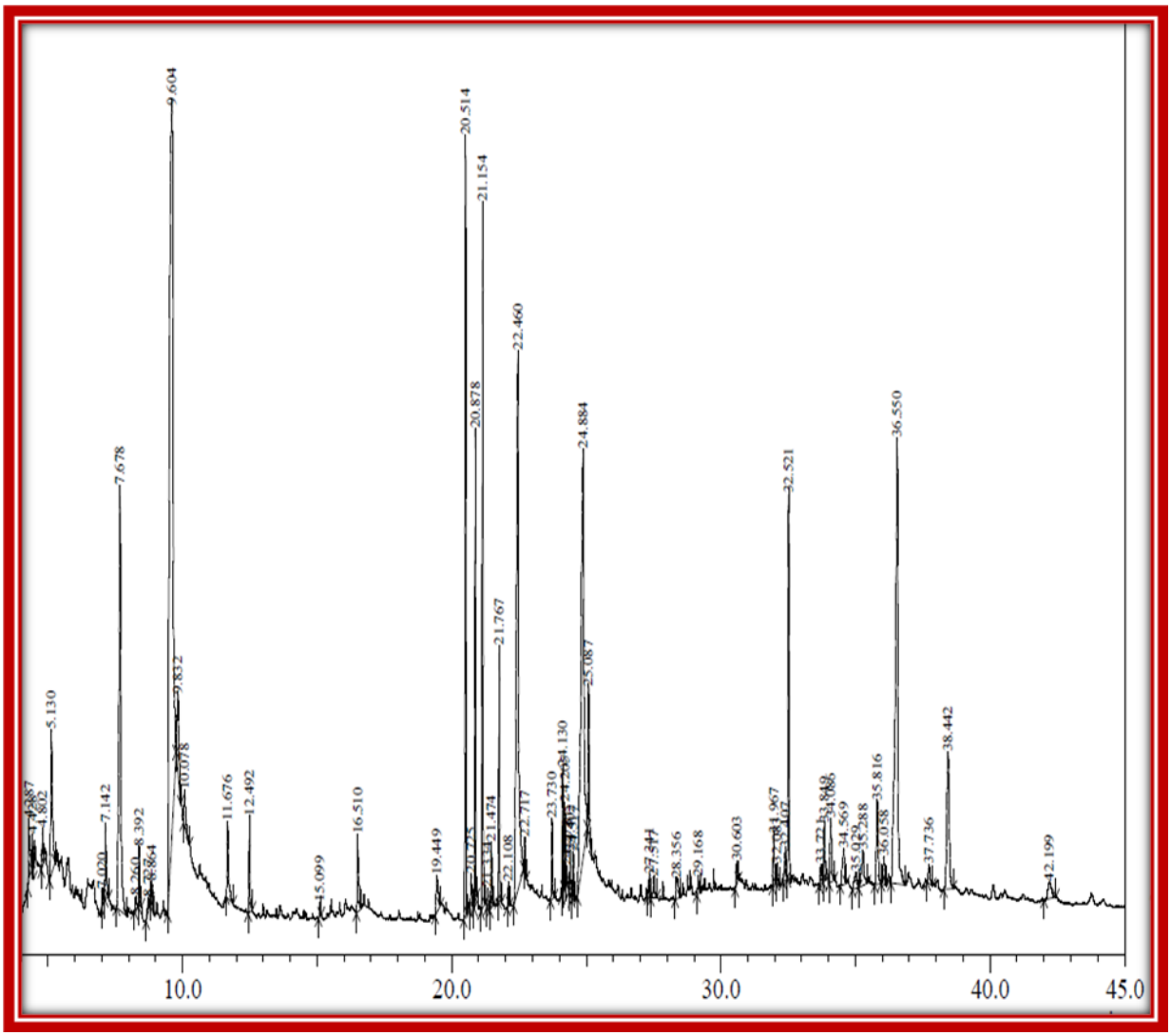
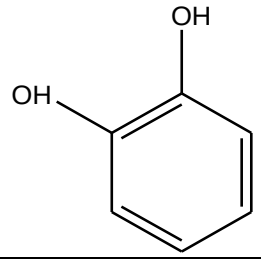
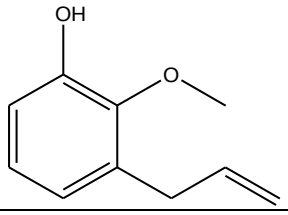
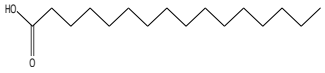
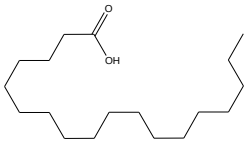
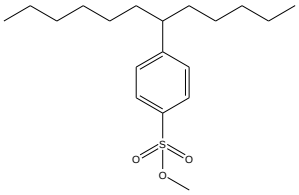
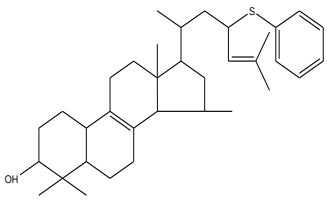
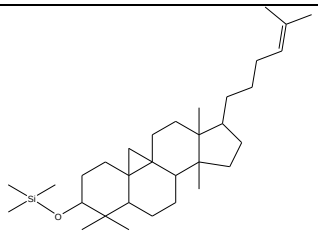
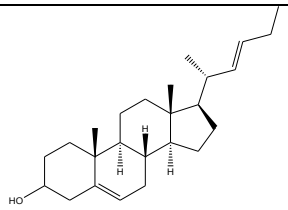
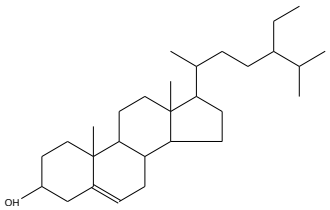
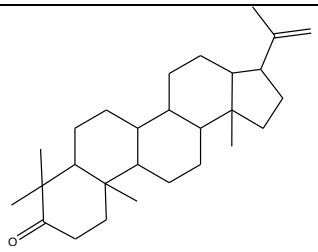
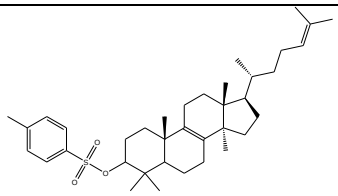
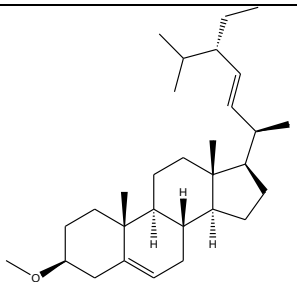


Figure 3 GC-MS Chromatogram of methanolic leaf extract from *Gymnema sylvestre*

Table 1 Phytoconstituents identified in the methanolic leaf extract of *Gymnema sylvestre* by GC-MS analysis

Sl.N0	Molecular weight	Name of the compound	Structure
1	110	Benzene-1,2-diol	
2	164	3-Allyl-2-methoxy-phenol	
3	242	Hexadecanoic acid	
4	284	Octa decanoic acid	
5	340	4-(1-Pentyl-heptyl)-benzene sulfonic acid methyl ester	
6	534	17-(1,5-Dimethyl-3-phenyl-sulfanyl-hex-4-enyl)-4,4,13,15-teta methyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetra decahydro-1-H-cyclopenta[α] phenanthren-3	

7	498	Tri methyl-[4,4,13,14-tetram ethyl-17-(5-methyl-hex-4-enyl)-tetradeca hydro-cyclopropa[9,10]cyclo penta[α]phenanthn-3-yloxy]-Silane	
8	356	26,27-Dinorcholesta-5,22-Dien-3-ol	
9	414	17-(4-Ethyl-1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetra decahydro-1H-cyclopenta[α]phenanth en-3-ol	
10	424	3-Isopropenyl-8,8,11a,13b-tetram ethyl-icosahydro-cyclopenta[α]chrysen-9-one	
11	580	Lanosta-8,24-Dien-3-yl 4-methyl Benzene sulfonate	
12	426	Stigmasta-5,22-Diene,3-methoxy-(3-Beta,22E)	

4. Conclusion

This study revealed the identification of different types of phytochemical constituents especially alkaloids, tannins, saponins, phenols, glycosides, flavonoids etc. The presence of various components in the methanolic extract of *Gymnema sylvestre* leaves was further confirmed by TLC and FT-IR spectroscopy. Even GC-MS analysis of the methanolic extract of *Gymnema sylvestre* reveals the presence of 41 phytoconstituents belonging to the type acids, esters, alcohols, ethers, etc. Thus, the medicinal plant *Gymnema sylvestre* is found to possess significant phytoconstituents. The analysis carried out on this plant shows that the plant rich in secondary metabolites (Flavonoids) which could be explored as potential drug in phytomedicine. Thus this type of spectral analyses is the first step towards understanding the nature of active principles in this medicinal plant which will be helpful for further detailed study.

5. References

[1] Neha Grover, Rishikesh Meena, Vidya Patni, Physiochemical evaluation, phytochemical screening and chromato-graphic fingerprint profile of *Woodfordia fruticosa* (L.) Kurz extracts, International Journal of Pharma Sciences and Research, 2014, 5(7), 2772-2782.

[2] Paul A. Keddy, Wet land Ecology: Principles and conservation, 2nd ed.; Cambridge University Press: USA, 2010, 175.

[3] RNS. Yadav and Munin Agarwala, Phytochemical analysis of some medicinal plants, Journal of Phytology, 2011, (3(12), 10-14.

[4] Anjali Soni, Sheetal Sosa, Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts, Journal of Pharmacognosy and phytochemistry, 2013, 2(4), 22-29.

[5] S. Vimal, S. Rajesh Kumar, Preliminary phytochemical and antimicrobial studies on the leaf extracts of Actinodaphne lanata Meissner, International Journal of Pharma Sciences and Research, 2015, 6(9), 1201-1205.

[6] R. Hegnaur, Phytochemistry and plant taxonomy-an essay on the chemotaxonomy of higher plants, Phytochemistry, 1986, 25(7), 1519-1535.

[7] Pulak Majumder, M. Paridhavi, An ethno-phytochemical and pharmacological review on novel Indian medicinal plants used in herbal formulations, International Journal of Pharmacy and Pharmaceutical Sciences, 2013, 5(4), 74-83.

[8] Arunkumar, Gymnema sylvestre: Qualitative analysis of phytochemicals and its medicinal importance in different medicinal system, Asian Journal of Biochemical and Pharmaceutical Research, 2016, 6(1), 146-150.

[9] M. Wani, F.A. Sarvar, J. Agrawal, J. Deshpande, S. Mathew, M. Khetmalas, Qualitative phytochemical analysis and antimicrobialactivity studies of Gymnema sylvestre R. Br, Acta Biologica Indica, 2012, 1(1), 121-124.

[10] Muhammad Dawood Shah, M. Amzad Hossain, Total flavonoids content and biochemical screeningof the leaves of tropical endemic medicinal plant Merremia borneensis, Arabian Journal of Chemistry, 2014, 7, 1034–1038.

[11] M. Swetha, N. Saritha, N. Devanna, Review article on HPLC-RP HPLC technique on the development and validation of Gymnema sylvestre, International Journal of Research in Pharmaceutical Sciences, 7(1), 95-97.

[12] C. Gopi, T.M. Vatsala, In vitro studies on effects of plant growth regulators on callus and suspension culture biomass yield from Gymnema sylvestre R.Br, African Journal of Biotechnology, 2006, 5(12), 1215-1219.

[13] M.S. Subashini, P. Rajendran, G. Ashok, B.M. Kanthesh, TLC, FTIR and GCMS analysis of leaves of Gymnema sylvestre R.Br from Kolli Hills, Tamil Nadu, India, International Journal of Current Microbiology and Applied Sciences, 2015, 4(7),757-764**.**