International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 1, 2016

Short Communication

UNRAVELING THE POTENTIAL PHYTOCHEMICAL COMPOUNDS OF GYMNEMA SYLVESTRE THROUGH GC-MS STUDY

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Received: 28 Apr 2015 Revised and Accepted: 18 Nov 2015

ABSTRACT

Objective: To profile the chemical composition of ethanolic extract of *Gymnema sylvestre* through Gas Chromatography-Mass Spectrometry technique.

Methods: The chemical compositions of the plant leaf extracts of *G. sylvestre* were investigated using Gas Chromatography–Mass Spectroscopy (Scion 436-GC Bruker model coupled with a Triple, quadruple mass spectrophotometer) and NIST-MS library.

Results: GC-MS analysis of leaf extracts revealed the existence of Terpenes, alcohols, fatty acids, amine and sterols. The highest % Peak area is hexadecanoic acid, α-Santoline alcohol, recorded the next highest % peak area of 9.05. Major of the compounds belongs to terpeneoid group, namely 6-Octen-1-ol, 3,7-dimethyl, Isophytol, Squalene, Nerolidol, β-Amyrin and Cedrene-V6 which constitutes 30.7% of the peak area. The presence of α-Tocopherol-β-D-mannoside and Vitamin E also identified through this study.

Conclusion: From the above finding we can interpret that the *G. sylvestre* contained a considerable amount of phytoconstituents especially terpenoids**.** In future, this study will be helpful for the quantitative analysis of phytochemicals as well as formulation studies.

Keywords: Diabetes, GC-MS, *Gymnema sylvestrae*, Terpenoids, Phytoconstituents.

The prevalence of diabetes, cardiovascular diseases, and other lifestyle diseases is associated with unhealthy eating habits. One of the basic amendments to avoid lifestyle disease is consuming a balanced diet with potential herbals identified in the Indian indigenous system of medicine. According to World Health Organization (WHO) criteria, approximately 438 million people (7.8%) of the adult population are expected to have diabetes by 2030. *G. sylvestre* R. Br. has been used as a traditional medicine plant in Africa, Australia and Asia especially in India. It is commonly known as "Gur-mar" in India and widely used in indigenous system of medicine for treatment of Diabetes mellitus. It also has stomachic, diuretic and coughs suppressant activity [1, 2]. The phytoconstituents accountable for sweet suppression activity comprises triterpene saponins known as gymnemic acids, gymnema saponins, and a polypeptide, gurmarin. The plant has been reported to possess antimicrobial [3], antieruodonic [4] and antiviral effects [5]. The leaves are reported to contain gymnemic acid, which on hydrolysis yields a D-glucuronides of a hexahydroxyolean-12-ene (gymnemagenin) [6], and other triterpenoid saponins, like GA-I to XVIII [7-10], damarane saponins [11] and some flavonoid glycosides [12]. The phytochemicals in leaf extract were also analyzed through gas chromatography coupled to mass spectrometry and identified for the presence of terpenoids, glycosides, saturated and unsaturated fatty acids, and alkaloids in three different leaf extract, namely, petroleum ether and chloroform as solvents used for extraction [13]. The bioactive constituents present in the plant were found to be mixture of diverse phytomolecules such as gymnemic acids, gymnemosides, gymnemasaponins, gurmarin, gymnemanol, stigmasterol, d-quercitol, *β*-amyrin related glycosides, anthraquinones, lupeol, hydroxycinnamic acids, and coumarols group. *G. sylvestre* is considered to be a very effective antidiabetic plant for masking sweet taste. The GC-MS study of the ethanol extract of this plant is not reported so far which needs to be unraveled. In this study, an attempt has been made to profile the chemical composition of the ethanol extract through Gas Chromatography Mass Spectrometry technique.

The leaves of *G. sylvestre* were collected from the herbal garden, Tamil University, Thanjavur, India and authenticated by Head, Department of Herbal and Environmental Sciences, Tamil University where a voucher specimen was submitted (fig. 1)

Fig. 1: ***Gymnema sylvestre***

Around 25 g powdered leaf of the selected plant was soaked in 30 ml of ethanol overnight and then filtered through filter paper. The filtrate is then concentrated by flushing nitrogen gas into the solution and was concentrated to 1 ml. The concentrate was again filtered in the Whatmann No.41 filter paper along with 2 g Sodium sulfate to remove the sediments and traces of water in the filtrate.

The chemical composition of *G. sylvestre* was investigated through Gas Chromatography-Mass Spectrometry/Mass Spectrometry Electron Ionization (GC-MS/EI) mode. The GC-MS/MS is a Scion 436-GC Bruker model coupled with a Triple quadruple mass spectrophotometer with fused silica capillary column BR-5MS (5% Diphenyl/95% Dimethyl polysiloxane) and Length: 30m; Internal diameter: 0.25 mm; Thickness: 0.25 µm. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 2 µl was employed (split ratio of 10:1). The column oven temperature program was as follows: 80 °C hold for 2 min, Up to 160 °C at the rate of 20 °C/min-No hold, Up to 280 °C at the rate of 5 °C/min-No hold, Up to 300 °C at the rate of 20 °C/min-10 min hold, Injector temperature 280 °C and total GC running time was 41 min [14]. This last increase was to clean the column from any residues. The mass spectrometer was operated in the positive

electron ionization (EI) mode with ionization energy of 70eV. The solvent delay was 0-3.0 min. A scan interval of 0.5 seconds and fragments from m/z 50 to 500 Da was programmed. The inlet temperature was set at 280 °C, source temperature 250 °C. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was MS Work station 8. The NIST Version 2.0 library database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for identifying the chemical components. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained. The GC-MS/MS was performed by Food Safety and Quality Testing Laboratory, Institute of crop processing technology, Thanjavur.

The result of the GC-MS analyses on the ethanolic extract of the leaves of *G. sylvestre* is presented in [table 1]. A total of 34 compounds were identified from the Mass Spectrometry chromatogram. The identified compounds were of different types of terpenes, alcohols, fatty acids, amine and sterols. The highest % Peak area of 9.85 is hexadecanoic acid (Retention time 16.19), α-Santoline alcohol, recorded the next highest % peak area of 9.05. Major of the compounds belong to terpeneoid group, namely Bicyclo[2.2.1] heptane, 1,3,3-trimethyl-, 6-Octen-1-ol, 3,7-dimethyl, Bicyclo[3.1.1] heptane, 2,6,6-trimethyl-, (1α,2β,5α)-formate, Isophytol, Squalene, Nerolidol, β-Amyrin and Cedrene-V6 which constitutes 30.7% of the peak area.

Other major phytochemical compounds are Catechol, Tetradecanoic acid, n-Hexadecanoic acid, α-Santoline alcohol, DL-Ephedrine, Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester, δ-Tocopherol, Vitamin E, Stigmasterol, Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methyl ethenyl)-, [1S-(1α,7α,8aβ)]-, Hop-22(29)-en-3β-ol. Fig. 2 shows the chromatogram of the ethanolic extract of *G. sylvestrae.* The chemical classification and its therapeutic activity were given in table. 2.

Table 1: Chemical compounds identified in ethanol extract of leaves of *G. sylvestrae* through GC-MS Study

S. No.	RT	Name of the compound	MF	MW	Peak area %
1.	4.73	Propane, 1,1-diethoxy-	C7H16O2	132	1.82
2.	6.05	Catechol	C6H6O2	110	4.00
3.	6.88	3-Methoxyacetophenone	C9H10O2	150	0.98
4.	9.56	2,3,5,6-Tetrafluoroanisole	C7H4F4O	180	0.89
5.	10.21	1,2,3,4-Cyclohexanetetrol	C6H12O4	148	1.47
6.	12.49	Tetradecanoic acid	C14H28O2	228	1.92
7.	13.75	Bicyclo[2.2.1]heptane, 1,3,3-trimethyl-	C10H18	138	7.43
8.	14.13	6-Octen-1-ol, 3,7-dimethyl-, formate	C11H20O2	184	2.78
9.	14.48	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1α,2β,5α)-	C10H18	138	3.45
10.	16.19	n-Hexadecanoic acid	C16H32O2	256	9.87
11.	18.56	Isophytol	C20H40O	296	7.70
12.	19.32	α-Santoline alcohol	C10H18O	154	9.06
13.	23.40	DL-Ephedrine	C10H15NO	165	1.81
14.	25.00	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C19H38O4	330	2.35
15.	25.29	Phthalic acid, di(hept-3-yl) ester	C22H34O4	362	1.31
16.	27.82	Spiro[cyclopropane-1,2'-[6.7]diazabicyclo[3.2.2]non-6-ene]	C9H14N2	150	6.00
17.	29.47	Squalene	C30H50	410	5.10
18.	30.917	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R*(4R*,8R*)]]-) (. δ-Tocopherol)	C27H46O2	402	0.47
19.	31.50	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-(Nerolidol)	C15H26O	222	0.93
20.	31.96	γ-Tocopherol	C28H48O2	416	1.06
21.	32.18	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-	C15H24	204	0.94
22.	32.61	1-Docosene	C22H44	308	0.96
23.	32.90	Vitamin E	C29H50O2	430	3.90
24.	33.32	Cholane-5,20(22)-diene-3b-phenoxy	C30H42O	418	3.70
25.	33.80	β-Amyrin	C30H50O	426	3.07
26.	34.06	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-	C30H50O	426	3.45
27.	34.49	Stigmasterol	C29H48O	412	5.40
28.	35.48	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1α,7α,8aβ)]-	C15H24	204	1.10
29.	36.19	A-Norcholestan-3-one, 5-ethenyl-, (5β)-	C28H46O	398	1.61
30.	36.67	Hop-22(29)-en-3β-ol	C30H50O	426	1.44
31.	37.27	α-Tocopherol-β-D-mannoside	C35H60O7	592	2.02
32.	38.29	A'-Neogammacer-22(29)-ene	C30H50	410	1.31
33.	39.53	Phytol, acetate	C22H42O2	338	0.33
34.	40.01	Cedrene-V6	C15H24	204	0.34

Note: RT-Retention Time, MW-Molecular Weight, MF–Molecular Formulae

Among the compounds identified in *G. sylvestre*, the notable ones were terpenoids, essential fatty acids and alcohol compounds. It was found that the foods originating from plants having an increased level of triterpenes are thought to have a cholesterol lowering effect. Triterpenoid saponins are a class of plant secondary metabolites originated via the isoprenoid pathway by cyclization of 2, 3-oxidosqualene precursor in which one or more sugar residues are added [16] and leading to the formation of the triterpenoid skeleton of b-amyrin and related glycosides. Vitamin E [4.9 %] might not have a role in the antimicrobial activity, but is an excellent free radical scavenger involved in the prevention of prostate cancer [17]. Stigmasterol, found in both aerial and root extracts of *G. sylvestre* may not be directly involved in exhibiting antimicrobial activity, but could cause the synergistic effect of the compounds present in the extracts [18]. The synergism exhibited by all the compounds in *G. sylvestre* extracts could be responsible for the antimicrobial and antidiabetic activity in a big way. The phytochemical composition through mass spectrometry is usually the proper springboard to

convey the pharmacological importance and therapeutic nature of the plant species. It is very clear from the present study, the ethanolic extract of the *G. sylvestre* contain terpenes, fatty acid, alcohols, amine and vitamin which have various pharmacological activities such as anti-inflammatory, antiallergic, antioxidant, antidiabetic, antimicrobial and many more. Phytochemical composition investigation is one of the tools for the standardization of the herbal drug. In future, this study will be used to check the genuine nature of the plant powder, thus it plays an important role in preventing the possible steps of adulteration.

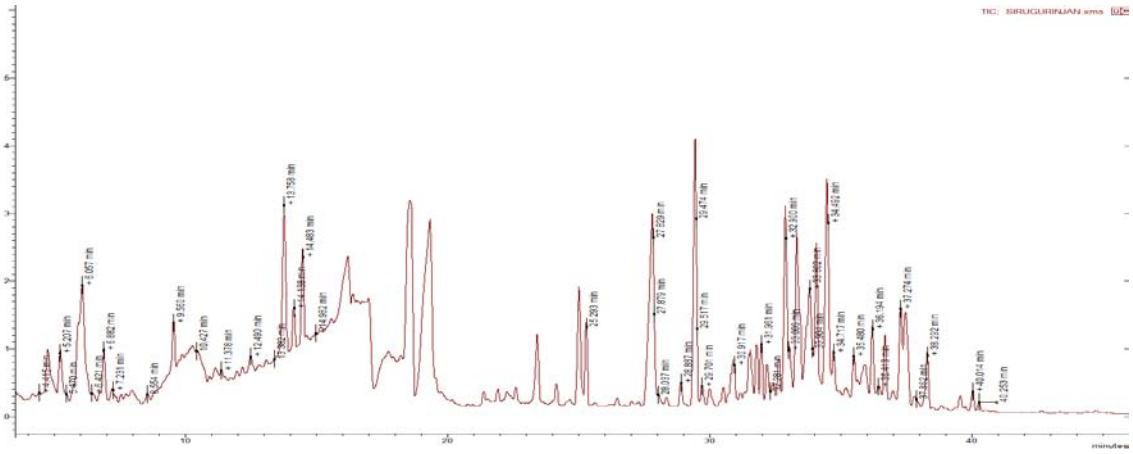
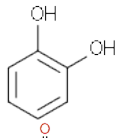
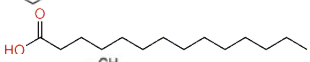
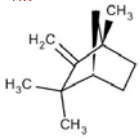
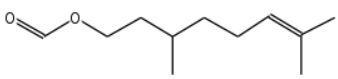
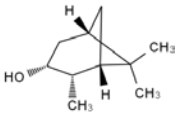
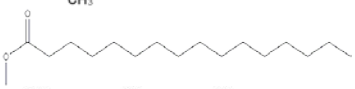
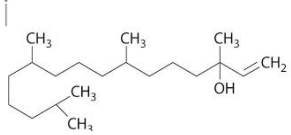
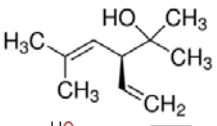
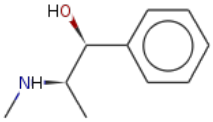
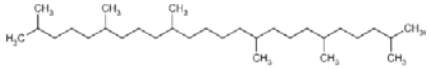
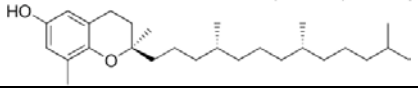
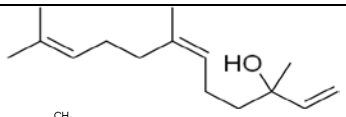
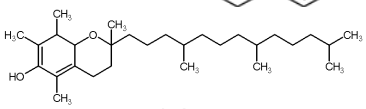
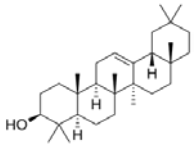
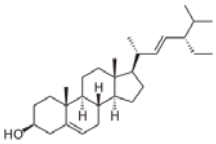
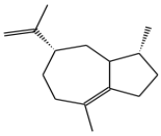
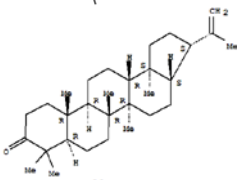
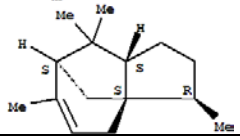


Fig. 2: GC-MS Chromatogram of ethanolic extract of *G. sylvestre*

Table 2: Therapeutic activity of compounds identified in the leaves of *G. sylvestre*

S. No.	Name of the compound	Structure	Nature and Therapeutic Activity*
1.	Catechol		Aromatic alcohol-Antioxidant, Anticancer, Pesticide
2.	Tetradecanoic acid		Fatty Acid-Antioxidant, Anticancer, hypercholesterolemic
3.	Bicyclo[2.2.1]heptane, 1,3,3-trimethyl-		Monoterpene-Antialzheimeran, Anticholinesterase
4.	6-Octen-1-ol, 3,7-dimethyl-, formate		Monoterpenoid-Antiallergenic, Antimicrobial, pesticide
5.	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1α,2β,5α)-		Terpene-Antimicrobial
6.	n-Hexadecanoic acid		Fatty Acid-Antioxidant, Antiandrogenic, Hypercholesterolemic, Pesticide
7.	Isophytol		Acyclic diterpene alcohol-Anticancer, Antidiabetic
8.	α-Santoline alcohol		Alcohol-Antimicrobial, Insecticidal
9.	DL-Ephedrine		Amine-Antiasthmatic, Antiepileptic, Antiinflammatory, Antirhinitic, Diuretic, Cardiotonic, Pesticide
10.	Squalene		Triterpene-Antioxidant, Anticancer, Pesticide
11.	δ-Tocopherol		Vitamin-Antidiabetic, Antinfertility, Antiinflammatory, Antioxidant, Antitumor, Cardioprotctive

12.	Nerolidol		Sesquiterpene-Antimicrobial, Pesticide, Antiacne
13.	Vitamin E		Vitamin-Antidiabetic, Antinfertility, Antiinflammatory, Antioxidant, Antitumor, Cardioprotctive
14.	β-Amyrin		Triterpene-Antitumor
15.	Stigmasterol		Sterol-Antihepatotoxic, Antioxidant, Hypocholesterolemic, Antiinflammatory, Anticancer, Antiviral
16.	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1α,7α,8aβ)]-		Naphthalene-Antiinflammatory, Antiulcer, Pesticide
17.	Hop-22(29)-en-3β-ol		Sterol-Antioxidant, Hypocholesterolemic,
18.	Cedrene-V6		Sesquiterpene-Anticancer

*Source: Dr. Duke's Phytochemical and Ethnobotanical Databases [15]

CONFLICT OF INTERESTS

Declared None

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