Journal of Current Chemical & Pharmaceutical Sciences

J. Curr. Chem. Pharm. Sc.: 3(1), 2013, 6-15
ISSN 2277-2871

GC-MS ANALYSIS OF BIOACTIVE CONSTITUENTS OF *HYPERICUM MYSORENSE* (HYPERICACEAE)

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(*Received : 28.11.2012; Accepted : 08.12.2012*)

ABSTRACT

The investigation was carried out to determine the possible bioactive components of leaf and bark of *Hypericum mysorense* using GC-MS. The chemical compositions of the ethanol extract of leaf and bark of *Hypericum mysorense* were investigated using Perkin-Elmer Gas Chromatography-Mass spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. 17 compounds from leaves were identified of which lupulon, 1,2,4-Cyclopentanetrione, 3,3-bis (3-methyl-2-butenyl)-5-(3-methyl-1-oxobutyl)- [synonyms: Hulupone] and deoxyartemisinin were found to be predominant compounds whereas there are 8 compounds were detected from the bark of *Hypericum mysorense* of which Sucrose, Diazoprogesterone and Squalene were found to be major compounds identified.

Key words: *Hypericum mysorense*, GC-MS, Squalene, Phytol, Lupulon.

INTRODUCTION

The genus *Hypericum mysorense* belongs to the family Hypericaceae and consists of approximately 20 species exclusively of tropical origin. *Hypericum mysorense* is a plant native to the Nilgiri Hills in India. It is closely related to *Hypericum perforatum*. *Hypericum mysorense* is mentioned in Ayurvedic texts as having anti-viral and nerve calming properties came into the spotlight. Hypericum *mysorense* has been used to treat wounds as part of the Ayurvedic system of traditional medicine[1]. Some research into the possibility of antiherpetic properties in *Hypericum mysorense* extracts has been performed[2,3].

Taking into consideration of the medicinal importance of the plant, the ethanol extract of *Hypericum mysorense* was analyzed for the GC-MS. This work will help to identify the compounds of therapeutic value. GC-MS is one of the technique to identify the bioactive constituents of long chain branched chain hydrocarbons, alcohols, acids, esters, etc.

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EXPERIMENTAL

Materials and methods

The leaf and bark of *Hypericum mysorense* were collected from the Kothagiri, Nilgiri Biosphere Reserve, Western Ghats, Tamil Nadu. The plants were shaded dried and pulverized to powder in a mechanical grinder. Required quantity of powder was weighed and transferred to stoppered flask, and treated with ethanol until the powder is fully immersed. The flask was shaken every hour for the first 6 hours and then it was kept aside and again shaken after 24 hours. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using a vacuum distillation unit. The final residue thus obtained was then subjected to GC-MS analysis.

GC-MS Analysis

GC-MS analysis of these extracts were performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-I, fused silica capillary column (30 mm X 0.25 mm 1D X 1 μMdf, composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 mL/min and an injection volume of 2 μL was employed (split ratio of 10 : 1); Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % 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 a Turbomass.

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and technology (NIST) having more than 62,000 patterns. 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.

RESULTS AND DISCUSSION

Seventeen compounds were identified in *Hypericum mysorense* leaf by GC-MS analysis. The active principles with their retention time (RT), molecular formula (MF), molecular weight (MW) and concentration (%) are presented in Table 1 and Fig. 1. The prevailing compounds were lupulon (69.41%), 1,2, 4-Cyclopentanetrione, 3,3-bis(3-methyl-2-butenyl)-5-(3-methyl-1-oxobutyl)-[synonyms: Hulupone] (21.57%), deoxyartemisinin (2.44%), vitamin E (0.94), α-sitosterol (0.92), 2-Benzofurancarboxylic acid, 2,4,5,6,7,7a-hexaahydro-4,4,7a-trimethyl-, methyl ester, cis- (0.70), 1H-Indole-2,3-dione, 5-pentyl-1-(trimethylsilyl)-, 3-(O-methyloxime) (0.70%), p-nitrophenyl p-decycloxybenzoate (0.69%), 1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)- (0.43%), caryophyllenyl alcohol (0.40), 3,7,11,15-tetramethyl-2-2hexadecen-1-ol (0.40%), cholan-24-oic acid, 3,12-dioxo-, (5a)- (0.40%), naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)- (0.38%), phytol (0.23%), 1,2-benzenedicarboxylic acid, diisooctyl ester (0.14%), bicycle [3,3,1] nonan-9-one, 1.2.4-trimethyl-3-nitro-, (2-endo, 3-exo, 4-exo)-(.+ -.)- (0.14%), 1,6,10-dodecatrine-3-ol, 3,7,11-trimethyl-, [S-(Z)]- (0.09%). Figs. 3, 4, 5, 6, 7, 8, 9 and 10 show the mass spectrum and structures of important constituents of ethanol extract of *Hypericum mysorense* leaf viz: 1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)-, Caryophyllenyl alcohol, 1,6,10-Dodecatrine-3-ol, 3,7,11-trimethyl-, [S-(Z)]-, 1H-Indole-2,3-dione, 5-pentyl-1-(trimethylsilyl)-, 3-(O-methyloxime), deoxyartemisinin, 1,2,4-Cyclopentanetrione, 3,3-bis (3-methyl-2-butenyl)-5-(3-methyl-1-oxobutyl)- [synonyms: Hulupone], lupulon, and α-sitosterol. Table 2 listed the various phytochemical constituents which contribute to the medicinal activity of ethanol extract of *Hypericum mysorense* leaf.

Table 1: Components identified in the ethanol extract of leaf of *Hypericum mysorense*

S. No.	RT	Name of the compound	Molecular formula	MW	Peak area %
1	6.77	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)-	$C_{15}H_{24}$	204	0.43
2	7.92	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	$C_{15}H_{24}$	204	0.38
3	8.70	Caryophyllenyl alcohol	$C_{15}H_{26}O$	222	0.40
4	8.99	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-	$C_{15}H_{26}O$	222	0.09
5	9.22	Bicyclo[3.3.1]nonan-9-one, 1,2,4-trimethyl-3-nitro-, (2-endo,3-exo,4-exo)-(.+-.)-	$C_{12}H_{19}NO_3$	225	0.14
6	11.34	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.40
7	14.61	Phytol	$C_{20}H_{40}O$	296	0.23
8	19.65	Cholan-24-oic acid, 3,12-dioxo-, (5á)-	$C_{24}H_{36}O_4$	388	0.40
9	20.41	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	0.14
10	20.72	1H-Indole-2,3-dione, 5-pentyl-1-(trimethylsilyl)-, 3-(O-methyloxime)	$C_{17}H_{26}N_2O_2Si$	318	0.70
11	20.99	p-Nitrophenyl p-decyloxybenzoate	$C_{23}H_{29}NO_5$	399	0.69
12	21.91	2-Benzofurancarboxylic acid, 2,4,5,6,7,7a-hexahydro-4,4,7a-trimethyl-, methyl ester, cis-	$C_{13}H_{20}O_3$	224	0.70
13	22.88	Deoxyartemisinin	$C_{16}H_{26}O_3$	266	2.44
14	23.33	1,2,4-Cyclopentanetrione, 3,3-bis(3-methyl-2-butenyl)-5-(3-methyl-1-oxobutyl)- [Synonyms: Hulupone]	$C_{20}H_{28}O_4$	332	21.57
15	24.39	Lupulon	$C_{26}H_{38}O_4$	414	69.41
16	28.42	Vitamin E	$C_{29}H_{50}O_2$	430	0.94
17	31.56	á-Sitosterol	$C_{29}H_{50}O$	414	0.92

Table 2: Activity of components identified in the ethanol extract of leaf of *Hypericum mysorense*

S. No.	Name of the compound	Molecular formula	Nature of compound	**Activity
1	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)-	$C_{15}H_{24}$	Sesquiterpene	Anti-tumor, analgesic antibacterial, anti-inflammatory sedative, fungicide
2	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	$C_{15}H_{24}$	Sesquiterpene	Anti-tumor, analgesic antibacterial, anti-inflammatory sedative, fungicide
3	Caryophyllenyl alcohol	$C_{15}H_{26}O$	Sesquiterpene alcohol	Anti-tumor, analgesic antibacterial, anti-inflammatory sedative, fungicide

Cont…

S. No.	Name of the compound	Molecular formula	Nature of compound	**Activity
4	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-	$C_{15}H_{26}O$	Sesquiterpene alcohol	Anti-tumor, analgesic antibacterial, anti-inflammatory sedative, fungicide
5	Bicyclo[3.3.1]nonan-9-one, 1,2,4-trimethyl-3-nitro-, (2-endo,3-exo,4-exo)-(.+-.)-	$C_{12}H_{19}NO_3$	Nitrogen compound	Antimicrobial
6	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	Terpene alcohol	Antimicrobial anti-inflammatory anticancer diuretic
7	Phytol	$C_{20}H_{40}O$	Diterpene	Antimicrobial anti-inflammatory anticancer diuretic
8	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	Plasticizer compound	Antimicrobial antifouling
9	1H-Indole-2,3-dione, 5-pentyl-1-(trimethylsilyl)-, 3-(O-methyloxime)	$C_{17}H_{26}N_2O_2Si$	Alkaloid	Antimicrobial anti-inflammatory
10	Deoxyartemisinin	$C_{16}H_{26}O_3$	Sesquiterpenoids	Anti-tumor, analgesic antibacterial, anti-inflammatory sedative, fungicide
11	1,2,4-Cyclopentanetrione, 3,3-bis(3-methyl-2-butenyl)-5-(3-methyl-1-oxobutyl)- [Synonyms: Hulupone]	$C_{20}H_{28}O_4$	Triketone compound	Antimicrobial anti-inflammatory anti-diabetic anticancer
12	Lupulon	$C_{26}H_{38}O_4$	Resinous substance	Sedative flavor in beer
13	Vitamin E	$C_{29}H_{50}O_2$	Vitamin	Antiageing, analgesic, antidiabatic anti-inflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, hepatoprotective, hypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, anti-bronchitic, anticoronary
14	á-Sitosterol	$C_{29}H_{50}O$	Steroid	Antimicrobial anticancer antiarthritic antiasthma diuretic

**Activity source: Dr. Duke's Phytochemical and Ethnobotanical Database

Eight compounds were identified in *Hypericum mysorense* bark by GC-MS analysis. The active principles with their retention time (RT), molecular formula (MF), molecular weight (MW) and concentration (%) are presented in Table 3 and Fig. 2. The prevailing compounds were Sucrose (73.09%), Diazoprogesterone (8.36%), Squalene (5.45%), Tridecane, 1-iodo-(3.27%), 1-Iodo-2-methylundecane (2.91%), Tetradecane, 1-iodo- (2.55%), 1,2-Benzenedicarboxylic acid, diisooctyl ester (2.18%), Dodecane, 1-iodo- (2.18%). Figs. 11, 12, 13, 14, 15 and 16 show the mass spectrum and structures of important constituents of ethanol extract of *Hypericum mysorense* bark viz: Sucrose, 1-Iodo-2-methylundecane, Dodecane, Tridecane, Tetradecane and Diazoprogesterone. Table 4 listed the various phytochemical constituents which contribute to the medicinal activity of ethanol extract of *Hypericum mysorense*.

Table 3: Components identified in the ethanol extract of bark of *Hypericum mysorense*

S. No.	RT	Name of the compound	Molecular formula	MW	Peak area %
1	7.69	Sucrose	$C_{12}H_{22}O_{11}$	342	73.09
2	19.88	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	296	2.91
3	20.42	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	2.18
4	21.28	Dodecane, 1-iodo-	$C_{12}H_{25}I$	296	2.18
5	22.66	Tridecane, 1-iodo-	$C_{13}H_{27}I$	310	3.27
6	24.03	Tetradecane, 1-iodo-	$C_{14}H_{29}I$	324	2.55
7	24.20	Squalene	$C_{30}H_{50}$	410	5.45
8	31.57	Diazoprogesterone	$C_{21}H_{30}N_4$	338	8.36

Table 4: Activity of Components identified in the ethanol extract of bark of *Hypericum mysorense*

S. No.	Name of the compound	Molecular formula	Nature of compound	**Activity
1	Sucrose	$C_{12}H_{22}O_{11}$	Sugar compound	Preservative
2	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	Iodo compound	Antimicrobial
3	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O4$	Plasticizer compound	Antimicrobial antifouling
4	Dodecane, 1-iodo-	$C_{12}H_{25}I$	Iodo compound	Antimicrobial
5	Tridecane, 1-iodo-	$C_{13}H_{27}I$	Iodo compound	Antimicrobial
6	Tetradecane, 1-iodo-	$C_{14}H_{29}I$	Iodo compound	Antimicrobial
7	Squalene	$C_{30}H_{50}$		Antibacterial, antioxidant, antitumor, cancer preventive, immunostimulant, chemo preventive, lipoxygenase-inhibitor, pesticide
8	Diaz progesterone	$C_{21}H_{30}N_4$	Nitrogen compound	Anti HIV

**Activity source: Dr. Duke's Phytochemical and Ethnobotanical database

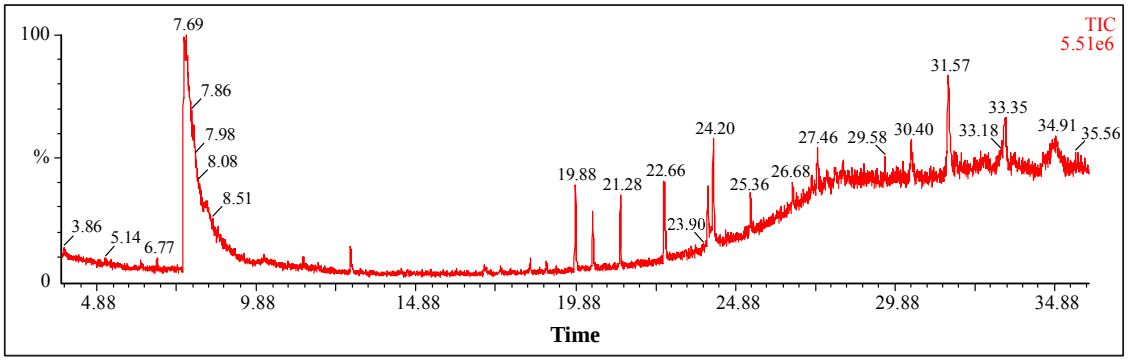

Fig. 1: GC-MS chromatogram of the ethanol extract of leaf of *Hypericum mysorense*

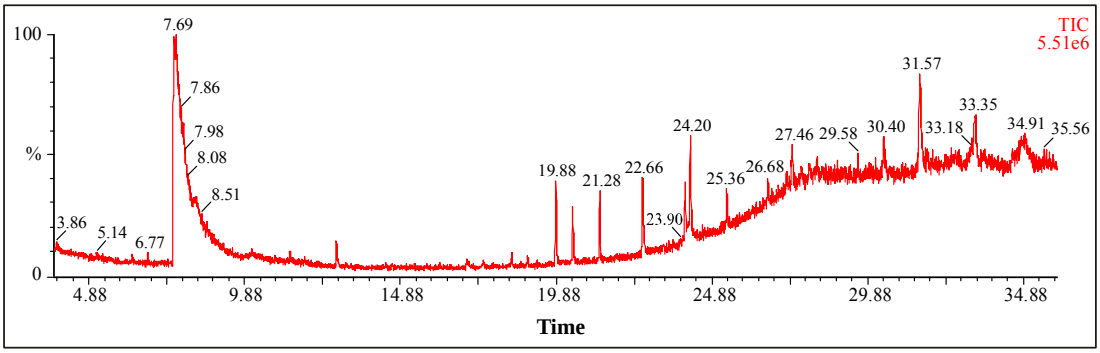

Fig. 2: GC-MS chromatogram of the ethanol extract of bark of *Hypericum mysorense*

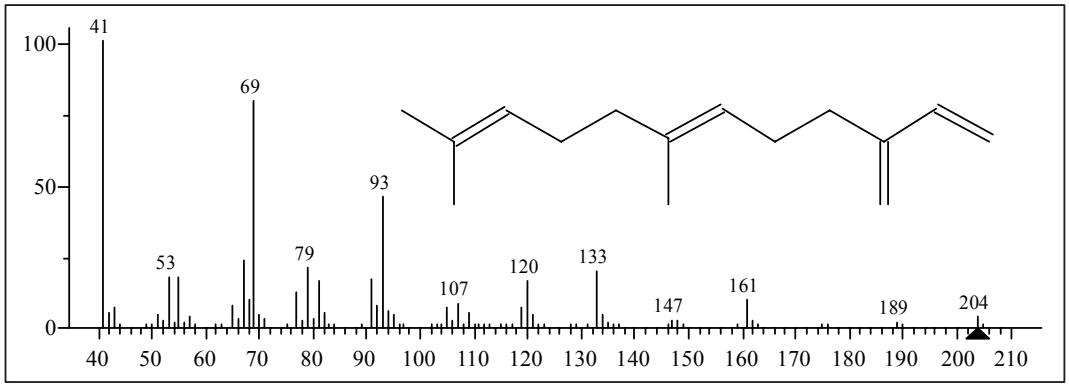

Fig. 3: Mass spectrum of 1, 6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)-

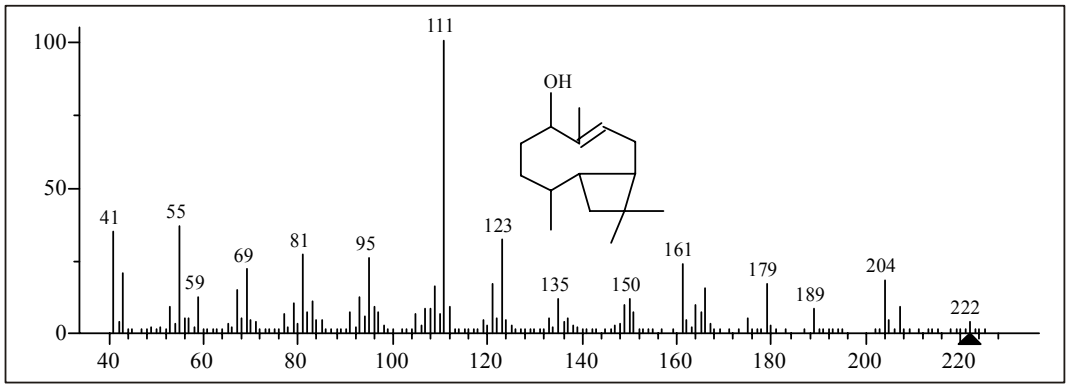

Fig. 4: Mass spectrum of Caryophyllenyl alcohol

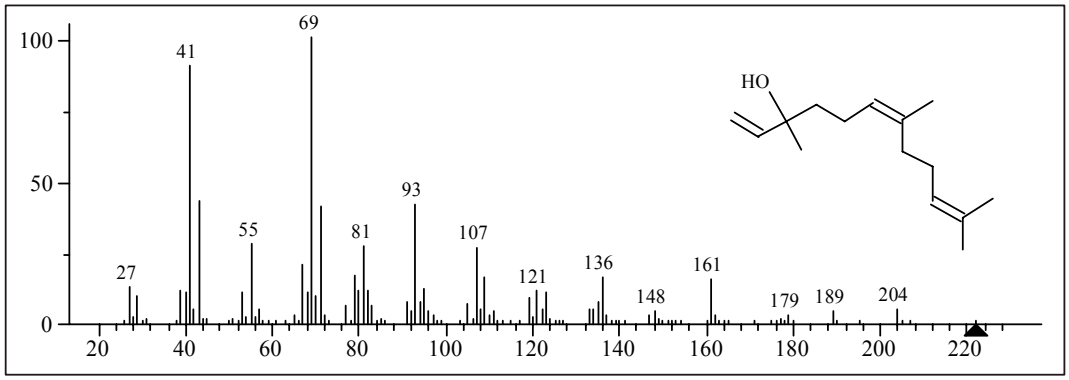

Fig. 5: Mass spectrum of 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-

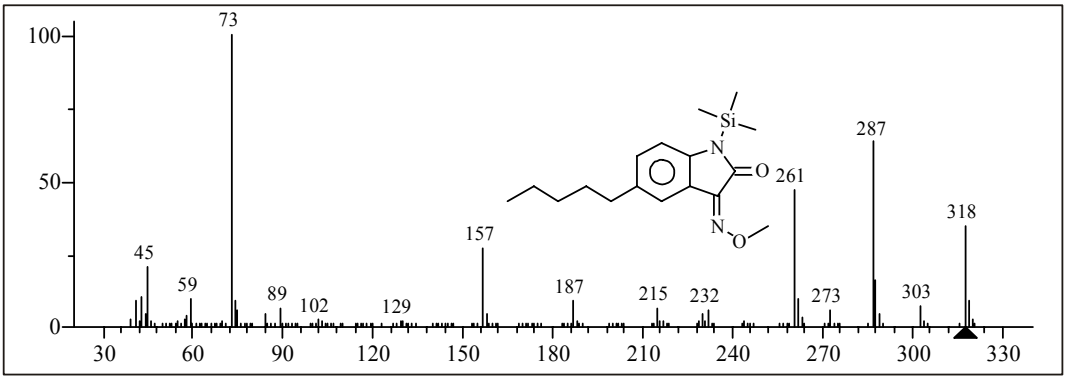

Fig. 6: Mass spectrum of 1H-Indole-2,3-dione, 5-pentyl-1-(trimethylsilyl)-, 3-(O-methyloxime

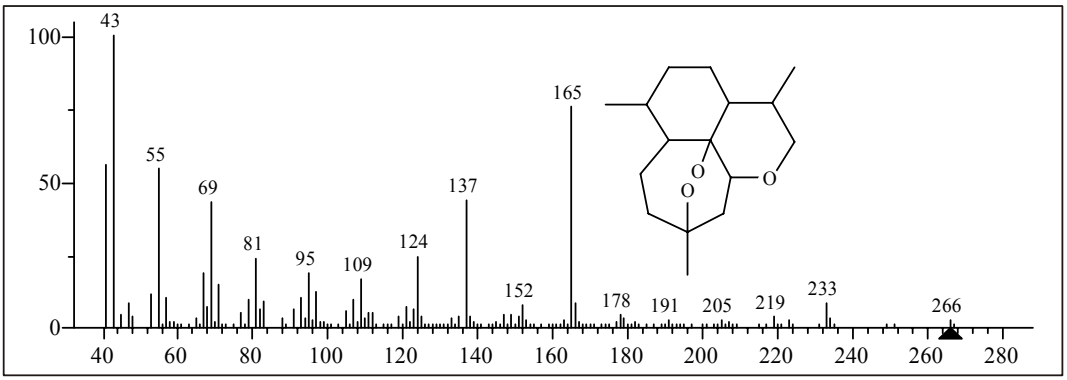

Fig. 7: Mass spectrum of Deoxyartemisinin

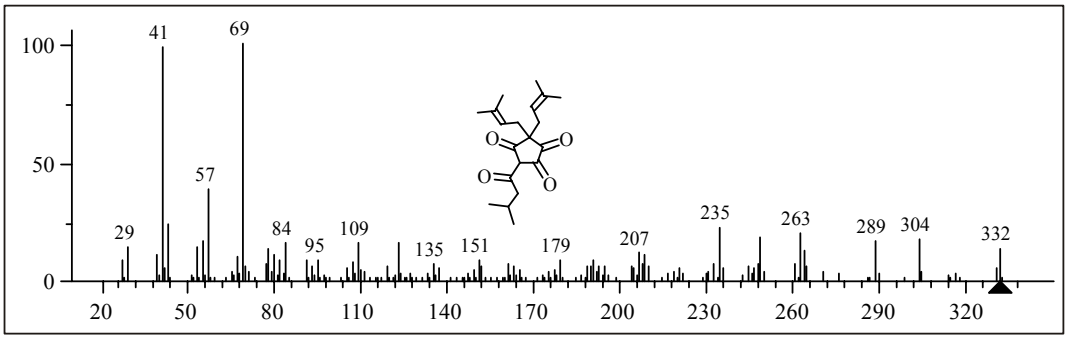

Fig. 8: Mass spectrum of 1,2,4-Cyclopentanetrione, 3,3-bis(3-methyl-2-butenyl)-5-(3-methyl-1-oxobutyl)-[Synonyms: Hulupone]

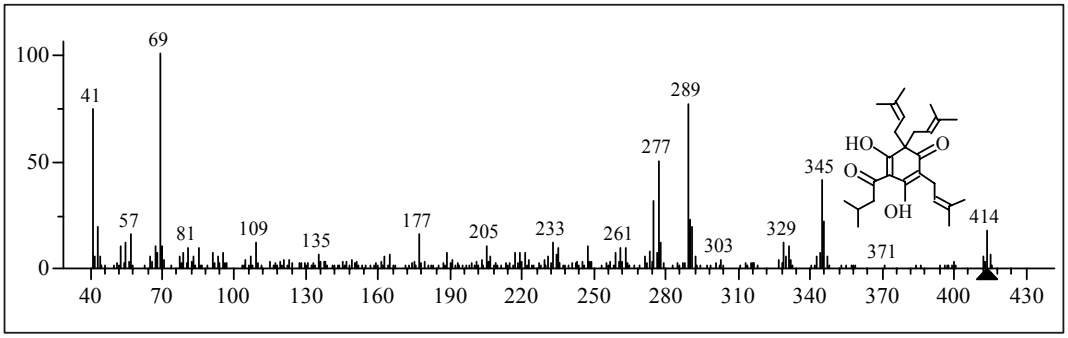

Fig. 9: Mass spectrum of Lupulon

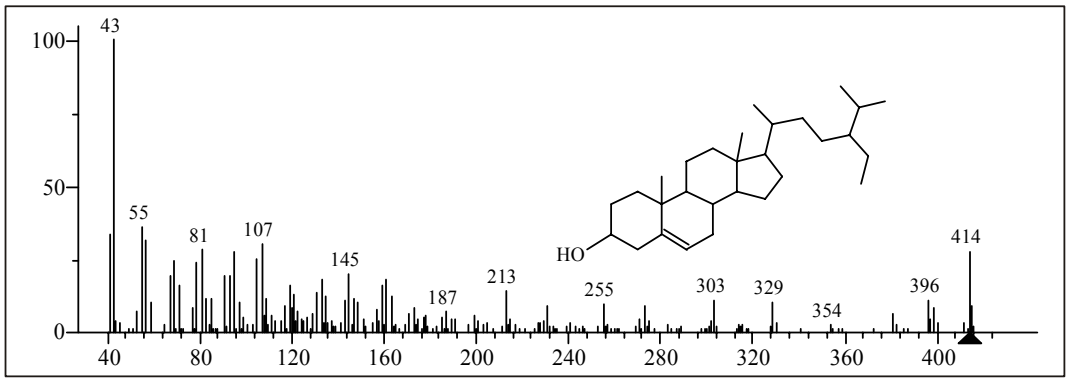

Fig. 10: Mass spectrum of á-Sitosterol

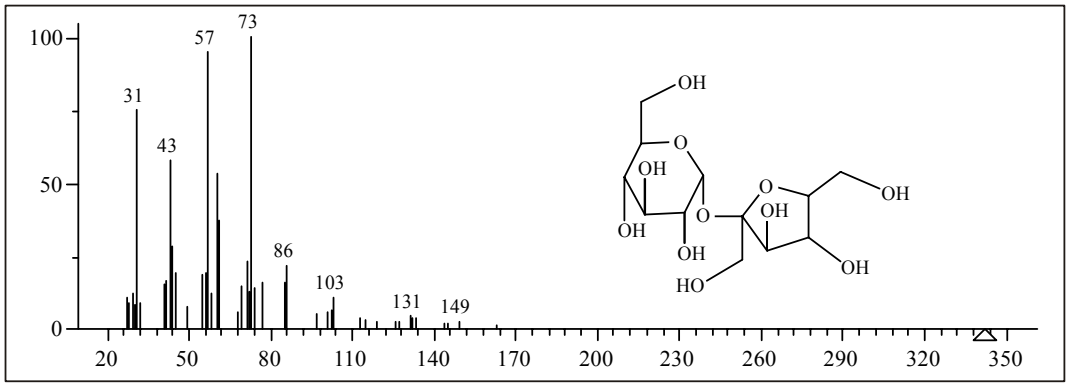

Fig. 11: Mass spectrum of Sucrose

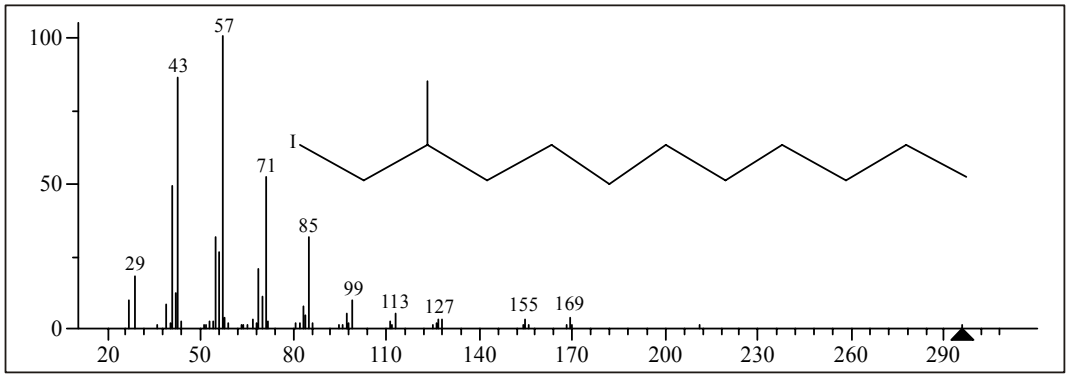

Fig. 12: Mass spectrum of 1-Iodo-2-methylundecane

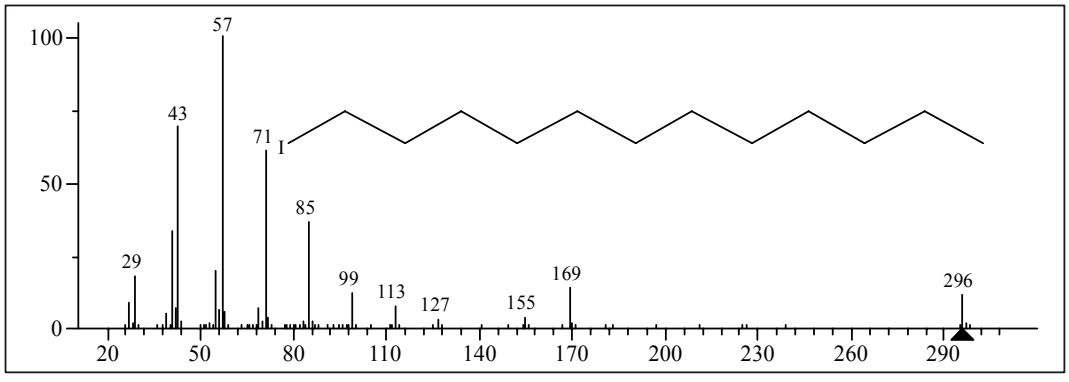

Fig. 13: Mass spectrum of Dodecane, 1-iodo-

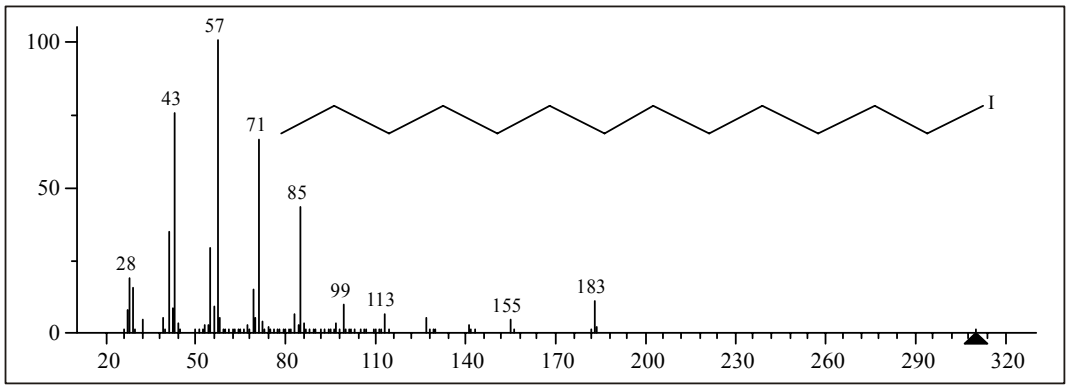

Fig. 14: Mass spectrum of Tridecane, 1-iodo-

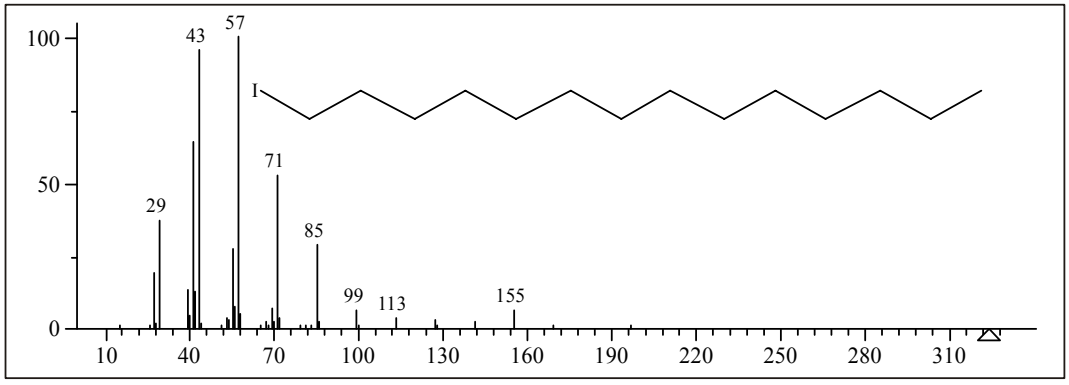

Fig. 15: Mass spectrum of Tetradecane, 1-iodo-

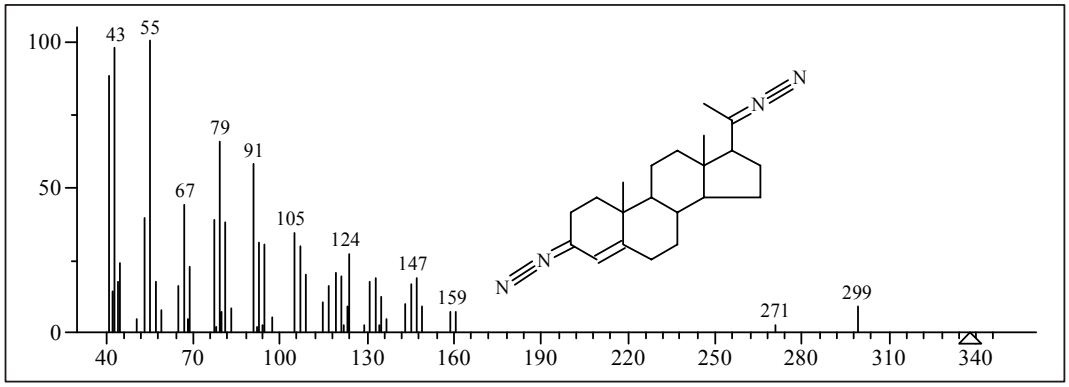

Fig. 16: Mass spectrum of Diazoprogesterone

Among the identified phytochemicals, phytol is detected in *Hypericum mysorense* whole plant which was also found to be effective at different stages of the arthritis. It was found to give good as well as preventive and therapeutic results against arthritis. The results show that reactive oxygen species promoting substances such as phytol constitute a promising novel class of pharmaceuticals for the treatment of rheumatory diseases and possibly other chronic inflammatory diseases[4,5]. Squalene has antioxidant activity. Recently, it has been found that, squalene possesses chemopreventive activity against the colon carcinogenesis[6,7]. Sitosterol limits the amount of cholesterol entering the body by inhibiting cholesterol absorption in the intestines, therefore decreasing the levels of cholesterol in the body. It is helpful with benign prostatic hyperplasia (BPH), due to its anti-inflammatory effects and its ability to improve urinary symptoms and flow. Lupulon, its antituberculous activity both *in vitro* and *in vivo*. These findings and its

relatively low toxicity indicated that lupulon may be an effective chemotherapeutic agent. It becomes, therefore, desirable to investigate some important factors which may influence its activity either *in vitro* or *in vivo*[8, 9]. Lupulon inhibits the growth of most common fungi and many gram-positive bacteria; it also inhibits several acid-fast organisms, including the tubercle bacillus. Vitamin-E is thought to be important chain breaking antioxidant, which plays an important role in various stages of carcinogenesis through its contribution and immunocompetence, membrane and DNA repair and decreasing oxidative DNA damage[10]. *In vivo* studies showed that vitamin-E can prevent oxidation of DNA by inhibiting activated neutrophils. Vitamin-E can protect the conjugated double bond of β-carotene from oxidation[11]. The above said phytocompounds were found in the ethanol extract of *Hypericum mysorense* which are being used for the pharmacological work.

In the present study, 17 and 8 compounds have been identified from the leaf and bark of *Hypericum mysorense* respectively by gas chromatography-mass spectrometry (GC-MS analysis). The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the plant. The mass spectrometry analyzes the compounds eluted at different times to identify the nature and structure of the compounds. These mass spectra are fingerprint of that compound which can be identified from the data library. Thus GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

ACKNOWLEDGEMENT

We would like to mark Mr. S. Kumaravel, Senior Scientist, Indian Institute of Crop Processing Technology (Ministry of Food Processing Industries, Government of India), Tanjavore, Tamil Nadu for providing all the facilities and support used to carry out the work.

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