GC-MS profile of bioactive compounds from medicinally important *Periploca hydaspidis*

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Abstract: The aim of the present study was to investigate bioactive compounds in different solvent extracted samples from the stem tissues of *P. hydaspidis* using Gas Chromatography and Mass Spectroscopy techniques. GC-mass spectrum was compared with the data base of National Institute of Standards and Technology (NIST) containing more than 62000 patterns of the mass spectrum. During matching with NIST library the match factor greater than 700 was considered only for better and pure results. The results revealed that different solvent extracted samples analyzed through GC-MS contained appreciable quantities of different bioactive molecules including antibiotics, fatty acids and protein which have important pharmacological significance.

Keywords: Pharmacological compound, GC-MS, *P. hyaspidis*, antibiotic, fatty acids.

INTRODUCTION

Throughout the history of mankind plants were used as medicine for curing and preventing diseases. From the analysis of the fossil records of humans it is shown that humans have been using the plants as medicine as early as 60,000 years. More than 65% of the world's population is using traditional medicinal plants for the treatment of various health related issues (Farnsworth, 1985). The search for medicinal plants, both as potential antimicrobial crude drugs as well as a source for natural compounds has attracted many research groups in the area of ethno-pharmacology (Bilal and Bakht, 2016; Bilal *et al*., 2017; Khaleeq *et al*., 2018' Shehla *et al*., 2018).

Plants having medicinal activities produce different bioactive chemical compounds which are used in the treatment of different human and animal diseases. Herbal medicine represents one of the most important fields of traditional medicine in rural areas. Thus, phytotherapy is practiced by a large section of people for the treatment of several physical, physiological, mental and social ailments. To encourage the proper use of herbal medicine and to find out their potential as sources for new drugs it is indispensable to study medicinal plants in a more intensified and scientific way (Awadh *et al*., 2001; Ayaz *et al.,* 2017, 2018). A renewed curiosity has occurred in the last two decades to investigate for phytochemicals of native and naturalized plants for pharmaceutical and nutritional purposes (Wangensteen *et al*., 2004).

MATERIALS AND METHODS

Plant material

The present research was conducted out at the Institute of Biotechnology and Genetic Engineering, The University of Agriculture Peshawar Pakistan. Plant materials were obtained from various sites of District Swat, KPK. The plant materials (stems) were thoroughly washed with distilled water to remove any dust particles, dried for eight days and pulverized to powder using a mechanical grinder.

Crude extract preparation

One thousand gram of powdered materials was soaked separately in six liters of methanol in extraction flasks and kept at 24 C in dark for 7 days and shaken three times daily. The samples were passed through Whatman filter paper No.1. Three liters of fresh methanol was mixed with the remaining residue, filtered and the whole procedures were adopted thrice. The filtered sample was dried below 45ºC under vacuum pressure in rotary evaporator. The resulting extract was taken and kept in dark for drying in a glass vial at room temperature.

Fractionation of crude extract

Major portion of the crude extract (80g) was dissolved in 500 ml sterile distilled water and mixed with analytical grade n-hexane (300ml) in separatory funnel, shaken gently and allowed to stand for 15 minutes to separate the two phases. The upper n-hexane phase was collected and the lower aqueous phase was re-fractionated three times with n-hexane. All fractions of n-hexane were pooled together and dried at 45°C under vacuum pressure with a rotary evaporator. The same procedures were followed for ethyl acetate and butanol. The lower aqueous phase at the end of the process was dried at 45ºC under vacuum pressure via rotary evaporator.

GC-MS analysis

The GC-MS analysis of the plant extracted with different solvents was carried out through Agilent Mass Hunter

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work station Gas chromatograph interfaced to mass spectrometer (GC-MS). Triple Quadrupole Acquisition method was followed during the process. The machine was equipped with a non polar column packed with film made of 95% Dimethylepolysiloxane and 5% phenyl (Agilent USB393752HHP-5MS-30m length × 250µm diameter × 0.25µm film thickness). For the detection of the compounds an electron ionization source with 70eV energy was used. Ultra pure Helium gas (99.99%) was used as a carrier gas for mobile phase with split mode at septum purge flow rate of 3ml/min. the employed injection volume was 1µL with split ratio of 20:1. The temperature of the injector was 250°C. The temperature strategy in the oven was programmed as, 50°C for 5 min, then 10°C/min to 180°C for 15 min, then 10°C/min to 280°C for 20 min and lastly 20°C/min to 300°C for 1 min. The pressure was 9.7131 psi and the constant flow rate was 1.1929 ml/min with average velocity of 39.805 cm/sec. the total run time was 65min. The solutions were prepared by taking 1gm of each extract and making it soluble in 20ml of their respective solvents. The solutions were filtered through Whatman no. 1 filter paper to remove any solid particles. All the solutions used were clean and transparent. All the chemicals used were highly pure analytical grade. The mole percent peak area was calculated according to the following formula (Hossain *et al*., 2011)

Mole % component (Peak area) = area under peak/total area of all peaks×100

STATISTICAL ANALYSIS

Data are shown as mean values of three replications. MSTATC computer software was used for statistical analysis (Russel and Eisensmith, 1983).

RESULTS

The phytochemical screening of the crude methanolic extract obtained from the stems tissues and analysed through GC-MS is presented in table 1 (fig. 1). The data revealed that stem tissues contain some important compounds containing clindamycin which is a broad spectrum antibiotic having the peak area of 0.47%. Dietary compounds like 1-(4-Methoxy-2-nitroanilino)-1-deoxy-a-d-arabinofuranose and 3-O-Methyl-d-glucose were also present with peak areas of 5.39% and 80.86% respectively. An important Triterpenoid, Lupeol was also detected in the extract having important pharmacological activities. The sharpness of the peaks indicated that the compounds were in pure form.

The GC-MS analysis of the n-hexane extracted sample is exhibited in table 2 (fig. 2). The chromatogram showed 26 peaks indicating the presence of 26 compounds. The aldehyde group containing compounds and fatty acids were predominant in n-hexane extracted samples. The aldehyde group was represented by, -Heptenal, (Z)-, 2,4-Heptadienal, (E,E)-, 2-Octenal, (E)-, 2-Decenal, (Z)-, 2,4-Decadienal, (E,E)-, 2,4-Decadienal, (E,E)- with 0.046%, 0.13%, 0.05%, 0.410%, 0.027%, 0.041% peak area respectively. The biologically active fatty acids detected were 9-Oxononanoic acid (0.03%), Tetradecanoic acid (0.032%), n-Hexadecanoic acid (10.12%),Oleic Acid (0.82%), cis-13-Eicosenoic acid (1.69%), Erucic acid (0.054%). γ-Sitosterol with peak area of 3.83% is a phytosterol, a bioactive compound similar to cholesterol in its chemical structure and pharmacological importance was also detected in the extract. Lupeol and Lup-20(29)-en-3-ol, acetate, (3β)- with 24.63% and 30.65% peak area respectively were also reported in the same extract.

The phytochemical constituents detected in stem ethyl acetate extracted samples are shown in table 3 (fig. 3). Analysis of spectrogram revealed 13 compounds in the extract. The data showed the presence of n-hexadecanoic acid (palmitic acid) with peak area of 0.17%. This compound is a saturated fatty acid showing anti-inflammatory activities. Geranyl- isovalerate (0.010%) was also detected in the tested extract. Geranyl-isovalerate is usually used in the synthesis of potential flavonoidic modulators. GC-MS analysis also showed the presence of α-Amyrin with peak area of 1.01%. α-Amyrin is a pentacyclic triterpene having well known pharmacological activities. 2-Heptenal,(Z)- (0.04%) and 2,4-Decadienal, (E,E)- (0.03%) representing the aldehyde group were also present in the extract. Some esteric and thiol compounds were also detected in the same extract during the GC-MS analysis. Analysis of the data indicated the presence of amino acid D-alanine, N-propargyloxycarbonyl-, decyl ester with peak area of 0.046% (table 4; fig. 4). It is one of the twenty amino acid present in the building block of proteins. The matching factor for this compound in NIST library was found to be 739. 2-furancarboxaldehyde-5-(hydroxymethyl)- was also detected in water extracted sample which is an organic compound. It contain a furan ring, which have both aldehyde and alcohol functional groups. It has a wide use in baking industry. The peak area of this compound was found to be 0.109%. The other phytochemicals detected were 2-myristynoyl pantetheine and 3-O-methyl-d-glucose with peak area of 0.66% and 99.18% respectively.

DISCUSSION

GC-MS analysis of the stem tissues of *Periploca hydaspidis* extracted with different solvents. The mass spectrum obtained was analysed by comparing them with the data base of National Institute of Standards and Technology (NIST). This data base contains more than 62000 patterns of the mass spectrum (Peter *et al*., 2012).

Table 1: Phytochemical constituents of crude methanolic extracts from stem

Compound name	Molecular formula	Molecular weight	Retention time	Match factor	NIST number	ID number	Peak area (%)
Clindamycin	C18H33ClN2O5S	424	10.351	765	247789	17369	0.47
2-Furancarboxaldehyde, 5-(hydroxymethyl)-	C6H6O3	126	12.896	798	60544	12795	4.19
1-(4-Methoxy-2-nitroanilino)-1-deoxy-a-d-arabinofuranose	C12H16N2O7	300	17.415	626	226091	27291	5.39
3-O-Methyl-d-glucose	C7H14O6	194	20.216	736	127259	35456	80.86
n-Hexadecanoic acid	C16H32O2	256	24.699	904	335494	6723	1.13
1,2-Benzenedicarboxylic acid, diisooctyl ester	C24H38O4	390	41.035	800	113206	20061	0.14
Lupeol	C30H50O	426	51.896	854	124852	7814	2.69
Lup-20(29)-en-3-ol, acetate, (3β)-	C32H52O2	468	54.344	890	194307	12142	5.98

Table 2: Phytochemical constituents of n-hexane extract from stem

Compound name	Molecular formula	Molecular weight	Retention time	Match factor	NIST number	ID number	Peak area (%)
-Heptenal, (Z)-	C7H12O	112	7.667	940	57732	1853	0.046
2,4-Heptadienal, (E,E)-	C7H10O	110	8.516	921	235281	43455	0.13
2-Octenal, (E)-	C8H14O	126	9.920	897	2492	4131	0.05
2-Decenal, (Z)-	C10H18O	154	13.366	885	53572	2230	0.410
2,4-Decadienal, (E,E)-	C10H16O	152	13.846	905	235040	9886	0.027
2,4-Decadienal, (E,E)-	C10H16O	152	14.173	905	235040	9886	0.041
9-Oxononanoic acid	C9H16O3	172	16.364	759	144254	35130	0.03
Methyl 10-oxo-8-ecenoate	C11H18O3	198	18.326	797	293161	2619	0.410
Tetradecanoic acid	C14H28O2	228	19.953	896	189107	8471	0.032
Hexadecanoic acid, methyl ester	C17H34O2	270	23.613	934	333716	38248	5.47
n-Hexadecanoic acid	C16H32O2	256	25.082	932	335494	6723	10.12
9,12-Octadecadienoic acid, methyl ester, (E,E)-	C19H34O2	294	30.373	883	35798	28494	0.054
Oleic Acid	C18H34O2	282	30.758	787	228066	4483	0.82
9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	280	32.911	873	333207	7212	0.54
,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C18H30O2	278	33.294	832	333201	41695	0.957
4,8,12,16-Tetramethylheptadecan-4-olide	C21H40O2	324	38.607	869	110183	61522	0.068
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	C16H22O4	278	41.058	954	291384	110647	2.73
cis-13-Eicosenoic acid	C20H38O2	310	41.185	851	333523	18357	1.69
Erucic acid	C22H42O2	338	43.108	805	233026	4647	0.054
9,19-Cyclochloestene-3,7-diol, 4,14-dimethyl-, 3-acetate	C31H52O3	472	46.253	722	186587	138012	0.54
3-Hydroxy-5a,9,9-trimethyldecahydro-3,9a-methano-2-benzazepin-1-one	C14H23NO2	237	46.898	648	243680	160944	0.904
γ-Sitosterol	C29H50O	414	50.633	895	151558	6743	3.83
4,4,6a,6b,8a,11,11,14b-Octamethyl-octa decahydro-2H-picen-3-one	C30H48O	424	51.197	796	194624	153809	5.74
Lupeol	C30H50O	426	52.160	900	124852	7814	24.63
12-Oleanen-3-yl acetate, (3α)-	C32H52O2	468	53.289	875	244056	153829	7.52
Lup-20(29)-en-3-ol, acetate, (3β)-	C32H52O2	468	54.686	891	194307	12142	30.65

During matching with NIST library the match factor greater than 700 was considered only for better and pure results (Raja *et al*., 2011). The phytochemical analysis of crude methanolic extract of stem revealed that this part of the plant contain some important compounds like clindamycin which is a broad spectrum antibiotic having the peak area of 0.47%. Some dietary compounds 1-(4-methoxy-2-nitroanilino)-1-deoxy-a-d-arabinofuranose and 3-O-methyl-d-glucose were present with peak areas of 5.39% and 80.86% were also detected which suggests that the subject plant can be used for dietary purposes especially for animals as fodder (Hossain *et al*., 2011). An important triterpenoid, lupeol was also detected in the extract which has some important pharmacological activities. The sharpness of the peaks indicated that the compounds were in pure form (Peter *et al*., 2012).

Table 3: Phytochemical constituents of stem ethyl acetate extracted sample

Compound name	Molecular formula	Molecular weight	Retention time	Match factor	NIST number	ID number	Peak area %
2-Heptenal, (Z)-	C7H12O	112	7.670	806	57732	1853	0.04
Furan, 2-pentyl-	C9H14O	138	8.513	818	237570	43229	0.01
Z-8-Methyl-9-tetradecenoic acid	C15H28O2	240	13.376	756	130845	17223	0.05
2,4-Decadienal, (E,E)-	C10H16O	152	14.173	749	235040	9886	0.03
Geranyl isovalerate	C15H26O2	238	16.313	773	127736	46745	0.010
Tetradecanoic acid	C14H28O2	228	19.914	818	189107	8471	0.05
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296	21.418	818	114703	43206	0.050
2-Pentadecanone, 6,10,14-trimethyl-	C18H36O	268	21.551	822	337240	2225	0.01
tert-Hexadecanethiol	C16H34S	258	23.471	725	234966	22634	0.070
n-Hexadecanoic acid	C16H32O2	256	24.741	914	335494	6723	0.17
4,8,12,16-Tetramethylheptadecan-4-olide	C21H40O2	324	38.584	758	110183	61522	0.050
1,2-Benzene dicarboxylic acid, diisooctyl ester	C24H38O4	390	41.038	855	113206	20061	1.25
3-(1,5-Dimethyl-hexyl)-3a,10,10,12b-tetramethyl-tetra decahydro-benzo [4,5]cyclohepta[1,2-E]indene	C30H50	410	50.327	707	189358	188643	0.50
α-Amyrin	C30H50O	426	53.125	823	197289	25159	1.01
Lup-20(29)-en-3-ol, acetate, (3β)-	C32H52O2	468	54.328	847	194307	12142	3.5

Table 4: Phytochemical constituents of stem water extracted sample

Compound name	Molecular formula	Molecular weight	Retention time	Match factor	NIST number	ID number	Peak area %
D-Alanine, N-propargyloxycarbonyl decyl ester	C17H29NO4	311	10.403	739	347742	89422	0.046
2-Furan carboxaldehyde, 5-(hydroxymethyl)-	C6H6O3	126	12.957	919	60544	12795	0.109
2-Myristynoyl pantetheine	C25H44N2O5S	484	17.418	700	111636	4243	0.66
3-O-Methyl-d-glucose	C7H14O6	194	21.192	719	127259	35456	99.18

The GC-MS analysis of the n-hexane extracted samples revealed 26 different compounds. Some of these compounds were biologically active fatty acids which can be further analyzed for its potential to treat malnutrition especially in animal models while the other unsaturated fatty acids present can be used in biodiesel production (Senthamarai *et al*., 2012). The aldehyde group of compounds and fatty acids were predominant in n-hexane extracted samples. The aldehyde group was represented by, -heptenal, (Z)-, 2,4-heptadienal, (E,E)-, 2-octenal, (E)-, 2-decenal, (Z)-, 2,4-decadienal, (E,E)-, 2,4-decadienal, (E,E)- with 0.046%, 0.13%, 0.05%, 0.410 %, 0.027%, 0.041% peak area respectively. The biologically active fatty acids detected were 9-oxononanoic acid (0.03%), tetradecanoic acid (0.032%), n-hexadecanoic acid (10.12%), oleic Acid (0.82%), cis-13-eicosenoic acid(1.69%), erucic acid(0.054%). γ-sitosterol with peak area of 3.83% is a phytosterol and is a bioactive compound which is similar to cholesterol in its chemical structure and pharmacological importance was also detected in the extract (Raja *et al*., 2011). Lupeol and lup-20(29)-en-3-ol, acetate, (3β)- with 24.63% and 30.65% peak area respectively were also reported in the same extract. These compounds are pharmacologically active and have several potential medicinal properties (Senthamarai *et al*., 2012).

The phytochemical constituents detected in stem ethyl acetate extracted samples represented 13 different compounds in their extract. Hexadecanoic acid with peak area of 0.17% was detected. This compound is a saturated fatty acid and is highly bioactive showing anti-inflammatory activities. It is also called as palmitic acid. The said compound can also be used as biological weapon in combination with Al (Prabhu *et al*., 2013). Geranyl-isovalerate, another important compound detected in the said extract can be used in synthesis of potential flavonoidic modulators (Zhiheng *et al*., 2013). α-Amyrin with peak area of 1.01% was also detected in the GC-MS analysis which is a pentacyclic triterpene having well known pharmacological activities. 2-heptenal, (Z)- (0.04%) and 2,4-decadienal, (E,E)- (0.03%) representing

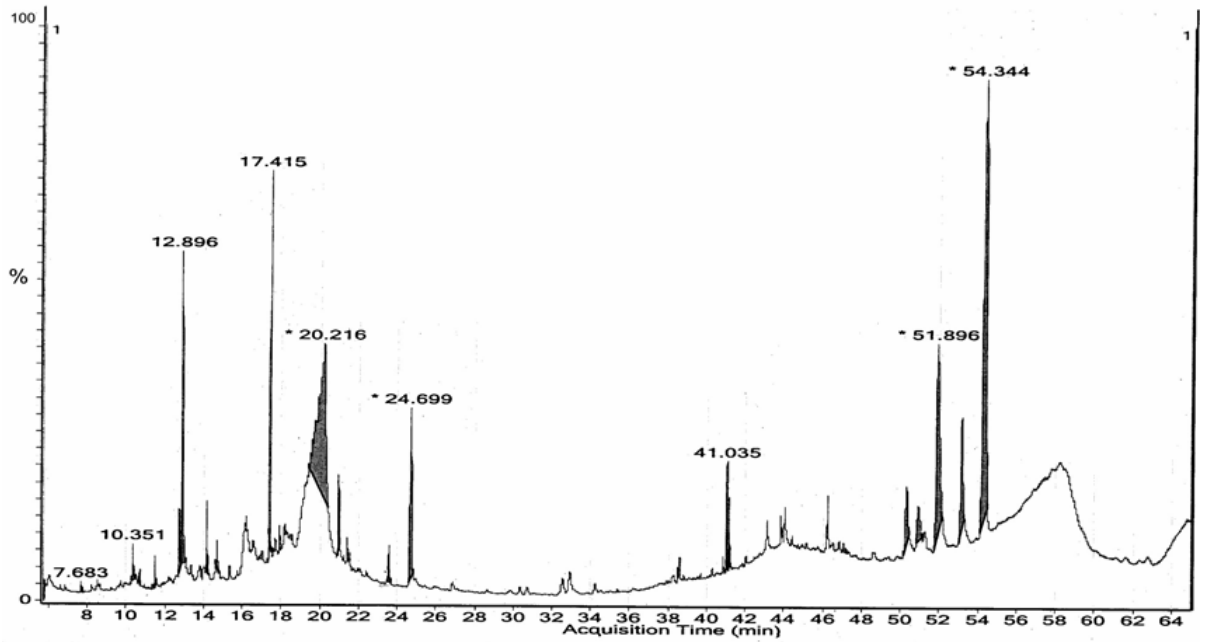

Fig. 1: Chromatogram obtained from stem crude methanolic extract

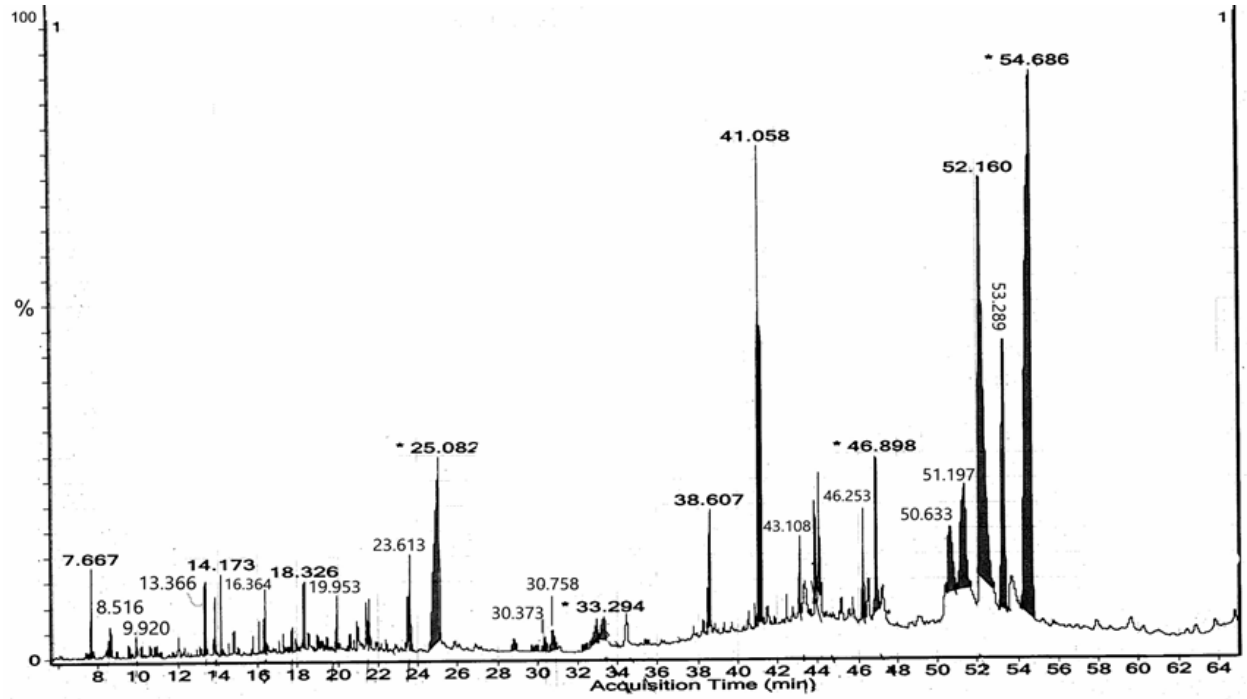

Fig. 2: Chromatogram obtained from n-hexane extract of stem

the aldehyde group were also present in the extract. Some esteric and thiol compounds were also detected in the same extract. The GC-MS analysis of stem water extracted samples demonstrated the presence of important compounds including amino acids like D-alanine, N-propargyloxycarbonyl-, decyl ester with peak area of 0.046%. It is one of the twenty amino acids of proteins, showing the dietary importance of the plant (Laila *et al*., 2013). 2-Furancarboxaldehyde-5-(hydroxymethyl)- an organic compound was also detected in water extracted samples. It contains a furan ring having both aldehyde and alcohol functional groups and has a wide use in baking industry (Senthamarai *et al*., 2012). The other phytochemicals detected were 2-myristynoyl pantetheine and 3-O-methyl-d-glucose with peak area of 0.66% and 99.18% respectively.

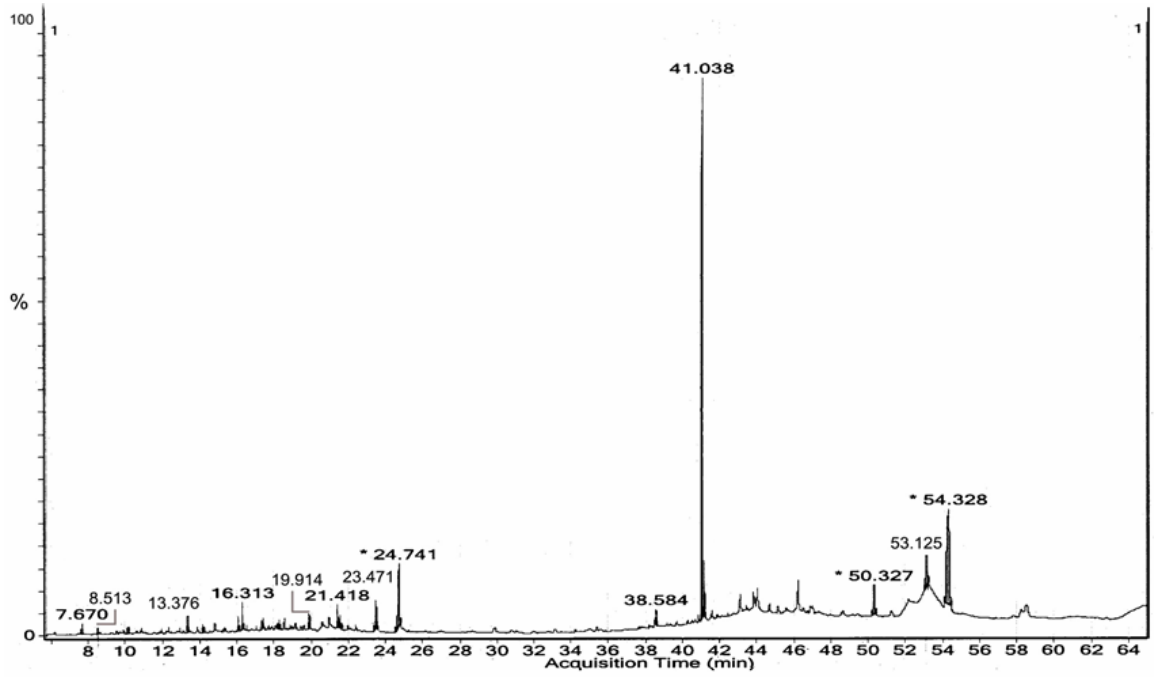

Fig. 3: Chromatogram obtained from stem ethyl acetate extracted sample

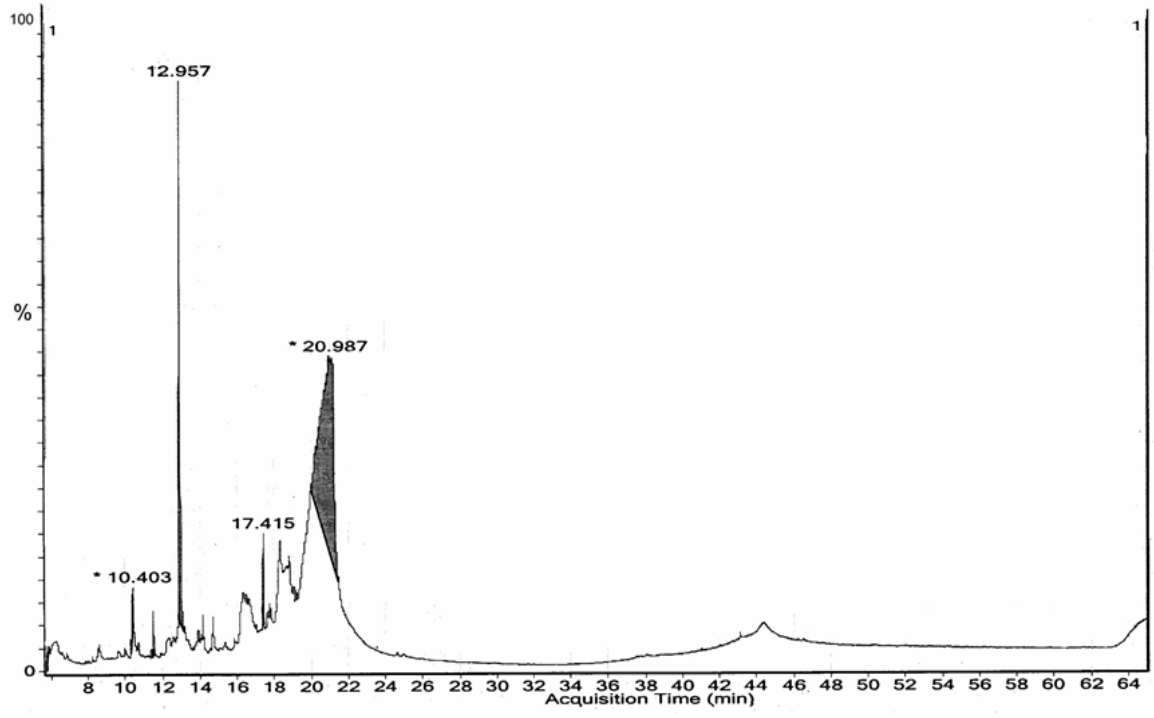

Fig. 4: Chromatogram obtained from stem water extracted sample

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