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Investigations on important secondary metabolites from aerial parts of *Artemisia absinthium* L. using GC-MS

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Abstract
The aim of the present study was to find the chemical constituents from aerial parts of *A. absinthium*- a medicinally important herb. The phytochemical analysis was carried out by using Gas Chromatography-Mass Spectroscopy instrument for the presence of active constituents. Three solvents viz. Methanol, Petroleum ether and Dichloromethane were used for extraction. The results showed the presence of 15 compounds in the Methanolic extract, 19 compounds in the Petroleum ether extract and 25 compounds in the Dichloromethane extract. Interpretation on mass spectrum of GC-MS was done using the National Institute Standard and Technology database comparing the spectral data of known compounds present in spectral library.

Keywords: antihelmintic, *Artemisia absinthium*, GC-MS, phytochemical, secondary metabolites

Introduction
The use of plants as medicines is a very ancient story and a traditional medical practice in all the passed civilizations [1]. Population rise, inadequate supply of drugs, the prohibitive cost of treatments, side effects of several *allopathic* drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. According to the World Health Organization, 80% of people in developing countries still depend on local medicinal plants to fulfill their primary health needs [2].

Artemisia absinthium L. of family Asteraceae, native to temperate regions of Eurasia and Northern Africa is commonly known as Wormwood. *Artemisia absinthium* is used to treat epilepsy, gastric problems, enlargement of spleen, urinary disorders and for wound healing [3]. It has some property like anthelmintic, antibacterial, antifungal, insect repellent for the flies and fleas [4], mosquitoes [5] and to kill house flies [6]. From the ethnopharmacological point of view, *A. absinthium* has been used for its antihelmintic, stomachic, antibacterial, antifeedent, antifertility, antipyretic, cytostatic, antitumor and malarial actions [7-8]. The plant was used as antidiarrhea [9], antihelmintic [10-12]. The plant is also described as anti-proliferative on human breast cancer cells [13].

Gas chromatography-mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. However, fewer reports are available with respect to the pharmacological properties of *Artemisia absinthium*. Keeping this in view, the present study has been undertaken to investigate the phytochemical constituents from aerial parts of *Artemisia absinthium* extracted in the solvents viz. Methanol, Petroleum ether and Dichloromethane.

Material and Methods
Collection of plant material
The aerial parts of *Artemisia absinthium* were collected from the village-Yadipora Pattan, District-Baramulla, Kashmir (India), situated along 34º11'16.24"N and 74º31'45.50"E at an elevation of 1596 meters. The plant was authenticated by Botanical Survey of India (BSI), Western Regional Centre, Pune, India. The voucher specimen (GMYAA1) was deposited in BSI, Pune, India as well as in the Department of Botany, Sant Gadge Baba Amravati University, Amravati as Voucher specimen 0252.

Preparation of Powder

The fresh aerial parts of *Artemisia absinthium* were washed with tap water and shade dried at room temperature (28±2 ˚C). The dried parts were powdered by electric blender. Finally, prepared powder was stored in air tight plastic bags and was used for further experimentation.

Preparation of the extract

The extraction was done by Soxhlet method using three solvents viz. Methanol, Petroleum ether and Dichloromethane. 10 grams of sample powder were extracted in 180 ml of solvent using Soxhlet apparatus by maintaining temperature of the boiling point for each solvent [14-15]. The extraction time required was 3-18 hours [16]. The extracts were filtered and concentrated to 5ml using rotatory vacuum evaporator at room temperature and then stored at -20˚C temperature until further analysis. These crude samples were then used for GC - MS analysis.

Gas Chromatography-Mass Spectrometric Analysis (GC-MS)

The GC-MS analysis was carried out at University Science Instrumentation Center (USIC), Shivaji University, Kohlapur, Maharashtra, India using gas chromatography – high resolution mass spectrophotometer. 2 µl of the prepared extracts was employed for GC-MS analysis. The GC-MS analysis was carried using Shimadzu Make QP-2010 with column of 60 meter length, with 0.25 mm internal diameter and 0.32 thickness. Helium gas was used as carrier gas at constant flow rate of 1ml/minute. Injector temperature was set at 50 C while as the Oven temperature was programmed from 10 ºC to 280 ºC at 10 ºC /minute to 200 ºC then 10 ºC/ 3 minutes to 250 ºC ending with a 5 minutes isothermal at 280 ºC. The sample was injected in split mode as 10:80.

Identification of compounds

GC-MS analysis provides chromatogram, retention time and mass spectrum. The identification is primarily based on two parameters, retention time and fragmentation pattern of compounds. Interpretation on mass spectrum of GC-MS was done using the National Institute Standard and Technology (NIST) database comparing the spectral data of known compounds present in spectral library NIST [17]. The information acquired through this was name of the compound, molecular weight, molecular formula and relative quantity of compounds (peak area %). The structures of important metabolites were drawn with the help of softwares Marvin Sketch and Chembiodraw.

Results and Discussion

Artemisia absinthium is medicinally very much important to cure various ailments. It is still used in the traditional medicinal practice in various parts of the globe including Jammu and Kashmir. In the present study attempts were made to find out the chemical constituents from aerial parts of *A. absinthium* by using GC-MS. The GC-MS analysis of Methanolic extract revealed the presence of 15 compounds (Figure 1, Table 1) among which the major peak area (18.61%) was shown by terpenoid derivative Retinol acetate at retention time 23.434 followed by quinic acid with peak area of 10.81% at retention time 17.396. Similarly, Petroleum ether extract has shown presence of 19 compounds (Figure 2, Table 2) which are mostly fatty acids and terpenes showing various biological activities like antimicrobial, anti-inflammatory, antioxidants etc. 25 compounds (Figure 3, Table 3) were detected in the Dichloromethane extract of *A. absinthium* aerial parts with major peak area of 29.04 % for the compound Cycloisolongifolene, 8,9-dehydro-9-formyl- at retention time 25.393. Some important compounds like Isogeraniol, d-verbenol, Globulol, Corymbolone were also detected in the Dichloromethane extract.

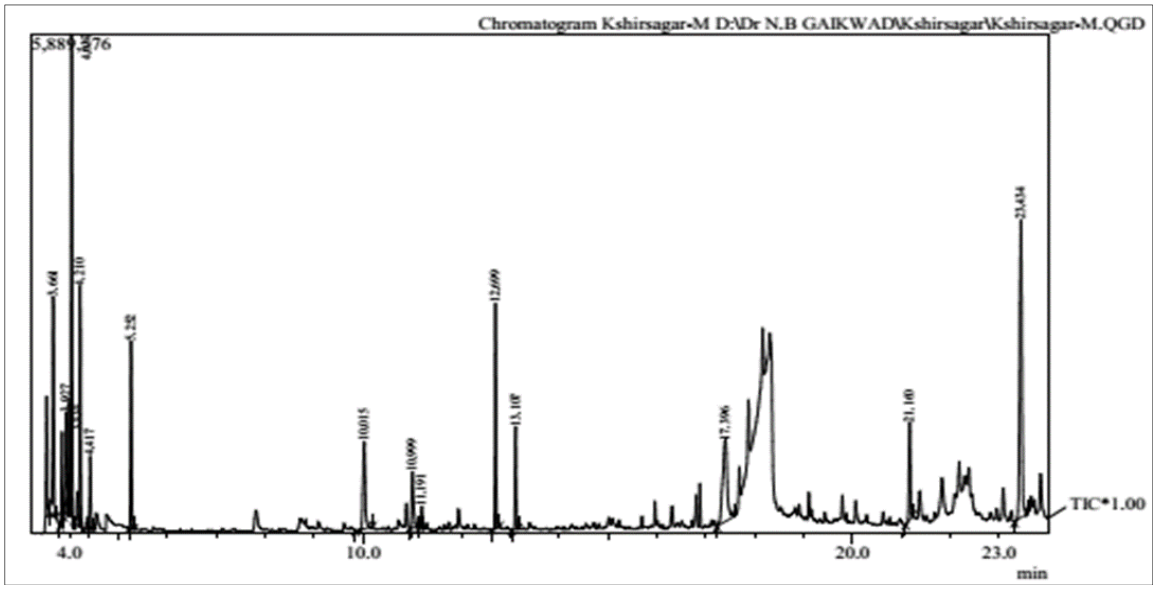


Fig 1: GC-MS chromatogram of Methanolic extract from aerial parts of *Artemisia absinthium*

Table 1: Identified compounds in Methanolic extract from aerial parts of *Artemisia absinthium*

S. No	Retention Time	Name of compound	Area (%)	Molecular weight	Molecular formula
1	3.661	Ethyl alcohol	9.44	46	C_2H_6O
2	3.838	Methylene Chloride	3.06	84	CH_2Cl_2
3	3.927	Pentane, 2-methyl-	2.79	86	C_6H_{14}

4	4.037	Butane, 2,2,3-trimethyl-	15.06	100	C_7H_{16}
5	4.210	Cyclopentane, methyl-	7.27	84	C_6H_{12}
6	4.417	Cyclohexane	2.33	84	C_6H_{12}
7	5.252	1,3,5-Cycloheptatriene	6.33	92	C_7H_8
8	10.015	Isopentyl alcohol, acetate	5.52	130	$C_7H_{14}O_2$
9	10.999	2,5-Furandione,3-(1,1-dimethylethyl)-	3.30	154	$C_8H_{10}O_3$
10	11.191	2,4,6-Trimethyl-3-cyclohexen-1-carboxaldehyde	0.73	152	$C_{10}H_{16}O$
11	12.699	Cyclohexanol, 2-methylene-3-(1-methylethenyl)-, acetate, cis-	7.11	194	$C_{12}H_{18}O_2$
12	13.107	Bicyclo[3.1.0]hex-3-en-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.alpha.,5.alpha.)-	3.26	152	$C_{10}H_{16}O$
13	17.396	(1R,3R,4R,5R)-(-)-Quinic acid	10.81	192	$C_7H_{12}O_6$
14	21.160	Pentadecanoic acid	4.39	242	$C_{15}H_{30}O_2$
15	23.434	Retinol, acetate	18.61	328	$C_{22}H_{32}O_2$

The major peak area (18.61 %) was shown by a terpenoid derivative Retinol acetate followed by Quinic acid with a peak area percentage of 10.81.

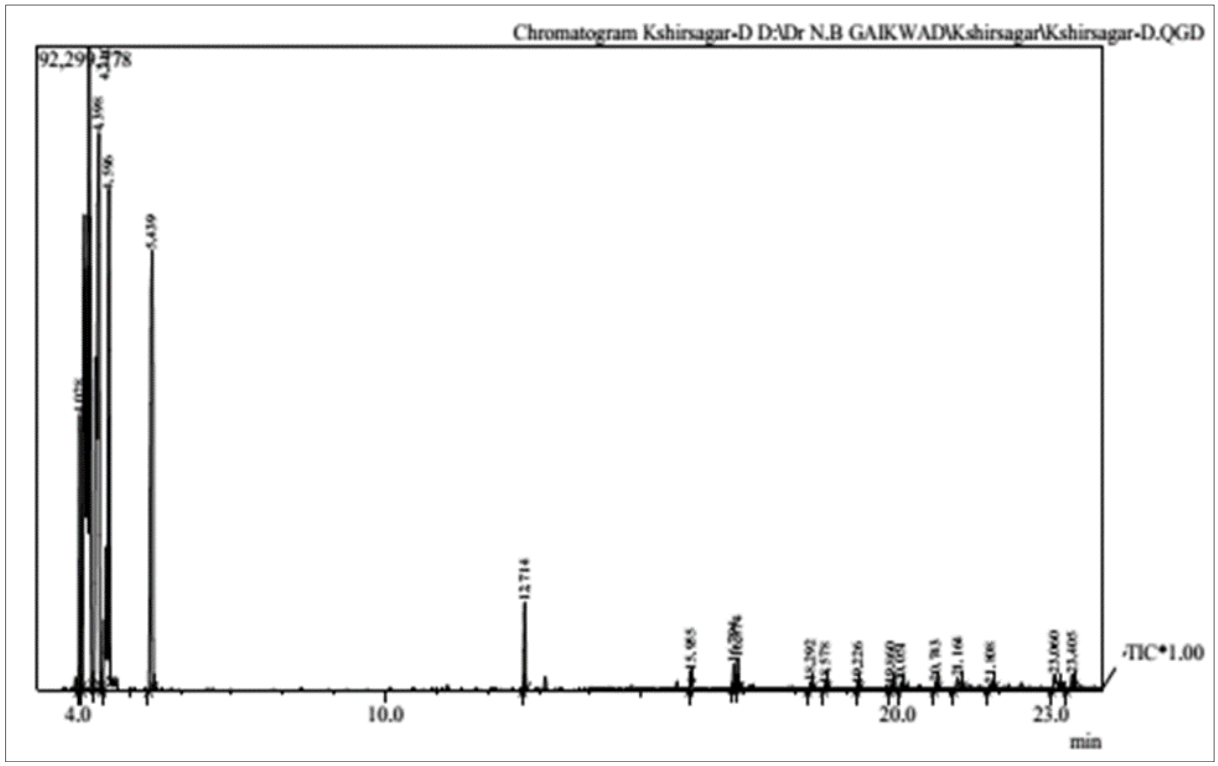


Fig 2: GC-MS chromatogram of Petroleum ether extract from aerial parts of *Artemisia absinthium*

Table 2: Identified compounds in Petroleum ether extract from aerial parts of *Artemisia absinthium*

Sr. No.	Retention Time	Name of compound	Area (%)	Molecular weight	Molecular formula
1	4.028	Methylene Chloride	4.40	84	CH_2Cl_2
2	4.210	Pentane, 2,3-dimethyl-	39.47	100	C_7H_{16}
3	4.398	1-Octanol	24.47	130	$C_8H_{18}O$
4	4.596	Pentane, 2-cyclopropyl-	13.30	112	C_8H_{16}
5	5.439	1,5-Heptadien-3-yne	13.88	92	C_7H_8
6	12.714	Cyclohexanol, 2-methylene-3-(1-methylethenyl)-, acetate, cis-	1.50	194	$C_{12}H_{18}O_2$
7	15.955	Pentanoic acid, 3,7-dimethyl-2,6-octadienyl ester, (E)-	0.27	238	$C_{15}H_{26}O_2$
8	16.794	2,6-Octadien-1-ol, 3,7-dimethyl-, propionate, (E)-	0.39	210	$C_{13}H_{22}O_2$
9	16.874	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate	0.45	196	$C_{12}H_{20}O_2$
10	18.292	Globulol	0.14	222	$C_{15}H_{26}O$
11	18.578	10-Methyl-8-tetradecen-1-ol acetate	0.05	268	$C_{17}H_{32}O_2$
12	19.226	1-Nonadecene	0.07	266	$C_{19}H_{38}$
13	19.860	2-Pentadecanone, 6,10,14-trimethyl-	0.08	268	$C_{18}H_{36}O$
14	20.051	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.11	296	$C_{20}H_{40}O$
15	20.743	Pentadecanoic acid, 14-methyl-, methyl ester	0.14	270	$C_{17}H_{34}O_2$
16	21.164	Octadecanoic acid	0.30	284	$C_{18}H_{36}O_2$
17	21.808	Limonen-6-ol, pivalate	0.12	236	$C_{15}H_{24}O_2$
18	23.060	2,6-Dimethyl-8-(tetrahydropyran-2-yloxy)-octa-2,6-dien-1-ol	0.51	254	$C_{15}H_{26}O_3$
19	23.405	Phytol	0.35	296	$C_{20}H_{40}O$

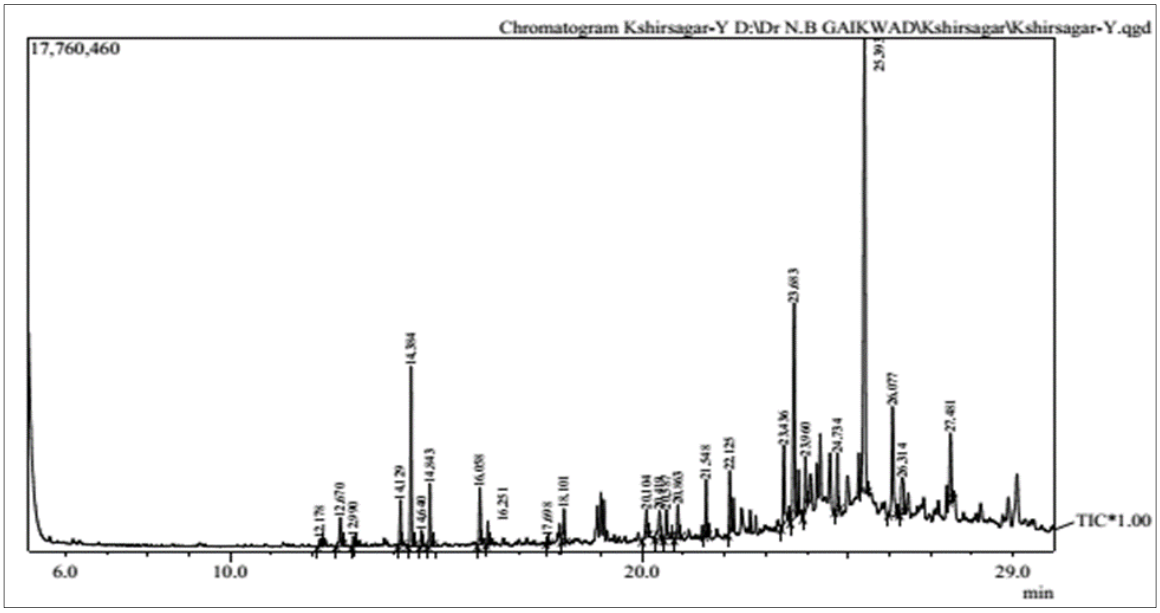
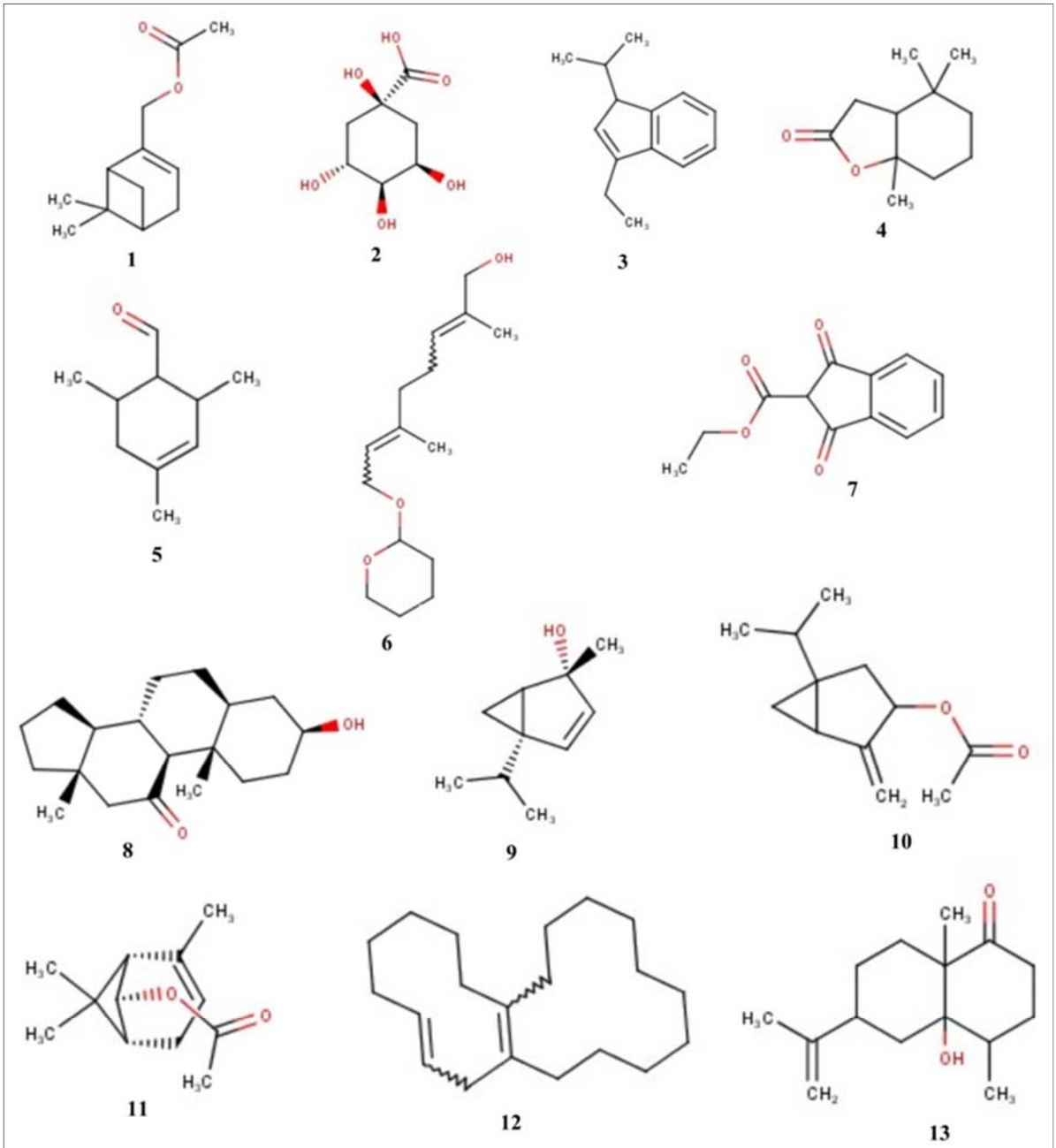


Fig 3: GC-MS chromatogram of Dichloromethane extract from aerial parts of *Artemisia absinthium*

Table 3: Identified compounds in Dichloromethane extract from aerial parts of *Artemisia absinthium*

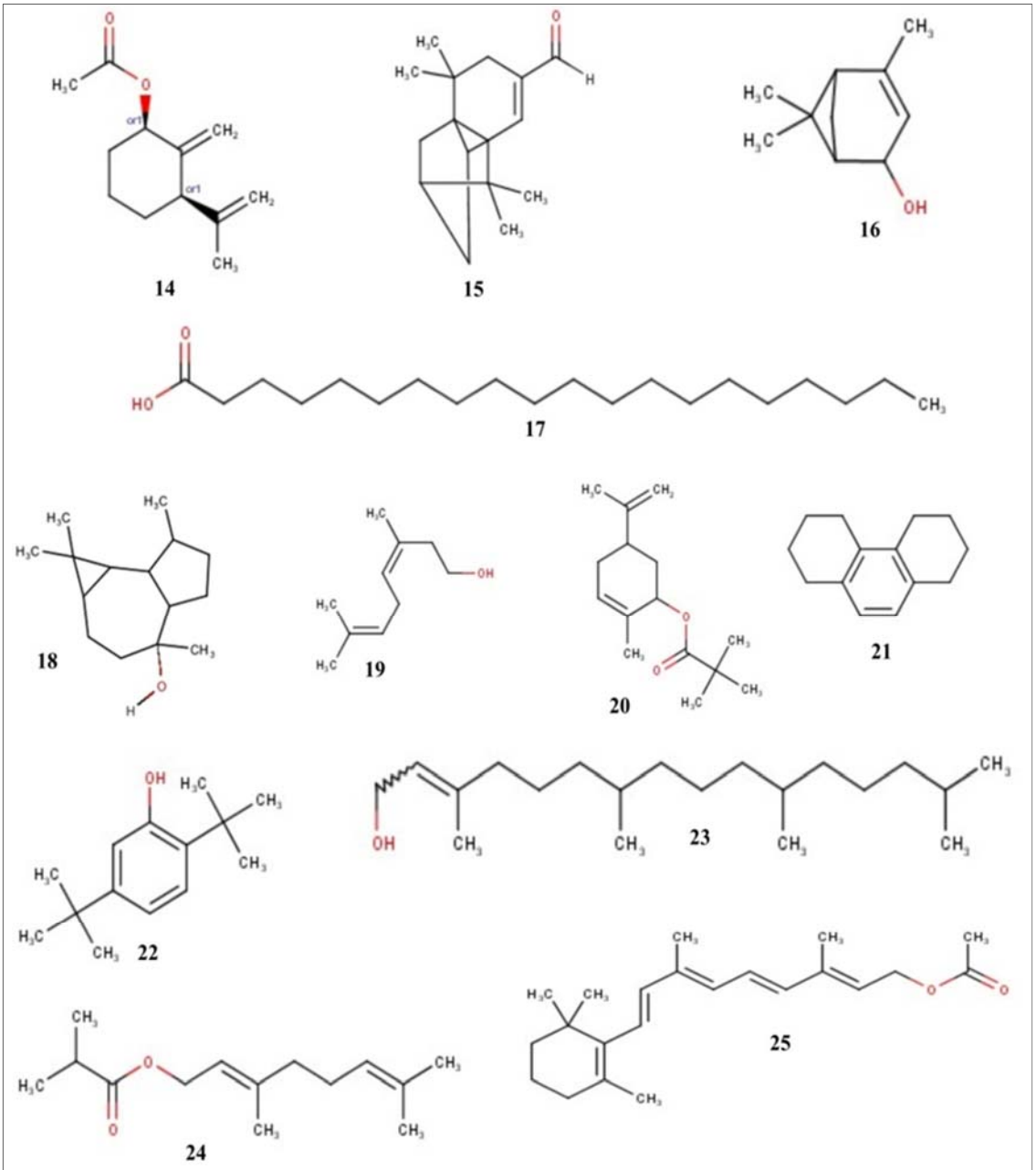
S. No	Retention Time	Name of compound	Area (%)	Molecular weight	Molecular formula
1	12.178	Isogeraniol	0.39	154	$C_{10}H_{18}O$
2	12.670	d-Verbenol	1.45	152	$C_{10}H_{16}O$
3	12.990	1-Dodecene	0.38	168	$C_{12}H_{24}$
4	14.129	Cyclohexanol, 2-methylene-3-(1-methylethenyl)-, acetate, cis-	1.96	194	$C_{12}H_{18}O_2$
5	14.384	Bicyclo[3.1.1]hept-2-en-6-ol, 2,7,7-trimethyl-, acetate, [1S-(1.alpha.,5.alpha.,6.beta.)]-	7.28	194	$C_{12}H_{18}O_2$
6	14.640	(-)-Myrtenyl acetate	0.71	194	$C_{12}H_{18}O_2$
7	14.843	Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1-methylethyl)-, acetate	2.43	194	$C_{12}H_{18}O_2$
8	16.058	2H-Indene-1,3-dione-2-carboxylic acid, ethyl ester	2.73	218	$C_{12}H_{10}O_4$
9	16.251	1-Hexadecene	0.91	224	$C_{16}H_{32}$
10	17.698	Propanoic acid, 2-methyl-, 3,7-dimethyl-2,6-octadienyl ester, (E)-	0.29	224	$C_{14}H_{24}O_2$
11	18.101	Phenol, 2,5-bis(1,1-dimethylethyl)-	1.71	206	$C_{14}H_{22}O$
12	20.104	Phenanthrene, 1,2,3,4,5,6,7,8-octahydro-	1.79	186	$C_{14}H_{18}$
13	20.419	1H-Indene, 3-ethyl-1-(1-methylethyl)-	2.34	186	$C_{14}H_{18}$
14	20.587	Globulol	1.76	222	$C_{15}H_{26}O$
15	20.863	Cyclopropane, 1-(1-hydroxy-1-heptyl)-2-methylene-3-pentyl-	1.78	238	$C_{16}H_{30}O$
16	21.548	E-15-Heptadecenal	2.22	252	$C_{17}H_{32}O$
17	22.125	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	2.68	296	$C_{20}H_{40}O$
18	23.436	Eicosanoic acid	4.41	312	$C_{20}H_{40}O_2$
19	23.683	Dibutyl phthalate	12.96	278	$C_{16}H_{22}O_4$
20	23.960	Androstan-11-one, 3-hydroxy-, (3.beta.,5.alpha.)-	2.91	290	$C_{19}H_{30}O_2$
21	24.734	Bicyclo[10.8.0]eicosa-1(12),14-diene	2.92	274	$C_{20}H_{34}$
22	25.393	Cycloisolongifolene, 8,9-dehydro-9-formyl-	29.04	230	$C_{16}H_{22}O$
23	26.077	2(3H)-Benzofuranone, hexahydro-4,4,7a-trimethyl-	7.55	182	$C_{11}H_{18}O_2$
24	26.314	Corymbolone	3.54	236	$C_{15}H_{24}O_2$
25	27.481	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	3.87	444	$C_{30}H_{52}O_2$

Some of the important compounds present in *A. absinthium* extracts having biologically active nature are described in Figure 4 and 5. Phenol, 2, 5-bis (1, 1-dimethylethyl)- is a Phenol with anti-bacterial activity [18], antifungal activity and antioxidant [19], anti-inflammatory activity [20], anticancerous [21]. Phytol (or 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol) is a Diterpene with anti-inflammatory [22], antimicrobial, diuretic [23], anti-cancer [24], cancer preventive [25-26], anti Joint dislocation, Hernia and antimalarial [27]. Globulol is a Tricyclic hydroazulene Sesquiterpene with antimicrobial [28], allelopathic [29], antibacterial [30], antioxidant activity, anti-inflammatory [31]. Also has reported as a constituent of essential oil by various authors. Limonen-6-ol, pivalate is a monoterpene which is a component of essential oil with antioxidant and insect repellent activity. Isogeraniol is a monoterpene and is present as a volatile oil in plants. It is used in skin infections [32], fragrances and cosmetics, Contact allergen. Corymbolone possess antiplasmodic activity [33], antifungal agent [34]. Cycloisolongifolene, 8,9-dehydro-9-formyl- is component of volatile oil which is used against cancers [35]. d-Verbenol is a monoterpene frequently a component of essential oils showing anti-ischemic activity, anti-oxidative and anti-inflammatory activities [36]; antiprotozoal activity [37]. (-)-Myrtenyl acetate is a component of essential oils and it possess anti inflammatory activity [38].



1. (-)-Myrtenyl acetate
2. (1R,3R,4R,5R)-(-)-Quinic acid
3. 1H-Indene, 3-ethyl-1-(1-methylethyl)-
4. 2(3H)-Benzofuranone, hexahydro-4,4,7a-trimethyl-
5. 2,4,6-Trimethyl-3-cyclohexen-1-carboxaldehyde
6. 2,6-Dimethyl-8-(tetrahydropyran-2-yloxy)-octa-2,6-dien-1-ol
7. 2H-Indene-1,3-dione-2-carboxylic acid, ethyl ester
8. Androstan-11-one, 3-hydroxy-, (3.beta.,5.alpha.)-
9. Bicyclo[3.1.0]hex-3-en-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.alpha.,5.alpha.)-
10. Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1-methylethyl)-, acetate
11. Bicyclo[3.1.1]hept-2-en-6-ol, 2,7,7-trimethyl-, acetate, [1S-(1.alpha.,5.alpha.,6.beta.)]-
12. Bicyclo[10.8.0]eicosa-1(12),14-diene
13. Corymbolone

Fig 4: Molecular structures of identified compounds from aerial parts of *A. absinthium*



14. Cyclohexanol, 2-methylene-3-(1-methylethenyl)-, acetate, cis-
15. Cycloisolongifolene, 8,9-dehydro-9-formyl-
16. d-Verbenol
17. Eicosanoic acid
18. Globulol
19. Isogeraniol
20. Limonen-6-ol, pivalate
21. Phenanthrene, 1,2,3,4,5,6,7,8-octahydro-
22. Phenol, 2,5-bis(1,1-dimethylethyl)-
23. Phytol or 3,7,11,15-Tetramethyl-2-hexadecen-1-ol
24. Propanoic acid, 2-methyl-, 3,7-dimethyl-2,6-octadienyl ester, (E)-
25. Retinol acetate

Fig 5: Molecular structures of identified compounds from aerial parts of *A. absinthium*

Conclusion

The aerial parts of *A. absinthium* contained higher amounts of secondary metabolites. Dichloromethane proved comparatively better solvent of extraction than Methanol and Petroleum ether. The various compounds isolated from aerial parts of *A. absinthium* has shown various medicinal properties like anti-bacterial, antifungal, antioxidant, anti-inflammatory, anticancerous, diuretic, anti-joint dislocation, anti-oxidative

etc. Further, various compounds isolated from *A. absinthium* are used in skin infections, fragrances and cosmetics. Gas chromatography and mass spectroscopy analysis showed the presence of various compounds with variable chemical structures. GC-MS analysis can open up new means for identification of natural drugs that can be employed for clinical trials which may generate successful results in future.

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