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COMPARATIVE PHYTOCHEMICAL INVESTIGATIONS FROM AERIAL PARTS OF *Artemisia pallens* Wall. USING GC-MS ANALYSIS

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Abstract: **The present study was carried out to find the chemical constituents from aerial parts of medicinal important plant *Artemisia pallens*. The Gas Chromatography-Mass Spectrometric technique was used to determine the presence of various chemical constituents. The present study revealed that the aerial parts of *Artemisia pallens* accumulate a considerable amount of monoterpenes, sesquiterpenes and fatty acid derivatives which are mostly constituents of essential oils. Three solvents viz. Methanol, Petroleum ether and Dichloromethane were used for extraction. The results showed the presence of 16 compounds in the Methanolic extract, 13 compounds in the Petroleum ether extract and 17 compounds in the Dichloromethane extract.**

Keywords: *Artemisia pallens, GC-MS, Phytochemical, Secondary metabolites.*

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I INTRODUCTION

Traditional medicine is an important source of potentially useful compounds for the development of chemotherapeutic agents [1]. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties [2].

Artemisia pallens also known as Davana, is an aromatic delicate, erect, branched annual herb about 60 cm in height. It is abundantly found in humid habitats in the plains all over India. It is native to the southern parts of India, especially in the states of Karnataka, Tamil Nadu, Andhra Pradesh and Maharashtra. It is ethnomedicinally used in Iraq and Indian tribal medicine for the treatment of Diabetes mellitus, healing of wounds, modulating immune system, antihelmintic, antimicrobial, stimulant and as a tonic [3]. The volatile oil has been reported as antispasmodic, antifungal, antibacterial and also as stimulant [4]. Essential oil of *Artemisia pallens* is used as antiseptic, disinfectant and commercially important due to its fragrance [5]. *Artemisia pallens* also possesses antibacterial activity [6].

Gas Chromatography Mass Spectroscopy is a very compatible technique used for the identification and quantification of chemical compounds present in an extract. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra [7]. The use of *Artemisia pallens* has been reported in ancient books like Ayurveda etc and its modern applications are receiving wide spread attention day by day. Hence, the aerial parts of *Artemisia pallens* were investigated in the present study to determine the possible chemical components by using technique GC-MS.

II MATERIALS AND METHODS

Collection of plant material:

Artemisia pallens was collected from village- Brahmanwada Chandurbazar, District- Amravati Maharashtra, the village is situated towards 21°19'0.77"N and 77°44'23.36"E at an elevation of 402 m. *Artemisia pallens* was identified by Botanical Survey of India (BSI), Western Regional Centre, Koregaon Road Pune (411001). Voucher specimens as voucher specimen number GMYAP2 (*Artemisia pallens*) was deposited in the BSI.

Powder Preparation

The fresh aerial parts of *Artemisia pallens* were washed with tap water and shade dried at room temperature (28 ±2 ˚C). These dried parts were powdered by electric

blender and finally stored in zip lock plastic bags until further experimentation.

Preparation of the extract

Soxhletion method was used for preparing of extracts using three solvents viz. Methanol, Petroleum ether and Dichloromethane. 10 grams of sample powder was extracted in 180 ml of solvent using Soxhlet apparatus. The temperature was maintained at the boiling point for each solvent [8], [9]. The time required is 3-18 hours [10]. The filtered extracts were concentrated to 5ml using rotatory vacuum evaporator at 28 ±2 ˚C and then analyzed by GC - MS.

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. Carrier gas Helium was used at constant flow rate of 1ml/minute. Injector temperature was set at 50 ^{o}C while as the Oven temperature was programmed from 10 ^{o}C to 280 ^{o}C at 10 ^{o}C /minute to 200 ^{o}C then 10 ^{o}C/ 3 minutes to 250 ^{o}C ending with a 5 minutes isothermal at 280 ^{o}C. The sample was injected in split mode as 10:80.

Identification of compounds:

The chromatogram, retention times, peak area, fragmentation pattern, m/z values etc are obtained by GC-MS analysis [11]. The identification in GC-MS is primarily based on retention times and fragmentation pattern of compounds. Interpretation on mass spectrum was done by comparing results with the spectral data of known compounds present in spectral library (National Institute Standard and Technology - NIST). 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.

III RESULTS AND DISCUSSION

The present study revealed that the aerial parts of *A. pallens* accumulate a considerable amount of constituents such as essential oils (monoterpenes and sesquiterpenes) and derivatives of fatty acids. In the present study 16 compounds were identified in the Methanolic extract (Figure 1, Table 1) and 13 compounds were identified in Petroleum ether extract (Figure 2, Table 2) while as 17 compounds were identified in Dichloromethane extract (Figure 3, Table 3). The obtained results will help in the phytochemical research of *A. pallens* for standardization of the herbal powdered drug and preventing possible adulteration. The identified compounds are of interest for further pharmacological studies too.

The molecular structure of some of the compounds are described in Figure 4. Some of the important identified compounds with their pharmacological importance are as 2(3H)-Furanone, 5-ethenyldihydro-5-methyl- has been reported by [12] in plant extracts and has been reported to show antimalarial activity [13]. 3-Butenoic acid, 2-oxo-4-phenyl- has been reported to show antifungal activity [14]. 4-Thujen-2.alpha.-yl acetate has been reported in the seed extract of *Piper nigrum* [15]; and also in volatile oils obtained from *Laurus nobilis* leaves [16]. Cinnamic acid, ethyl ester and its derivatives have been reported for anti-microbial, anti-fungal and nematicidal activities [17], [18], [19]. Longipinane, (E)- has been reported by [20], [21] and it possess anti-pedant and cytotoxic activity [22], [23]. Spathulenol has been reported as a component of essential oil by various authors and it possesses antibacterial, insect repellent activity [24]; immune modulatory activity [25].

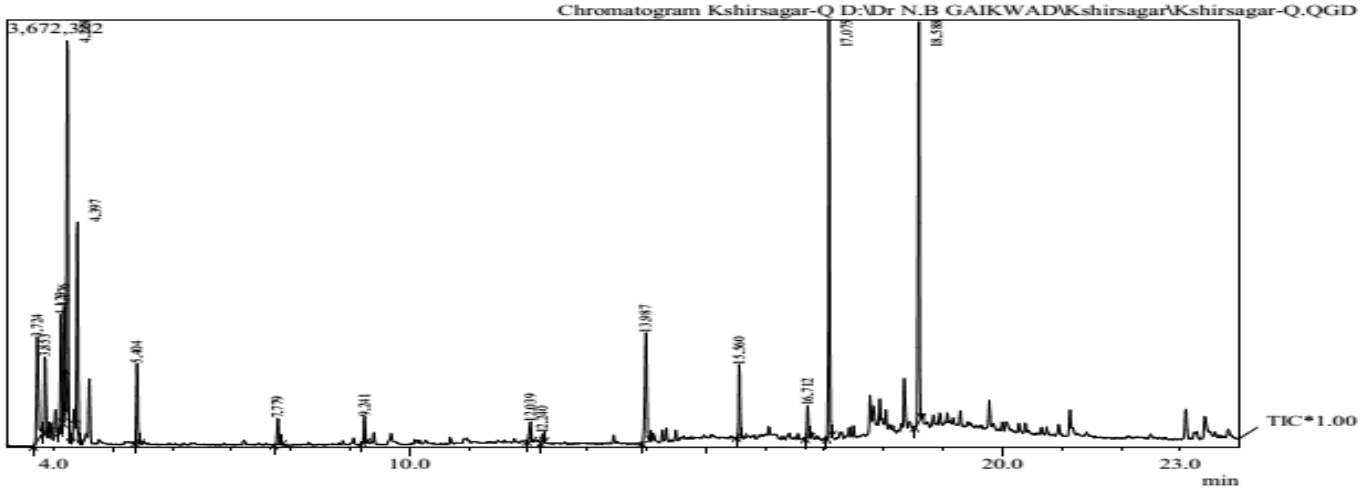


Figure 1: GC-MS chromatogram of Methanolic extract from aerial parts of Artemisia pallens.

Table 1: Identified compounds in Methanolic extract from aerial parts of Artemisia pallens.

Sr. No.	Retention Time	Name of compound	Area (%)	Molecular weight	Molecular formula
1	3.724	Nitrous Oxide	6.73	44	N_2O
2	3.853	Ethanol	3.46	46	C_2H_6O
3	4.123	Pentane, 2-methyl-	5.40	86	C_6H_{14}
4	4.176	Hexane, 2,2,3-trimethyl-	4.88	128	C_9H_{20}
5	4.228	Butane, 2,2,3-trimethyl-	15.55	100	C_7H_{16}
6	4.397	Cyclopentane, methyl-	9.62	84	C_6H_{12}
7	5.404	Toluene	3.87	92	C_7H_8
8	7.779	2(5H)-Furanone, 5,5-dimethyl-	1.53	112	$C_6H_8O_2$
9	9.241	3-Hepten-2-one, 4-methyl-	1.14	126	$C_8H_{14}O$
10	12.039	1,7-Heptanediol	1.14	132	$C_7H_{16}O_2$
11	12.240	Lilac aldehyde	0.36	168	$C_{10}H_{16}O_2$
12	13.987	Lilac alcohol C	6.82	170	$C_{10}H_{18}O_2$
13	15.560	Cinnamic acid, ethyl ester	3.06	176	$C_{11}H_{12}O_2$
14	16.712	+/-.-trans-Nerolidol	1.47	222	$C_{15}H_{26}O$
15	17.075	5-Hepten-3-one, 2-(5-ethenyltetrahydr+ o-5-methyl-2-furanyl)-6-methyl-, [2S-[2.alpha.(R),5.alpha.]]-	16.67	236	$C_{15}H_{24}O_2$
16	18.588	10-Methyl-8-tetradecen-1-ol acetate	18.23	268	$C_{17}H_{32}O_2$

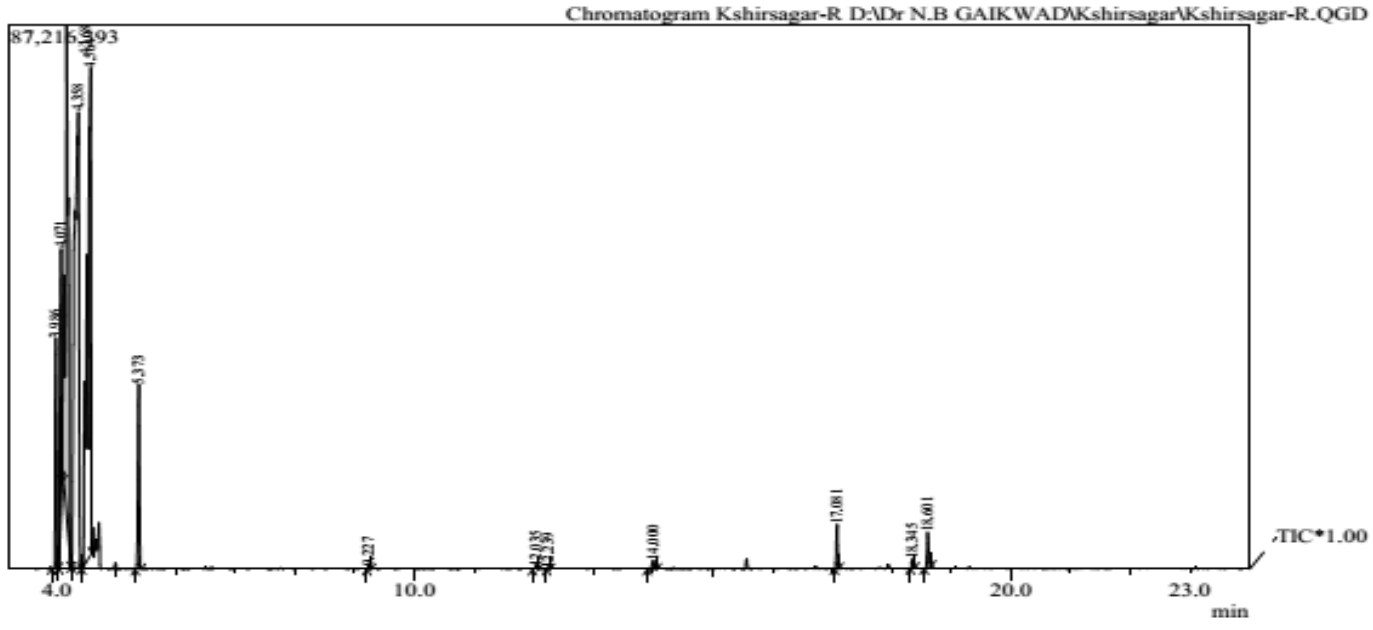


Figure 2: GC-MS chromatogram of Petroleum ether extract from aerial parts of Artemisia pallens.

Table 2: Identified compounds in Petroleum ether extract from aerial parts of Artemisia pallens.

Sr. No.	Retention Time	Name of compound	Area (%)	Molecular weight	Molecular formula
1	3.986	Dichloromethane	5.37	84	CH_2Cl_2
2	4.071	Furan, tetrahydro-2-methyl-	6.99	86	$C_5H_{10}O$
3	4.168	Pentane, 2,3-dimethyl-	20.87	100	C_7H_{16}
4	4.358	1-Octanol	32.20	130	$C_8H_{18}O$
5	4.564	1-Octanol	27.55	130	$C_8H_{18}O$
6	5.373	Toluene	4.48	92	C_7H_8
7	9.227	2(3H)-Furanone, 5-ethenyldihydro-5-methyl-	0.08	126	$C_7H_{10}O_2$
8	12.035	1,7-Heptanediol	0.09	132	$C_7H_{16}O_2$
9	12.239	Lilac aldehyde D	0.02	168	$C_{10}H_{16}O_2$
10	14.000	Lilac alcohol C	0.30	170	$C_{10}H_{18}O_2$
11	17.081	Cyclohexane, 1,4-dimethyl-2-(2-methylpropyl)-, (1.alpha.,2.beta.,5.alpha.)-	0.93	168	$C_{12}H_{24}$
12	18.345	5-Hepten-3-one, 2-(5-ethenyltetrahydro-5-methyl-2-furanyl)-6-methyl-, [2S-[2.alpha.5.alpha.]]-	0.20	236	$C_{15}H_{24}O_2$
13	18.601	Cyclopropane, 1-(1-hydroxy-1-heptyl)-2-methylene-3-pentyl-	0.93	238	$C_{16}H_{30}O$

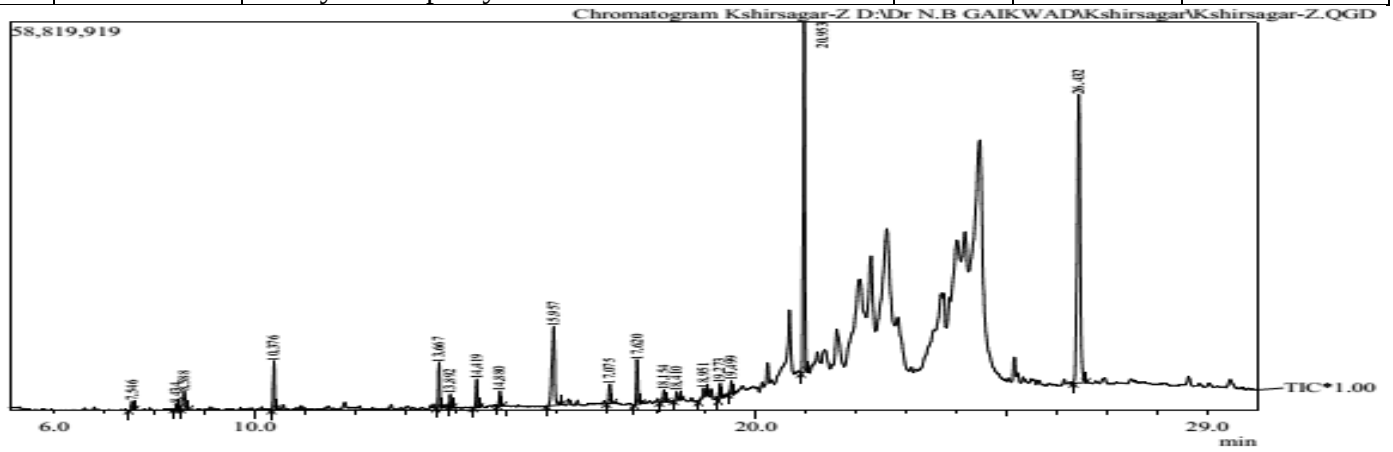
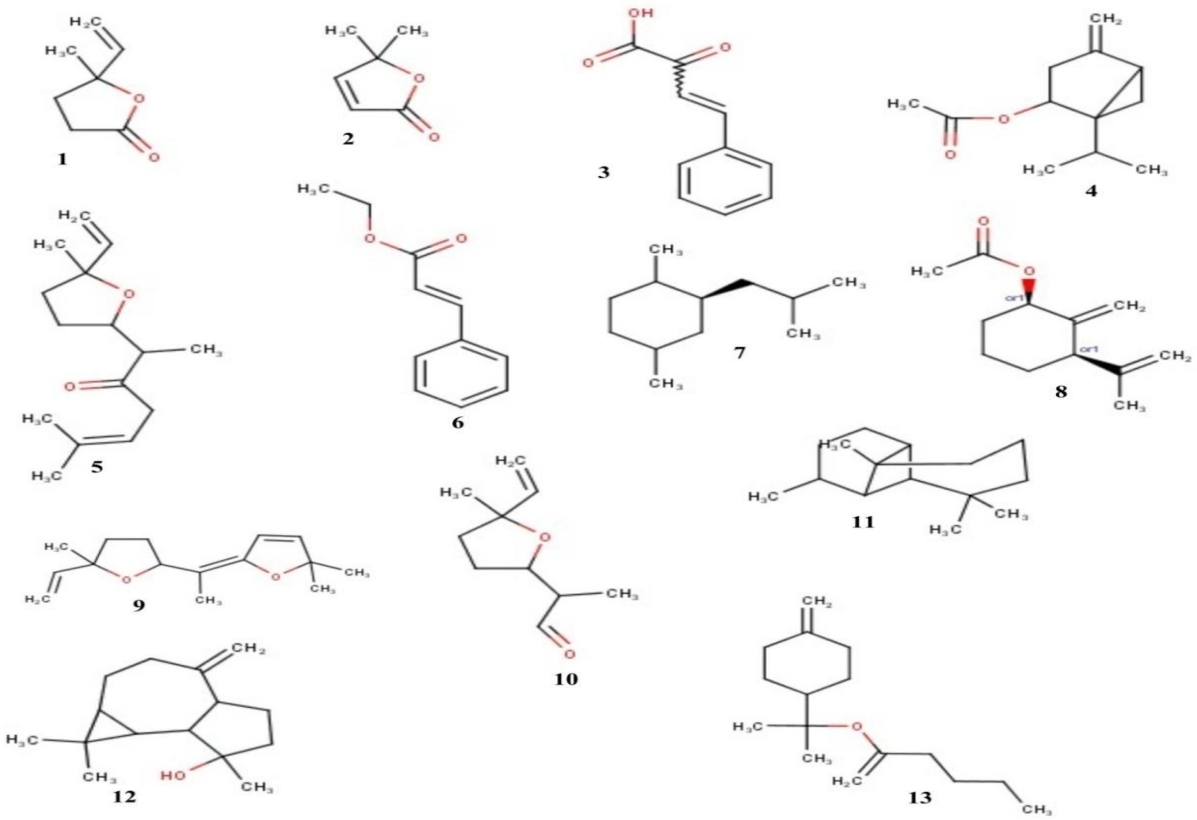


Figure 3: GC-MS chromatogram of Dichloromethane extract from aerial parts of Artemisia pallens.

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

Sr. No.	Retention Time	Name of compound	Area (%)	Molecular weight	Molecular formula
1	7.546	Cyclohexene,3-(1-methylpropyl)-	0.64	138	$C_{10}H_{18}$
2	8.434	1,3-Hexadiene, 3-ethyl-2,5-dimethyl-	0.28	138	$C_{10}H_{18}$
3	8.588	2(5H)-Furanone, 5,5-dimethyl-	2.05	112	$C_6H_8O_2$
4	10.376	3,5-Octadien-2-ol	3.26	126	$C_8H_{14}O$
5	13.667	1,7-Heptanediol	3.06	132	$C_7H_{16}O_2$
6	13.892	Lilac aldehyde D	0.76	168	$C_{10}H_{16}O_2$
7	14.419	Cyclohexanol, 2-methylene-3-(1-methylethenyl)-, acetate, cis-	1.88	194	$C_{12}H_{18}O_2$
8	14.880	4-Thujen-2.alpha.-yl acetate	0.95	194	$C_{12}H_{18}O_2$

9	15.957	Lilac aldehyde	10.03	168	$C_{10}H_{16}O_2$
10	17.075	Lilac alcohol C	1.82	170	$C_{10}H_{18}O_2$
11	17.620	3-Butenoic acid, 2-oxo-4-phenyl-	3.27	176	$C_{10}H_8O_3$
12	18.154	Longipinane, (E)-	0.91	206	$C_{15}H_{26}$
13	18.410	Davana ether	0.74	234	$C_{15}H_{22}O_2$
14	18.951	trans-.beta.-Terpinyl pentanoate	0.86	238	$C_{15}H_{26}O_2$
15	19.273	5-Hepten-3-one, 2-(5-ethenyltetrahydro-5-methyl-2-furanyl)-6-methyl-, [2S-[2.alpha.(R.5.alpha.]]-	1.07	236	$C_{15}H_{24}O_2$
16	19.499	Spathulenol	1.03	220	$C_{15}H_{24}O$
17	20.953	Cyclopropane, 1-(1-hydroxy-1-heptyl)-2-methylene-3-pentyl-	27.31	238	$C_{16}H_{30}O$



1. 2(3H)-Furanone, 5-ethenyldihydro-5-methyl-
2. 2(5H)-Furanone, 5,5-dimethyl-
3. 3-Butenoic acid, 2-oxo-4-phenyl-
4. 4-Thujen-2.alpha.-yl acetate
5. 5-Hepten-3-one, 2-(5-ethenyltetrahydro-5-methyl-2-furanyl)-6-methyl-, [2S-[2.alpha.5.alpha.]]-
6. Cinnamic acid, ethyl ester
7. Cyclohexane, 1,4-dimethyl-2-(2-methylpropyl)-, (1.alpha.,2.beta.,5.alpha.)-
8. Cyclohexanol, 2-methylene-3-(1-methylethenyl)-, acetate, cis-
9. Davana ether
10. Lilac aldehyde
11. Longipinane, (E)-
12. Spathulenol
13. trans-.beta.-Terpinyl pentanoate

Figure 4: Molecular structures of identified compounds from aerial parts of A. pallens.

IV CONCLUSION

The aim of the study was to analyze the chemical components present in the aerial parts of *A. pallens* widely grown in Maharashtra (India). The aerial parts of *A. pallens* 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. pallens* has shown various medicinal properties like antimalarial, antifungal activity, anti-microbial, anti-fungal, antibacterial, insect repellent activity etc. 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. Thus, this type of study may give information on nature of active principles present in the medicinal plants.

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