GC-MS study and anti - microbial activity of essential oils isolated from leaves of *Artemisia annua*

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Abstract- The chemical composition of the essential oil obtained from the leaves of *Artemisia annua* collected from the Garhwal region of Uttarakhand, was analyzed by GC-MS. The major constituent was found out to be (Z)-β-Farnesene (18.80%). The antibacterial and antifungal activity of the oil was determined by disc diffusion method. Results showed that the oil exhibited mild antimicrobial activity.

Keywords: *Artemisia annua Antibacterial, Antifungal, Asteraceae, Essential oil.*

Introduction

Artemisia annua L. is an annual, herbaceous, aromatic medicinal plant belonging to the family, Asteraceae. It is mentioned in traditional Chinese medicine as a cure for different diseases like fever, hemorrhoid, and malaria. It is native to the mild and temperate climate of Asia but has been naturalized to other countries outside Asia as well. The most common ethnobotanical usage of this plant involves the use of whole plant decoction for the treatment of cold, malaria, and cough. The whole flowering plant is known to be antipyretic, antihelminth, antispasmodic, antiseptic, and antimalarial. The antimalarial activity of this plant is due to artemisinin, a sesquiterpene lactone containing an endoperoxide moiety that acts as a key pharmacore[(1)]. Artemisinin forms an important part of combinatorial treatment therapy recommended for the treatment of malaria. Artemisinin and its derivatives like artesunate have also been reported to have potent anticancer properties as well. Besides artemisinin, certain other phytochemicals reported in this plant are monoterpenes, poly-phenols, coumarins, flavones, flavonols, phenolic acids, many sesquiterpenes properties. They have been reported to synergize the activity of artemisinin and its derivatives against malaria[(1)]. Considering the immense medicinal properties of this plant, it was selected for present investigations, The present paper deals with GC MS analysis and study of antimicrobial activity of the plant.

Material and Methods

Plants Materials

Whole plants of *Artemisia annua* was collected from the Bugani road, Srinagar Garhwal, Uttarakhand, India. The plant was identified from Department of Botany, HNB Garhwal University Srinagar, Uttarakhand. A Voucher Specimen (GUH-3354) was deposited in the Department of Botany.

About 10 kg sample of dried leaves of *Artemisia annua* were subjected to hydro distillation for 8 hours using a Clevenger-type apparatus. The oil was extracted over ether and dried over anhydrous Na_2 SO_4. The yield was 0.05% (v/w).

GC and GC/MS

GC/MS analysis were performed with a Perkin Elmer Clarus 500 gas chromatograph equipped with a split/splitless injector (split ratio 50:1) data handling system. The column was Rtx-5 capillary columns (60 m x 0.32 mm, 0.25 μm film thickness). Helium (He) was the carrier gas at a flow rate 1.0 mL/min. The GC was interfaced with (Perkin Elmer Clarus 500) mass detector operating in the EI+ mode. The mass spectra were generally recorded over 40-500 amu that revealed the total ion current (TIC) chromatograms. Temperature program was used as follows: initial temperature of 600C (hold: 2 min) programmed at a rate of 3⁰C /min to a final temperature of 2200C (hold: 5 min). The temperatures of the injector, transfer line and ion source were maintained at 210^0C, 210^0C and 200^0C, respectively.

Identification of Compounds

The components of the oils were identified by comparison of their mass spectra with those of computer library (NIST/ Pfleger /Wiley) or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds or with data published in literature[2-3]. Isolation of the compounds: The fractionation of the oil was carried over silica gel (230-400 mesh, Loba) by column chromatography using n-hexane (Qualigens) and varying percentages of diethyl ether (Qualigens) in n-hexane as mobile phase[4]. Monitoring was done on pre-coated silica gel TLC plates using iodine as visualizing agent. Repeated column chromatography of the column fractions gave one compound coded as C.

Microorganisms

3-gram negative bacteria viz. Pasteurella multicoda (MTCC 1348), Escherichia coli (MTCC 443), and Salmonella enterica (MTCC 1223), and 2-gram positive bacteria viz. Staphylococcus aureus (MTCC 637) and Bacillus subtilis (MTCC 541) were used for the study of antibacterial activity. Fungi used were Candida albicans (MTCC 854) and Aspergillus flavus (MTCC 771). Standard pure cultures of these bacteria were procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India as Microbial Type Culture Collection (MTCC) and maintained in the laboratory by regular sub culturing on to nutrient agar.

Antimicrobial activity

Antibacterial screening of the oil was done by Disc diffusion method as reported in literature, with slight modification[5]. Then minimum inhibitory concentration (MIC) of the essential oil is determined. Bacterial suspension of 0.1 ml (10 times diluted) was added to the previously prepared nutrient agar plate and bacterial strain was thoroughly spread on the surface of agar media, using a bent rod. The sterilized Whatmann filter paper No.1 disc (5mm in diameter) was thoroughly soaked with essential oil (15µL) and placed in the inoculated plates. Gentamycin and Nystatin were used as a reference drugs. Fine pointed forceps were used to place the disc on the previously inoculated plates with the maximum possible aseptic precautions. The discs were firmly pressed against the nutrient agar medium so that they come in complete contact with the agar surface. The discs were placed at equal distances from each other on the seeded plates and the plates were incubated at 370 C overnight, to observe the zone of inhibition around the disc. They were then compared with the zone of inhibition using standard antibiotic after overnight incubation on the nutrient agar plates.

Result and Discussion

Essential Oil Composition The essential oil (yield 0.1%; v/w) obtained from leaves of *Artemisia annua* s was analyzed by using GC-FID and GC-MS.

A total of 24 constituents, representing 74.47% of the total oil, have been elution from an HP-5 column. Essential oil showed the dominant presence of sesquiterpene hydrocarbons (56.86%) followed by oxygenated sesquiterpenes (15.58%) and oxygenated monoterpenes (13.08%). The major constituents of sesquiterpene hydrocarbons were (Z)-β-Farnesene (18.80%), Germacrene D (10.84%) and β-Caryophyllene (3.39%). Oxygenated monoterpenes comprised dihydro citronellol (15.08%) as the representative constituent while α-Cadinol (2.35%), Trans-Arteannuic identified. The composition of the essential oil obtained from leaves of *Artemisia annua* with the retention indices, retention time, percentage composition and identification methods. Compounds are listed in order of their alcohol (3.05%), Zerumbone (2.00%) and Humulene epoxide II (2.85%) were found as major Oxygenated sesqui-terpenes. Aldehyde and hydrocarbon were found in relatively smaller amounts consisting of n-Nonanal (2.79%) and Longicyclene (2.02%) respectively. To the best of our knowledge, this is the first report on the presence of dihydro citronellol (15.08%) in the genus. Earlier, the leaf oil of Senecio chrysanthemoides showed the presence of Germacrene D (10.84%) as main constituent. while in present study, oil was rich in (Z)-β-Farnesene (18.80%) with absence of β-thujone[(6)]. Chemical variation of essential oils has been attributed to difference in environmental and genetic factors[(7)]. Furthermore, ecological factors, particularly, light and temperature have also been reported to influences the production of essential oils as well as other active agents in plants.

Table- Essential oil composition of *Artemisia annua*

Compounds	RT	aLRI	Peak Area (%)	Identification
α-Zingiberene	31.418	1495	1.68	a.b
α-Muurolene	31.648	1499	1.25	a.b
γ-Cadinene	32.116	1513	0.37	a.b
Germacrene D-4-ol	32.279	1574	1.63	a.b
Spathulenol	32.410	1576	1.21	a.b
β-Caryophyllene	28.999	1418	3.39	a.b
(Z)-*β*-Farnesene	30.189	1443	18.80	a.b
γ-Muurolene	30.876	1477	0.69	a.b
Germacrene D	31.091	1480	10.84	a.b
n-Nonanal	8.492	1098	2.79	a.b
Dihydro citronellol	16.876	1196	15.08	a.b
Longicyclene	26.426	1373	2.02	a.b
α-Copaene	27.417	1376	1.40	a.b
β-Maalene	27.686	1380	0.24	a.b
Iso-longifolene	27.919	1387	2.81	a.b
Longifolene	28.005	1402	1.65	a.b
β-Isocomene	28.693	1403	1.74	a.b
Caryophyllene oxide	34.339	1581	1.98	a.b
Humulene epoxide II	35.208	1606	2.85	a.b
Trans-Arteannuic alcohol	36.112	1607	2.05	a.b
epi-*α*-Muurolol	36.466	1641	1.49	a.b
α-Cadinol	36.799	1653	2.35	a.b
Zerumbone	37.130			

Conclusions

The essential oils obtained from the leaves Artemisia *annua* containing (Z)-*β*-farnesene (18.80%), dihydro citronellol (15.08%) and germacrene D (10.84%) as major constituents showed interesting antibacterial and antioxidant activities which make this essential oil a potential industrial resource of new products. Therefore, isolation, characterization and biological activities of major constituents of essential oil will be the further research findings.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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