



RESEARCH ARTICLE

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Essential Oils of Aerial Parts of *Anaphalis margaritacea* (L.) Benth., *Salvia leucantha* Cav. and *Thymus linearis* Benth. From Uttarakhand Himalaya: Chemical Constituents and Antibacterial Activity

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ABSTRACT

The components present in the essential oils extracted from *Anaphalis margaritacea* (L.) Benth., *Salvia leucantha* Cav. and *Thymus linearis* Benth. were identified by GC and GC/MS analysis. The main compounds present in the oils were aromadendrene (20.0%) in *S. leucantha*, Phellandrene (12.5%), α -thujene (12.0%) in *A. margaritacea* and thymol (50.0%) in *T. linearis*. The antibacterial activity of all the three oils against *E. coli*, *P. aeruginosa*, *S. aureus* and *S. typhi*, has been studied. In the antibacterial activity *S. typhi* is the most resistant bacteria to all the tested oils whereas essential oil of *T. linearis* showed highest antibactericidal activity against *S. aureus* in diffusion method.

Keywords: Essential oil, Antibacterial activity, *Salvia leucantha*, *Anaphalis margaritacea*, *Thymus linearis*.

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INTRODUCTION

Phytochemical with biological activities have been used for a wide range of industrial, pharmaceutical and biological utility. In future biopharmaceuticals may create a new field in medicine and pharmaceuticals. The Himalayas have a great wealth of medicinal plants. Medicinal herbs are the main ingredients of local medicines and are thus of vital importance in traditional health-care. The plant species *Anaphalis margaritacea*, *Salvia leucantha* and *Thymus linearis* are rich in terpenoids and reported in medicinal used as herbal product. In some cases herbal remedies provide safe alternative to synthetic drugs. The extracts including

volatile constituents of many plants species have shown interesting biological activities leading to researches focused on the characterization of antimicrobial constituents of these plants. [1-3]

The species taken in the study have been used in the treatment of different diseases namely diarrhea, ulcers, antioxidant, antifungal, antiviral and fever [4-13] etc. Various species of genus *Salvia* have been documented for their antimicrobial, hepatoprotective and biting-deterrent activity. [14-16] The compound obtained from thyme is known to possess several biological activities such as antibacterial, analgesic, antipyretic, antiparasitic, alleopathic, antiviral, and anti-

inflammatory. [6-12] The compounds present in the essential oil obtained from *A. margaritacea* shown good pharmacological properties like antiseptic and sedative. [4-5] The oils extracted from the species *Anaphalis*, *Thymus* and *Salvia* have importance in biological activities. Thus, all the three species can serve as models for phytochemical studies and related biological aspects.

In the present study we have used the part of plant above ground from different climatic conditions as already reported. In order to assess the pharmaceutical importance of the compounds present in the essential oil obtained from the species. The target of the study is to obtain the biological activity of plants namely *A. margaritacea*, *S. leucantha* and *T. linearis* from Uttarakhand Himalaya from different locations for the optimum results. The essential oil obtained from any species with different percentage of constituents can give different activity against any microorganism as already reported for studied. The composition of the compounds may depend upon the climatic conditions of the naturally growing area of plant. It has been possible to predict activity of the compounds present in the essential oil of the plant. Thymol [10-12] which is the marker constituents of *T. linearis* is reported in the literature has been taken for the confirmation of the result in our study along with synthetic antibiotic gentamycin.

MATERIALS AND METHODS

Plant Collection

Anaphalis margaritacea from Binsar hill Almora at 29°40'09.55"N latitudes and 79°43'13.27"E longitudes, 1843m (KU/CHE/ALM-01), *Thymus linearis* from Hemkund Chamoli at 30°40'42.76"N latitudes and 79°35'20.45"E longitudes, 2763m (KU/CHE/ALM-02) and *Salvia leucantha* from Almora at 30°07'31.69"N latitudes and 79°50'02.91"E longitudes, 1860m (KU/CHE/ALM-03) were collected at the flowering stage in the month of August and September and specified the plant species by Kumaun University, Botany Department.

Chemicals

Anhydrous Na₂SO₄, dimethyl sulphoxide (DMSO) of analytical grade, Merk was used in the study. The media Mueller-Hinton broth (MHB) and Mueller-Hinton agar (MHA) of Hi-Media, India make was taken in the study of antibacterial activity.

Hydrodistillation of plant materials

The hydrodistillation of plant material was carried out with Clevenger type apparatus. Each material of 700 gm was extracted for three hours with 1 liter distilled water. The procedure followed for extraction is well established method and reported in literature. [17] The extract was dehydrated with by anhydrous Na₂SO₄. The estimation of oils was calculated from v/w in percentage. The temperature of the oil was maintained 4°C during storage.

Instrumental analysis

The samples were analyzed with the gas chromatography (GC) showed by the instrument Varian vista 6000 having D.B.-5 non polar silica capillary column 60 m × 0.40 mm, film thickness: 0.25µm. The 10% solutions of the oil in *n*-hexane were injected in a volume 0.5µL and the detector was allowed to record the chromatograph at 250°C. The oven temperature was maintained 40-250°C with the rate 3°C/min. GC/MS analysis was done by Thermo Quest Trace GC 2000 with a Finnigan MAT Polaris Q ion trap mass spectrometer. During the measurements the volume of injection was capped 0.10µL and split ratio 1:40 keeping other parameters constant as used in GC. The MS were taken at 70 eV with a mass range of 40-450 amu.

Compound identification of essential oils

The compounds of the oil were identified by the values of retention time (RT) and retention index (RI) obtained from GC relative to n-alkanes (C₈-C₂₃) series. The comparison between experimental and standard retention values reported in the MS library search (NIST and WILEY) was also considered. For further identification of mass spectra the values obtained in the spectra were confirmed with the values reported mass spectrum literature data. [18] The percentage of all the compounds was obtained from the experimentally measured value by GC FID without using correction factors.

Determination of antibacterial activity

Bacterial strains

The in vitro biological activity was carried out against bacterial strains namely [*Pseudomonas aeruginosa* (MTCC No. 424), *Escherichia coli* (MTCC No. 443), *Staphylococcus aureus* (MTCC No. 737) and *Salmonell typhi* (MTCC No. 531)] obtained from Microbiology laboratory, Sushila Tewari Forest Hospital Trust, Haldwani, Nainital, Uttarakhand which were purchased from the Institute of Microbial Technology, Chandigarh. The bacterial culture was maintained at 4°C on agar-agar media throughout the work.

Antibacterial activity evaluation

We have attempted well diffusion method for the evaluation of antibacterial activity of the oils obtained in the study. [19] The solutions of the oils in dimethyl sulphoxide (DMSO) were used for the evaluation process having known concentration. The petri dishes with the dimension 90 mm were used for the preparation of the media having volume 20 mL of Mueller-Hinton agar and microbial culture (1×10⁶ CFU/mL) spread along the surface of the hole media. The well having capacity 15µL/mL of sample by cutting it with dimension of 2 mm borer. The reference standard antibiotic gentamicin was taken as positive control and DMSO as negative control. The diffusion of samples was allowed for three hours at room temperature followed by incubation period 24 hours at 37±1°C. Antibacterial activity was observed in mm dimension of inhibition zone.

Table 1: Major compounds of essential oil from Asteraceae and Lamiaceae species analysed by GC and GC/MS data.

Major Compounds ^a	RI ^b	RI ^c	Compound %		
			<i>A. margaritacea</i>	<i>T. linearis</i>	<i>S. leucantha</i>
α -Thujene(2)	924	926	12.0	-	-
α -Pinene (3)	939	940	10.0	-	-
α -Phellandrene(1)	1002	1005	12.5	-	-
Thymol(5)	1306	1302	-	50.0	-
β -Caryophyllene(7)	1419	1421	-	-	12.0
Aromadendrene (6)	1441	1439	-	-	20.0
Germacrene-D-4-ol(4)	1574	1574	8.5	6.1	8.0
Caryophyllene oxide(8)	1583	1581	-	-	5.0

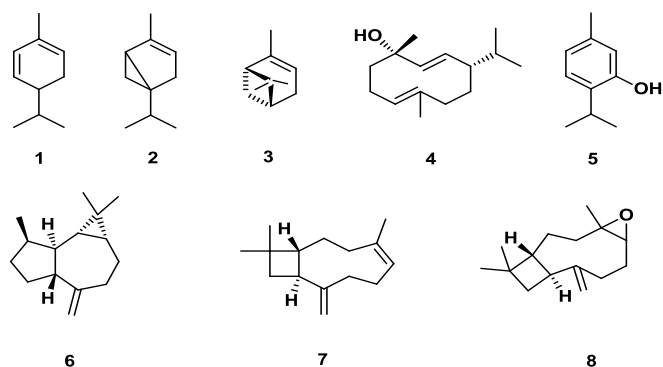
^aMS, NIST and WILEY libraries spectra and the literature. ^bLiterature retention index (RI) on DB-5 column.

^cCalculated retention index (RI) relative to homologous series of n-alkanes (C₈-C₂₃) on D.B.-5 non-polar fused silica capillary column.

Table 2: Antibacterial activity of essential oil from Asteraceae and Lamiaceae species.

Samples	Diameter of Inhibition Zone (mean \pm SD) mm ^a			
	Bacterial strains			
	<i>S. typhi</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>
Essential oils				
<i>A. margaritacea</i>	4.0 \pm 0.3	7.0 \pm 0.2	8.0 \pm 0.1	5.0 \pm 0.2
<i>T. linearis</i>	8.0 \pm 0.1	8.0 \pm 0.2	10.0 \pm 0.2	7.0 \pm 0.2
<i>S. leucantha</i>	3.0 \pm 0.1	5.0 \pm 0.1	6.0 \pm 0.1	3.0 \pm 0.1
Reference antibiotic				
Gentamicin	18.4 \pm 0.2	24.3 \pm 0.1	21.0 \pm 0.2	20.6 \pm 0.2

^aInhibition zone diameter includes well diameter

**Fig. 1: Chemical structure of major compounds.**

RESULTS AND DISCUSSION

The results obtained from the GC and GC/MS analysis for essential oils extracted from different plant are given in Table 1. The compounds and present in the oils were identified by comparing their retention time (RT) and retention index (RI) with the authenticated reported values. The isolation and characterization of terpenoid constituents obtained from *A. margaritacea*, *S. leucantha* and *T. linearis* showed that the percentage yield (v/w) of the oil was 0.1%, 0.1% and 0.2% respectively. The experimental results are mentioned in the Table 1 along with Fig. 1. In the study the major constituents obtained from *A. margaritacea* having α -Phellandrene (12.5%), α -thujene (12.0%), α -pinene (10.0%) and germacrene-D-4-ol (8.5%). Previous chemical investigation of this plant revealed the presence of α -pinene (2.22%) along with other compounds. [5] In plant species *T. linearis* oil was dominated by the presence of Thymol (50.0%) and germacrene-D-4-ol (6.1%) whereas reports on *T. linearis* revealed the presence of thymol, carvacrol, linalool and *p*-cymene. [10-12, 17, 20] The oil extract of *S. leucantha* have compounds Aromadendrene (20.0%) and β -

caryophyllene (12.0%) with other compounds in less quantity which is supported by the previous studies in which other compounds were bornyl acetate (40.92%), azulene (12.94%), germacrene-D (8.26%), borneol (8.11%), bicyclogermacrene (5.34%), limonene (35%) and α -pinene (17%) along with the compounds obtained in this study. [14-16, 21] The plant species studies *A. margaritacea*, *T. linearis* and *S. leucantha* may have quantitative changes only. Essential oils are volatile in nature the presence of any constituents may depends upon the atmospheric condition and temperature of the collection site of the plant. In view of the cause we have concern mainly with the quantitative variations of the constituents and search for any trace atmospheric conditions.

The extracted oils obtained from the plant species *A. margaritacea*, *S. leucantha* and *T. linearis* along with reference antibiotic gentamicin have shown antibacterial activity against bacterial strains of *S. typhi*, *E. coli*, *S. aureus* and *P. aeruginosa* with different inhibition zones. The values are listed in Table 2. On comparing inhibition zone values, *T. linearis* was found to be more effective than *A. margaritacea* and *S. leucantha* against all the bacterial species while the most resistant bacteria *S. typhi* to all the oil extract. The compounds characterized in the oil extract are responsible for the antibacterial activity during the course of investigation recorded. The single compound or the mixture of compounds in the oil extract has shown the resultant antimicrobial effect. A considerable number of compounds have been reported for their biological activity in literature *viz.* thymol and carvacrol [11], thymol, carvacrol, geraniol [22] and thymol or carvacrol. [23-24] The activity performance of oil extract depends on the compounds present in it are assumed to be bioactive as confirm by the presence in our study. From this brief account, the higher bactericidal activity of *T. linearis* oil than other oils might be due to presence of thymol as major constituents and synergistic effect of various other constituents in the oils.

It has been observed that in the biological screening optimum zone of inhibition for gentamicin is in the range (18.4-24.3 mm) for all the bacterial strains studied whereas thymol contained essential oil of *T. linearis* has optimum zone of inhibition (7.0-10.0 mm). Thus, the experimental results further support the biological

activities of essential oil quantitatively. Due to the increasing demands of herbal medicine in place of synthetic medicine the extracted essential oil is suitable drug in place of gentamicin which is safer in metabolic regulations like kinetic factors involving substrate co-factors and enzymes including alternate or separate pathway for catabolism and anabolism of a key substrate.

The present investigation provides useful information of the compounds present in the extracted oils of the plants. The quantity of the major constituents is different from the quantity reported earlier in same species collected from different sites. It may be concluded in the study the variation in the quantity of different compounds present in extracted oils are influence by atmospheric conditions including soil structure. The essential oil from *Anaphalis*, *Thymus* and *Salvia* species against bacterial strains has antibacterial activity. Thus, the species could be natural agents for fight against bacterial infections and can be a source of antimicrobial drugs.

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