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 antibacterial activity against *S. aureus* in diffusion method.

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

**DOI:** 10.25004/IJPSDR.2019.110514

**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. The species taken in the study have been used in the treatment of different diseases namely diarrhea, ulcers, antioxidant, antifungal, antiviral and fever etc. Various species of genus *Salvia* have been documented for their antimicrobial, hepatoprotective and biting-deterrent activity. The compound obtained from thyme is known to posses several biological activities such as antibacterial, analgesic, antipyretic, antiparasitic, alleopatic, antiviral, and anti-
The compounds present in the essential oil obtained from *A. margaritacea* shown good pharmacological properties like antiseptic and sedative. The compounds in the essential oil were identified by the values obtained in the MS library search comparison between experimental and retention factors.

**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 Na$_2$SO$_4$, dimethyl sulfoxide (DMSO) of analytical grade, Merck 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 Cleveenger 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. The extract was dehydrated with anhydrous Na$_2$SO$_4$. 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 × 0.25 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: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 (C$_n$-C$_3$) 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. 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. The solutions of the oils in dimethyl sulfoxide (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 (1×10$^6$ 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.
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 (22.2%) along with other compounds. [8] 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%), bicyclodermacrene (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.

ACKNOWLEDGEMENTS

Author’s thanks to Department of Microbiology, Sushila Tewari Forest Hospital Trust, Haldwani for antimicrobial activity and Department of Botany, Kumaun University.

REFERENCES

