Tanacetum longifolium is a medicinal herb belonging to the family Astereaceae (Compositae). Tanacetum species are the rich source of essential oils. The essential oil was isolated from plant by using a Clevenger type apparatus. The chemical composition of essential oil was analyzed by GC-MS. The antibacterial activity was tested against gram positive Bacillus subtilis and Staphylococcus aureus and gram negative Escherichia coli, and Pseudomonas aeruginosa. The major constituents identified were α-eudesmol (22.5 %), 1,4 dimethyl azulene (13.5%), germacrone (8.2%), trans chrysanthemol (5.3%), 1, 8-cineole (4.5%), and camphor (2.5%). The essential oil of T. longifolium showed strong in vitro activity against E. coli, Bacillus subtilis and Staphylococcus aureus. The results recommended that raw material and their constituents have commercial utility in incense (dhoop), fragrant, flavor, and pharmaceutical industries.

Keywords: Astereaceae, Tanacetum longifolium, antibacterial activity, essential oil, and chemical composition.

Introduction

Tanacetum longifolium is a medicinal herb has its place to the family Astereaceae (Compositae). The Astereaceae or Compositae (commonly stated to as the aster, daisy, or sunflower family), is an extremely large and well-known family of vascular plants (Kumar and Tyagi, 2013). The group has more than 22,750 presently recognized species, spread across 1620 genera and 12 subfamilies. The most members of Astereaceae family are herbaceous, but a significant number are also shrubs, vines and trees. The family is spread throughout the world and most common in the arid and semi-arid regions of subtropical and lower temperate latitudes (Kumar and Tyagi, 2013). Concern is increasing in species of Tanacetum due to its essential oils (cordial, stomachic, and used as a food preservative), bitter substances and the presence of sesquiterpene lactones, which revealed biological activities like growth regulating, cytotoxicity, antimicrobial activity (Coron et al., 1992).
The plants of genus *Tanacetum* have been used as expectorants, vermifuges, antiseptic and spasmolytics in popular medicine (Oksuz, 1990). The dry leaves and flowers of *T. vulgare* are used as antiseptic and spasmodic and for defensive against dandruff in Bulgaria, (Nano et al., 1979). The leaves of *T. parthenium* are used as a popular British traditional herbal therapy for the prophylaxis of migraine (Kubo and Kubo, 1995).

The genus *Tanacetum* is signified by six species viz. *Tanacetum nubigenum*, *Tanacetum tibeticum*, *Tanacetum longifolium*, *Tanacetum arteminiodes*, *Tanacetum gracile*, and *Tanacetum senecionis* in Kumaon and Garhwal regions at 3600-4300 m elevation (Strachey, 1852). These herbs grow in gardens wast area or along road sides, forest shades, creek banks, river-gravels, margins or fields, pasturelands, on mountain steeps, limestone rocks, slopes, crevices and screes.

Chemotherapeutic agents are widely used as antimicrobial agents. One of the major problems in antimicrobial chemotherapy is the increasing incidence of resistance to antibiotics, which leads to the deficiency of antimicrobial treatment. The overuse of antibiotics and consequent antibiotic selection pressure is thought to be the most important factor contributing to the appearance of different kinds of resistant microbes. Species of various plants are used as antimicrobial agents worldwide (Kumar and Tyagi, 2013). This study was an attempt to explore the antibacterial potential of *Tanacetum longifolium* essential oil.

The literature review of the genus *Tanacetum* shows that no previous work reported on antibacterial activities of essential oil of *Tanacetum longifolium* (Kumar and Tyagi, 2013). Therefore, present research work was undertaken to study the chemical composition, and antibacterial activity of *Tanacetum longifolium*.

**Materials and methods**

**Plant collection**

The plants were collected from the Alpine slope of Kumaun Himalaya of Uttarakhand State, India. The plant was identified as *Tanacetum longifolium* by the Department of Botany, D.A.V. (P.G.) College, Dehradun, Uttarakhand. Freshly picked tissue stored in dry plastic bags during the several days required for transport. The voucher specimen was also deposited in the Herbarium of Department of Botany, D.A.V. (P.G.) College, Dehradun, Uttarakhand.

**Isolation and analysis of essential oil**

Air dried plant material (500g) was hydrodistilled for 4 hours using a Clevenger type apparatus. The oil was dried over anhydrous sodium sulphate (Na$_2$SO$_4$) and then isolated oil was kept in a sealed vial in refrigerator until analysis. The chemical composition of essential oil was analyzed by GC-MS. The components of the oil of *T. longifolium* were recognized by comparison of their mass spectra with those of a computer library and with authentic compounds. Further they were also confirmed by comparison of their retention indices either with those of authentic compounds and with data published in literature (Davies, 1990, Ateya (1992), Shibamoto, 1987 and Adams, 2001).

**Antibacterial activity**

The antibacterial activity was determined with the agar well diffusion method (Kumar and Tyagi, 2013). The bacterial strains selected were gram positive *Bacillus subtilis*...
and Staphylococcus aureus and Escherichia coli, and Pseudomonas aeruginosa.

A loopful of 10^4-10^6 suspension of 24 hour old broth of each bacterium was streaked on the surface of Muller-Hinton agar plates. Wells were dug in the agar with the help of a sterile cork borer. Stock solutions of the compounds or crude extracts containing 2mg/ml were prepared in sterile dimethyl sulfoxide (DMSO). Dilutions of the stock solution containing 50, 100, 150, 200µg were prepared in DMSO. 100µl of each dilution was then added in the respective wells. The plates were then incubated at the 37°C for 24 hour and zones of inhibition were measured in millimeters. Penicillin was used as standard antibiotics to compare the extent of activity of the test samples.

Results and Discussion

Identification of essential oil constituents

The identified chemical constituents of the essential oil of T. longifolium are given in table 1 in order of their retention index. Total 37 compounds (86% of the total constituents of oil) were identified from the oil of T. longifolium. The major constituent was α-eudesmol (22.5 %), 1,4 dimethyl azulene (13.5%), germacrone (8.2%), trans chrysanthemol (5.3%), 1, 8-cineole (4.5%), and camphor (2.5%). The results recommended that raw material and their constituents have commercial utility in incense (dhoop), fragrant, flavor, and pharmaceutical industries.

The essential oil isolated from Tanacetum longifolium had shown antibacterial activity against tested bacterial species (Table-2 and Figure-1). The antimicrobial activity of essential oils of Tanacetum longifolium against some bacteria was found as moderate. The results indicated that the essential oils of both Tanacetum species essential oils used in this study inhibited development of bacteria at different ratios. In general, the antimicrobial activity of the essential oil tested was more pronounced against Gram-positive than against Gram negative bacteria. This type of results are also observed from the studies with essential oils from many other plant species (Ouattara et al., 1997 and Nostro, 2000).

| S. No. | Compounds               | RRI  | Percentage |
|-------|-------------------------|------|------------|
| 1     | Tricyclene              | 927  | 0.9        |
| 2     | α- pinene               | 936  | 0.1        |
| 3     | Camphene                | 950  | 0.2        |
| 4     | Sabinene                | 973  | 0.1        |
| 5     | β- pinene               | 978  | 0.3        |
| 6     | Verbenene               | 982  | 0.2        |
| 7     | α- terpinene            | 1013 | 0.2        |
| 8     | 1, 8- cineole           | 1038 | 4.5        |
| 9     | Limonene                | 1025 | 0.1        |
| 10    | (z)-β-ocimene           | 1029 | 1.5        |
| 11    | Trans- sabenene hydrate | 1053 | 0.5        |
| 12    | Furanoid                | 1058 | 0.7        |
| No. | Compound            | Retention Time | Rf Value |
|-----|---------------------|----------------|----------|
| 13  | Linalool            | 1086           | 2.3      |
| 14  | Camphor             | 1123           | 2.5      |
| 15  | Trans-chrysanthemol | 1153           | 5.3      |
| 16  | n-decanal           | 1180           | 0.1      |
| 17  | Geraniol            | 1235           | 0.2      |
| 18  | Decanol             | 1264           | 0.2      |
| 19  | Eugenol             | 1331           | 0.5      |
| 20  | α-isocome          | 1389           | 0.8      |
| 21  | Germacrene-D       | 1479           | 2.3      |
| 22  | β-Vetisperene      | 1486           | 0.2      |
| 23  | 2-tridecanol       | 1490           | 1.4      |
| 24  | α-salinene         | 1494           | 0.8      |
| 25  | Germacrene A       | 1503           | 0.3      |
| 26  | δ-Cadinene         | 1520           | 0.2      |
| 27  | 1, 4- dimethylazulene | 1532 | 13.5  |
| 28  | Caryophlene oxide  | 1578           | 3.1      |
| 29  | Valgarone A        | 1580           | 0.4      |
| 30  | Tetradecanol       | 1596           | 2.8      |
| 31  | Cedrol             | 1603           | 0.3      |
| 32  | Furanogermacrene   | 1624           | 2.5      |
| 33  | α-eudesmol         | 1653           | 22.5     |
| 34  | α-barbatenal       | 1659           | 2.5      |
| 35  | Germacrone         | 1684           | 8.2      |
| 36  | α-Santalol acetate | 1756           | 0.8      |
| 37  | Dihydrodiplophylline | 1896 | 3.0   |

**Table 2** Antibacterial activity of extracts and essential oil of *Tanacetum longifolium*

| Organisms              | Zone of inhibition (in mm) |  |
|------------------------|----------------------------|---|
|                        | control       | essential oil |
| **Gram positive**      |               |               |
| *Bacillus subtilis*    | 16            | 13            |
| *Staphylococcus aureus*| 14            | 15            |
| **Gram negative**      |               |               |
| *Escherichia coli*     | 15            | 14            |
| *Pseudomonas aeruginosa*| 12            | 12            |

Total identified 86 percent
Penicillin was used as standard antibiotics to compare the extent of activity of the test samples. It is interesting that *T. longifolium* essential oil was showed similar antimicrobial activity against the microorganisms as in antibiotic. The essential oil of *T. longifolium* showed strong in vitro activity against *E. coli*, *Bacillus subtilis* and *Staphylococcus aureus*.

In conclusion, the amount of essential oil obtained by hydrodistillation from *T. longifolium* in full flowering stage was 0.92 percent of the dried weight. Total 37 compounds (86% of the total constituents of oil) were identified from the oil of *T. longifolium*. The major constituent was α-eudesmol (22.5 %), 1,4 dimethyl azulene (13.5%), germacrone (8.2%), trans chrysanthenol (5.3%), 1, 8-cineole (4.5%), and camphor (2.5%). The results recommended that raw material and their constituents have commercial utility in incense (dhoop), fragrant, flavor, and pharmaceutical industries.

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