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Antibacterial activity of volatile oil of *Tanacetum longifolium* from western Himalayan region of Uttrakhand, India

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ABSTRACT

The genus Tanacetum, commonly known as tansy and traditionally, T. longifolium is used in preparation of incense and fragrant materials by the local inhabitants in Uttarakhand. The in vitro antibacterial activities of the essential oils were evaluated against a total of six bacteria,viz, Salmonellatyphi, Klebsiellapneumoniae, E.coli, Staphylococcusaureus, Streptococcusmutans, Bacillussubtilis.

Key words: *Tanacetum* volatile oil, antibacterial, zone of inhibition, MIC.

INTRODUCTION

The genus *Tanacetum*, commonly known as tansy is represented by six species viz. *T. nubigenum*, *T. tibeticum*, *T. longifolium*, *T. arteminiodes*, *T. gracile* and *T. senecionis* in Kumaun and Garhwal regions in 3600-4300 m elevation [1, 2]. *Tanacetum* species are reported to possess anthematic, carminative, stimulant, antispasmodic and anti-migrant properties [3-5] Some members of Asteraceae family particularly *Tanacetum* species have traditionally been used in the manufacture of balsams, cosmetics, dyes, insecticides, medicines and preservatives and have also been used in herbal remedies e.g. *T. vulgare*) [6,7]. α-Thujone, β-thujone, chrysanthenone, α-chrysanthenyl acetate, camphor and 1,8-cineole-borneol dominated chemotypes have been reported in *T. vulgare* [8-11]. *T. longifolium* Wall was shown to possess *trans*-sabinyl acetate (43.2 %) and *trans*-sabinol (12.7 %) as major components [12]. The essential oil compositions of four species of *Tanacetum* grown in Itlay have been analyzed. The leaf and flower oils of *T. argyrophyllum* var. *argyrophyllum* were dominated by α-thujone (52.0 %, 63.0 % respectively). Caryophyllene oxide and α-thujone were the major constituents of *T. argenteum* subsp. *canum* var. *canum*; while borneol (28.0 %) and 1, 8-cineole were reported as the major constituents of *T. praeteritum* sub. *praeteritum*. α-Thujone and β-thujone were the major constituents of *T. messicyticu* [13]. The essential oil from *T. corymbosum* was shown to be dominated by γ-cadinene and δ-cadinene [14]. Sesquiterpene lactones and coumarins have also been reported from various species of genus *Tanacetum* [15-19] *T. parthenium* (L.) Schultz Bip. is the most prominent and a known remedy used mainly in the prophylactics of migraine, as well as for the treatment of arthritis, fever, vertigo, menstrual disorders, stomach ache and psoriasis [20]. These activities mainly have been attributed to sesquiterpene lactone parthenolide, which has antimigraine, anti-inflammatory, gastric anti-ulcer and antinociceptive activity [21, 22].

Unlike previous report on *T. longifolium*, which showed the presence of long chain esters and monoterpenoids viz, *trans*-sabinyl acetate (43.2 %) and sabinol (12.7 %) as major constituents [23], but we have reported earlier an entirely different oil composition having sesquiterpenoids (72.1 %) as the major constituents. Further, *trans*-sabinyl acetate (43.2 %) and sabinol (12.7 %) reported as the major constituents earlier were not detected even in trace quantity [24]**.** The present study revealed the antibacterial activity of volatile oil of *Tanacetum longifolium*.

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MATERIALS AND METHODS

Collection of Plant material: The fresh aerial parts of *Tanacetum longifolium*, was collected from world famous Milam glacier of Western Himalaya. Plants herbaria were identified CHEM/DST/07/01 in Botany Department, Kumaun University, Nainital and Botanical Survey of India, Dehradun.

Oil isolation: The fresh plant materials (1.0 kg) were subjected to steam distillation using a copper electric still, fitted with spiral glass condensers. The distillates were saturated with NaCl and extracted with *n*-hexane and dichloromethane. The organic phase was dried over anhydrous Na_2SO_4 and the solvent was distilled off in a rotary vacuum evaporator at 30^o C.

Antibacterial activity: The *in vitro* antibacterial activities of the essential oils were evaluated against a total of six bacteria, *viz.*, *Salmonella typhi*, (Clinical isolated), *Klebsiella pneumoniae*, (MTCC 109), *E. coli* (MTCC 1610), *Staphylococcus aureus* (MTCC 96), *Streptococcus mutans* (MTCC 890), *Bacillus subtilis* (MTCC 121). The test strains were purchased from the Institute of Microbial Technology (IMTECH), Chandigarh. MTCC (Microbial Type Culture Collection) numbers represents the standard strain numbers assigned to these microorganisms. The cultures of bacteria and fungi were maintained on their appropriate agar slants at 4^o C throughout and used as stock cultures.

Determination of zone of inhibition (ZOI)

The antimicrobial activity of the essential oils was investigated by the disc diffusion method using 24-48 h grown strains reseeded on Nutrient Broth (bacterial strains) and Potato Dextrose Agar (PDA, fungal strains) [25]. The cultures were adjusted to 5 × 106 CFU/mL with sterile water. 100 µL of the suspensions were spread over Nutrient agar and PDA plates to obtain uniform microbial growth. Filter paper discs (6.0 mm in diameter) were impregnated with 20 µL of the oils and then placed onto the agar plates which had previously been inoculated with the test microorganism. The petri dishes were kept at 4^o C for 2 h. The plates were incubated at 37^o C (24 h) and at 30^o C (4 h) for bacterial and fungal strains, respectively. The diameter of the inhibition zones (mean values) were measured in millimeter and considered as the zone of inhibition (ZOI). All experiments were performed in triplicate.

Determination of the minimum inhibitory concentration (MIC)

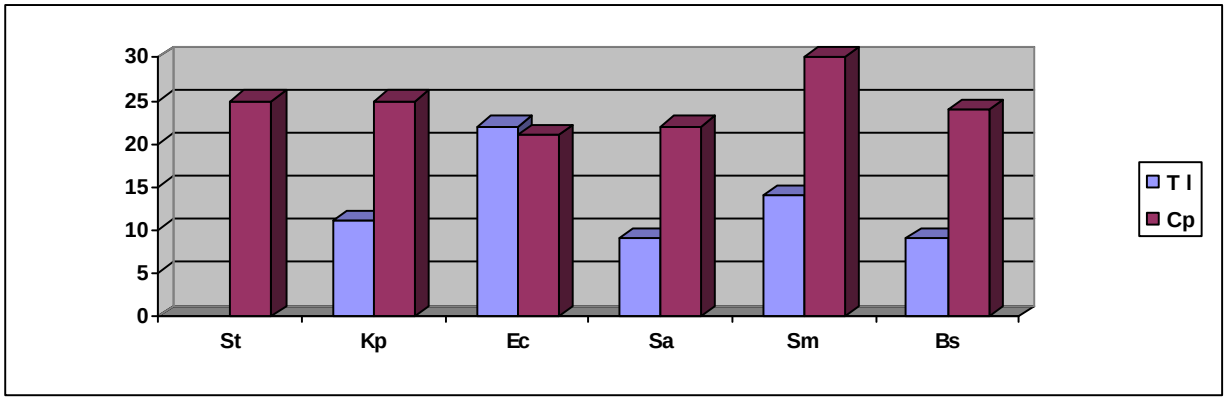
The minimum inhibitory concentration (MIC) values were determined using a modified agar-well diffusion method [25]. In the agar-well diffusion technique, two-fold serial dilutions of the essential oils were prepared by diluting oil with hexane to achieve a decreasing concentration range from 50 to 2.70 µL/mL (for the fungi) and 50 µL/mL to 3.20 µL/mL (for the bacteria), using 100 µl of a suspension containing 5 × 106 CFU/mL of bacteria spread on nutrient agar plates, whereas the fungal strains were reseeded on PDA. The wells were filled with 20 µL of essential oil solutions in the inoculated Nutrient PDA agar plates. The bacterial cultures were incubated at 37° C for 24 h, while fungal cultures were incubated at 30° C for 48 h. The least concentration of each essential oil showing a clear zone of inhibition was taken as the MIC. Hexane was used as the negative control. Chloramphenicol and amphotericin B were used as positive controls for bacteria and fungi, respectively. Antimicrobial (antibacterial and antifungal) activity of leaf essential oil of *T. longifolium* by disc diffusion assay (10µL of oil/disc) against different microorganisms shown in the table 3.2 and 3.3 respectively.

RESULTS AND DISCUSSION

The antimicrobial activity of essential oils of *Tanacetum* species against some bacteria and were found as moderate (Table 1). It is interesting that *Tanacetum* the essential oils were showed good antibacterial activity. It can be seen from the table that the oil showed very good antibacterial activity against *E. coli* ZOI 22 mm (MIC 3.54) with respect to standard viz., Chloramphenicol (10µg/disc) (ZOI 21mm).

Table 1 Antibacterial activity by disc diffusion assay (10 µl of oil/disc)
(Zone of inhibition and MIC)

Oil/antibiotic	*S. typhi*	*K. pneumoniae*	*E. coli*	*S. aureus*	*S. mutans*	*B. subtilis*
T. longifolium	Na	11 mm (9.42)	22 mm (3.54)	9 mm (7.20)	14 mm (3.20)	9 mm (7.50)
Cp.(10µg/disc)	25 mm	25 mm	21 mm	22 mm	30 mm	24 mm



Antibacterial activity of leaf essential oil of T. longifolium (Tl= Tanacetum longifolium, Cp= Chloramphenicol) Bacteria: St, Salmonella typhi ; Kp, Klebsiella pneumoniae ; Ec, Escherichia coli ; Sa, Staphylococcus aureus; Sm, Streptococcus mutans ; Bs , Bacillus subtilis; Na=not active No inhibition zone, Chloramphenicol (10 µg/disc).

CONCLUSION

The present investigation reveals that the antibacterial activity of volatile oil of *Tanacetum longifolium* and it is also found to be good natural antibacterial. One of the major problems in antimicrobial chemotherapy is the increasing occurrence of resistance to antibiotics, which leads to the insufficiency 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. In addition to the anti-inflammatory, antibacterial, antifungal and insecticidal effects of the *Tanacetum* essential oils, antimicrobial activities can be used in chemotherapy in the next future.

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