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Antimicrobial Activity of Volatile Oil of *Artemisia capillaris* Growing Wild in Utrakhand Himalaya

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The genus *Artemisia* (family: Asteraceae) is a source of valuable drugs and essential oils because of its intricate chemical composition comprising several chemotypes. The in vitro antibacterial activities of the essential oils were evaluated against a total of six bacteria, viz, *Salmonellatyphi*, *Klebsiellapneumoniae*, *E.coli*, *Staphylococcus aureus*, *Streptococcusmutans*, *Bacillus subtilis* and antifungal activity tested against *Candida albicans* and *Candida glabrata*

Keyword: Asteraceae, *Artemisia capillaris*, Essential Oil, Antibacterial.

1. Introduction

Utrakhand Himalaya is bestowed with a rich flora of aromatic plants. The climatic variations from subtropical to alpine have favored the growth of many aromatic plants. The genus *Artemisia* (family: Asteraceae) is a source of valuable drugs and essential oils because of its intricate chemical composition comprising several chemotypes. Reports on chemical composition of various *Artemisia* species from different origins show the presence of 1, 8-cineole, α -thujone, β -thujone, chamazulene, davanone, artemisia ketone, germacrene D, β -caryophyllene and caryophyllene oxide.^[1,2] In recent years, there has been increasing interest in healthy lifestyles and healthy aging. As a result, many people are involved in searches for natural compounds that can improve health, especially those of plant origins. A great number of aromatic, spicy, medicinal, and other plants belonging to the family Asteraceae contain chemical compounds exhibiting antimicrobial and antioxidant properties^[3]. Antimicrobial and

antioxidative plant oils and extracts have been used for many purposes, including raw and processed food preservation, pharmaceuticals, alternative medicines, and natural therapies. Natural products are perceived as having fewer negative impacts than synthetic agents; natural products may be effective, selective, biodegradable, and less toxic to the environment. The genus *Artemisia* is one of the most important genera in the family Asteraceae and is widespread throughout the world^[4]. Previous reports on *A. capillaris* showed that it has antifungal effects^[5] and allelopathic effects⁶ stimulates immune activities in human cells, and has anticancer activity^[7]. The major active components of *A. capillaris* are scoparone and capillarisin, and the concentrations of these compounds are related to the season of harvest^[8]. Many members of the genus *Artemisia* (Asteraceae) are important medicinal plants. For example, *A. vulgaris* (mugwort), native of Britain and Europe, has been used as a tonic, febrifuge, anthelmintic, women's menstrual troubles and

infertility, anti-nervous disorders, against complaints of the gastrointestinal tract (e.g. stomach ulcers and indigestion). The essential oil shows the antimicrobial activity and contains 1,8-cineole, camphor and thujone as major constituents^[9]. We have already reported the phenyl alkynes rich essential oil from leaves and roots of *A. capillaris*^[10]. Present communication reveals the antimicrobial activity of leaf volatile oil of *A. capillaris*.

2. Materials and Methods

2.1 Plant Material

The plant material was collected from Milam glacier at an altitude of 3,600 m in the month of August. The identifications were done separately from Botany Department, Kumaun University, Nainital and Botanical Survey of India (BSI) Dehradun. The voucher specimens (No.CHEM/DST/06/01) have been deposited in the Phytochemistry laboratory of the Chemistry Department, Kumaun University, Nainital.

2.2 Oil Isolation

The fresh leaves (2.5 kg) were subjected to steam distillation in a copper electric still fitted with spiral glass condensers obtaining 5L water distillate each. The distillates were saturated with NaCl and the oils were extracted with hexane followed by dichloromethane. The organic phases were then dried over anhydrous Na₂SO₄ and the solvent distilled off in a thin film rotary vacuum evaporator at 30^oC.

2.3 Antimicrobial 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 antifungal activity of the oils was performed against *Candida albicans* (MTCC 1637) and *Candida glabrata* (MTCC 3019). The test strains were purchased from the Institute of Microbial Technology (IMTECH), Chandigarh. MTCC (Microbial Technology 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.

2.4 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)^[11]. 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.

2.5 Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) values were determined using a modified agar-well diffusion method^[1]. 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 1.26 μL/mL (for the fungi) and 50 μL/mL to 2.20 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^oC for 2 hrs, while fungal cultures were incubated at 30^oC for 48 hrs. 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 *A. capillaris* leaf oil by disc diffusion assay (10 μ L of oil/disc) against different microorganisms shown in the table 1 and 2 respectively.

3. Results and Discussion

Antibacterial activity against *Staphylococcus aureus*, ZOI 25 mm, (MIC, 4.25) with respect to standard, viz chloramphenicol, ZOI 22 mm showed very good activity. Also antifungal

activity showed by leaf oil of *A. capillaris* against *Candida albicans* and *Candida glabrata*, exhibit largest ZOI 29 mm and 20 mm (MIC, 1.26, 2.08) with respect to standards viz amphotericin B (20 μ g), ZOI 16 mm and 11 mm respectively. Earlier work on *A. capillaris* showed antiplatelet and anti-Hiv activity, insecticidal activity against *Sitophilus zeamais*, and antimicrobial activity^[12]. This species was recorded to control cabbage white butterfly (*Pieris rapae*), cotton aphids (*Aphis gossypii*), cucurbit leaf beetle (*Aulacophora femoralis*) and other vegetable pests in china^[13].

Table: 1 Antibacterial activity of leaf essential oil *A. capillaris* (by disc diffusion assay (10 μ L of oil/disc) (Zone of inhibition and MIC)

Oil/antibiotic	<i>S. typhi</i>	<i>K. pneumoniae</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>S. mutans</i>	<i>B. subtilis</i>
<i>A. capillaris</i>	9.0 mm (15.54)	10 mm (14.16)	9.0 mm (10.2)	25 mm (4.25)	11 mm (3.20)	14 mm (2.20)
Cp.(10 μ g/disc)	25 mm	25 mm	21 mm	22 mm	30 mm	24 mm

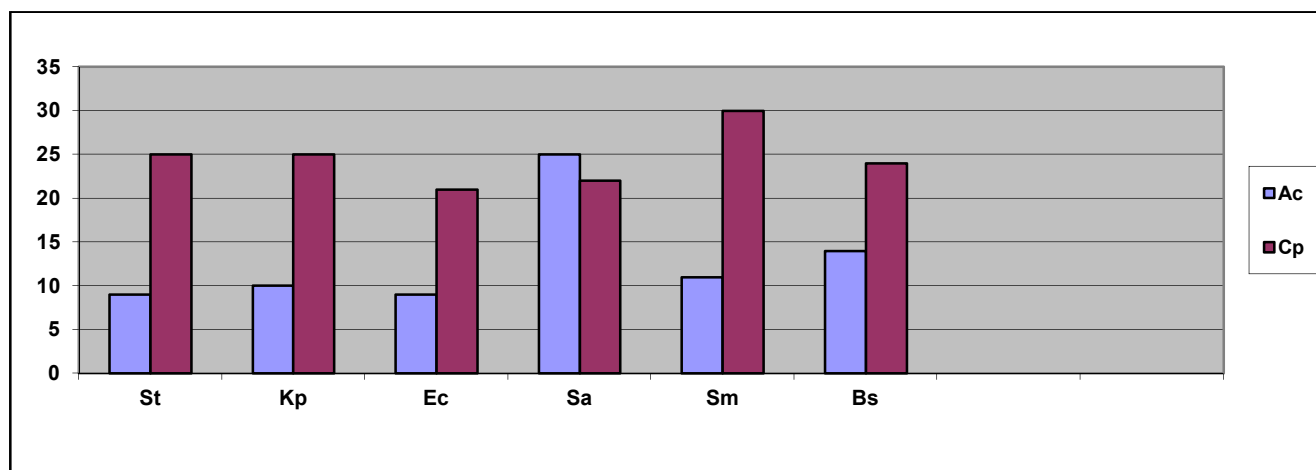
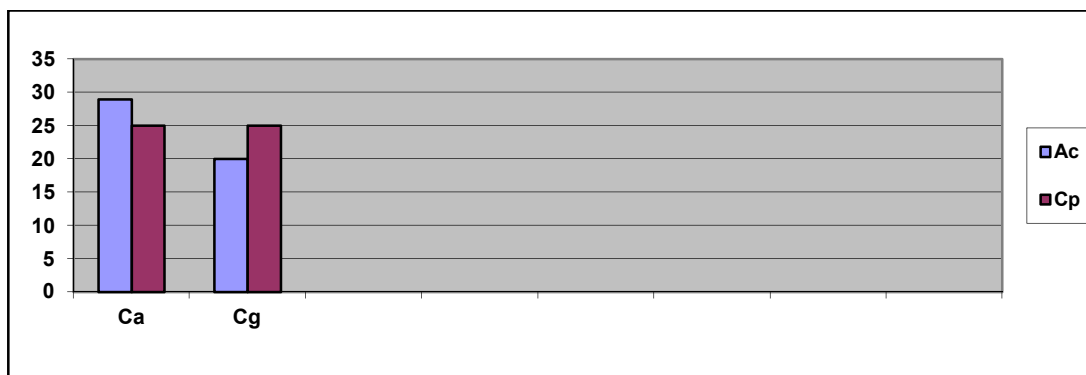


Fig: 1 Antibacterial activity of leaf essential oil of *A. capillaris* (Ac=*Artemisia capillaris*, Cp= Chloramphenicol) Bacteria: St, Salmonella typhi; Kp, Klebsiella pneumoniae ; Ec, Escherichia coli ; Sa, Staphylococcus aureus; Sm, Streptococcus mutans ; Bs Bacillus subtilis No inhibition zone. Chloramphenicol (10 μ g/disc)

Table: 2 Antifungal activity of leaf essential oil *A. capillaris* (by well diffusion assay (40µL of oil/well) (Zone of inhibition and MIC)

Oil /antifungal	<i>Candida albicans</i>	<i>Candida glabrata</i>
<i>A. capillaris</i>	29 mm (1.26)	20 mm (2.08)
Amphotericin B (20µg)	16 mm	11 mm

**Fig: 2** Antifungal activity of leaf essential oil of *A. capillaris*

(Ac =*Artemisia capillaris*, Am= Amphotericin B) Fungal strains: Ca, *Candida albicans*; Cg, *Candida glabrata*; No inhibition zone. Amphotericin B 20µg

4. Conclusion

The present investigation reveals that the essential oil of *Artemisia capillaris* is found to be good natural antibacterial and antifungal agent. Attempts will be made in future to isolate the huge amount of oil to use this purpose.

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