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# Antimicrobial activity of leaf essential oil of *Chaerophyllum villosum* Wall. ex DC. from Kumaun Himalayan of Uttrakhand

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**ABSTRACT**

The objective of the present study was to evaluate the antimicrobial activity of leaf essential oil of *Chaerophyllum villosum.* The essential oils composition of leaves of *Chaerophyllum villosum* Wall. ex DC. (family: Apiaceae) were analyzed and compared using capillary GC and GC-MS. The leaf essential oil of *C. villosum* was dominated by monoterpene hydrocarbons (91.34%) represented by γ-terpinene (74.93%) as single major constituent followed by p-cymene (10.00%), terpinolene (2.93%) and β-pinene (2.54%). Among the various micro organisms, the oil was more active against *Staphylococcus aureus* and *Streptococcus mutans*. In antifungal activity of the essential oil shows good results against *Candida albicans* and *Candida glabrata.*

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## INTRODUCTION

The genus *Chaerophyllum*, belonging to Apiaceae family, comprised of about 110 species which includes annual and perennial herbal plants widely distributed in temperate and sub temperate zones of Asia, Africa and Europe[1-3]. *Chaerophyllum villosum* Wall ex DC was widely distributed in E. Asia Himalayas from India to Bhutan, Nepal and China and widely grows in moist shady places, road sides or open grassy places at elevations of 2100-3500 m. In high altitude tribes of Uttarakhand Himalaya (India) it was commonly known and sold in the name of 'Ganjari' widely used by people in food, spice and also as medicine[4-6]. Although the various species in the genus *Chaerophyllum* were known to possess toxin chaerophyllin capable of causing diarrhea and incoordination, yet these were also used as antimicrobial, antioxidant, stimulant and expectorant[7-10]. Literature survey revealed that there were few reports on the essential oils of *Chaerophyllum* species which report wide variety of terpenoid constituents. Sabinene (30.0%), *p*-cymen-8-ol (16.0%) and terpinolene (11.5%) were reported as major constituents from essential oil of *C. byzantinum.* The hydro distilled essential oils from flower, leaf and stem of *C. macropodum* were characterized by myristicin (15.7%-42.5%) and *trans*-β-ocimene (24.9%-54.2%) as major constituents. The principal constituents identified in *C. libanoticum*, used as a food plant in Turkey, were β-phellandrene (17.6%), limonene (15.9%), β-pinene (8.8%), and sabinene (8.5%). While the essential oil isolated from aerial parts of *C. macrospermum* showed (E)-β-ocimene (55.9%), terpinolene (9.8%), α-pinene (7.5%), β-phellandrene (4.3%) and β-pinene (4.2%) [11-15] Literature survey also revealed that there were few reports on the antimicrobial activity as the essential oil of *Chaerophyllum libanoticum* Boiss. et Kotschy was evaluated for its antimicrobial activity using a micro dilution assay resulting in the inhibition of a number of common human pathogenic bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and the yeast Candida albicans.[16] The essential oils of the aerial parts and fruits of *Chaerophyllum aureum L.*, collected and the oils were tested against six bacterial strains and one strain of yeast, Candida albicans. The highest antimicrobial activity was observed against the Gram-positive bacteria *Staphylococcus aureus*, *S. epidermidis* and Micrococcus luteus, while of the Gram-negative strains, Escherichia coli was the most sensitive.[17]

## MATERIALS AND METHODS

### Plant collection and identification

The fresh leaves of *C. villosum* were collected from Milam glacier (Uttarakhand, India) at an altitude of 3600 m in the month of August at mature stage in 2008. The identification was done from Botany Department, Kumaun University, Nainital and BSI, Dehradun. The voucher specimens (No.Chem08/DST/CV) have been deposited in the Phytochemistry lab of the Chemistry Department, Kumaun University, Nainital.

### Isolation of essential oil

The fresh planting materials (2 kg each) 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 sodium sulfate and the solvents were distilled off in a rotary vacuum evaporator at $30^0$C and the percentage oil content was calculated on the basis of fresh weight of plant materials.

**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 4o 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).[18] 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.[18] 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 1.70 µL/mL (for the fungi) and 50 µL/mL to 2.60 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 *C. villosum* by disc diffusion assay (10µl of oil/disc) against different microorganisms shown in the table 1 and 2 respectively.

## RESULTS AND DISCUSSION

Antibacterial activity against *Staphylcoccus aures*, ZOI 21 mm, and *Streptococcus mutans* 23mm of essential oil *Chaerophyllum villosum* with respect to standard, *viz* chloramphenicol, ZOI 22 mm and 30 mm showed very good activity. Also antifungal activity showed by leaf oil of *Chaerophyllum villosum* against *Candida albicans* and *Candida glabrata*, exhibit largest ZOI 25 mm and 13 mm with respect to standards viz amphotericin B (20 µg), ZOI 16 mm and 11 mm respectively. To the best of our knowledge, this is the first time the antimicrobial activity of essential oil of leaves *C. villosum* is being reported. Earlier work on *Chaerophyllum* species showed that remarkable antibacterial activity shown by *Chaerophyllum libanoticum* Boiss.[16] The essential oil of *Chaerophyllum aureum* was evaluated for its antimicrobial activity using a microdilution assay resulting in the inhibition of a number of common human pathogenic bacteria including methicillin resistant Staphylococcus aureus (MRSA) and the yeast Candida albicans.[17]

**Table 1: Antibacterial activity of leaf essential oil of *C. villosum* by disc diffusion assay (10µl of oil/disc) (Zone of inhibition in mm)**

| Oil/antibiotic | *Salmonella typhi* | *Klebsiella pneumoniae* | *E.coli* | *Staphylococcus aureus* | *Streptococcus mutans* | *Bacillus subtilis* |
|---|---|---|---|---|---|---|
| *Chaerophyllum villosum* | 16 mm | 17 mm | 16 mm | 21 mm | 23 mm | 14 mm |
| Chloramphenicol (10µg/disc) | 25 mm | 25 mm | 21 mm | 22 mm | 30 mm | 24 mm |

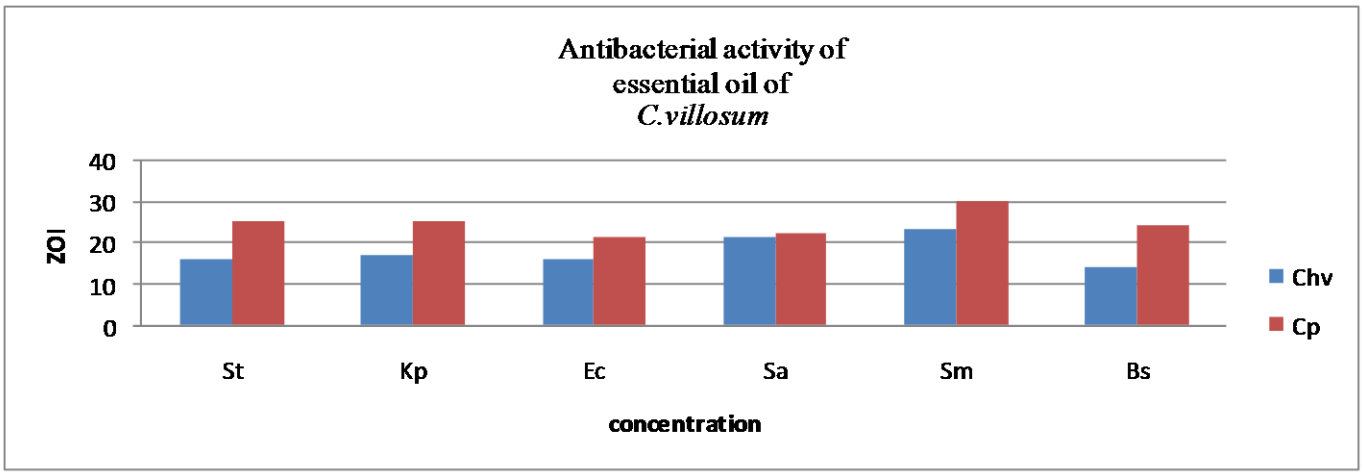


**Fig 1: Antibacterial activity of leaf essential oil of *Chaerophyllum villosum* Chv= *Chaerophyllum villosum*, Cp= Chloramphenicol Bacteria: St, *Salmonella typhi* ; Kp, *Klebsiella pneumoniae* ; Ec, *Escherichia coli* ; Sa, *Staphylococcus aureus*; Sm, *Streptococcus mutans* ; Bs, *Bacillus subtilis* No inhibition zone. chloramphenicol (10 µg/disc)**

**Table 2: Antifungal activity of leaf essential oil of *C. villosum* by well diffusion assay (40µl of oil/well)**

| Oil /antifungal | *Candida albicans* | *Candida glabrata* |
|---|---|---|
| *Chaerophyllum villosum* | 25 mm | 13 mm |
| Amphotericin B (20µg) | 16 mm | 11 mm |

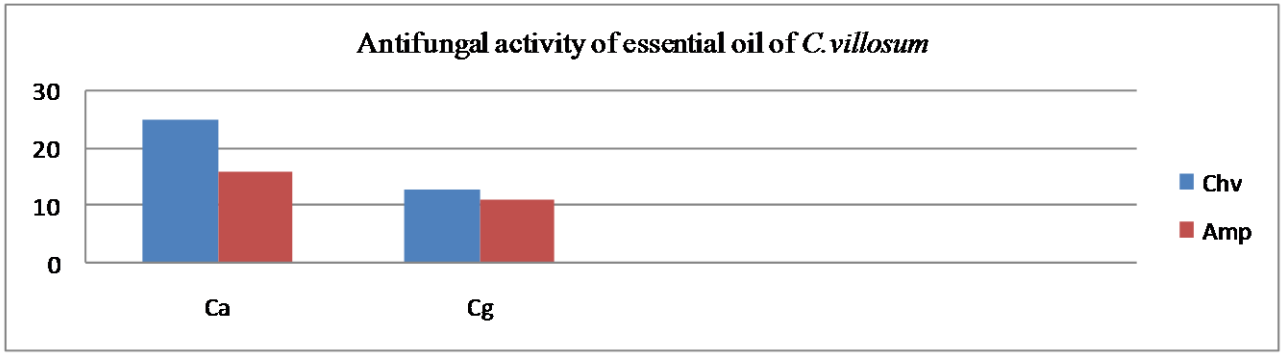


**Fig 2: Antifungal activity of leaf essential oil of *C. villosum* Chv = *Chaerophyllum villosum*, Amp= amphotericin B Fungal strains: Ca, *candida albicans*; Cg, *candida glabrata*; No inhibition zone.**

## CONCLUSION

The present investigation reveals that the antimicrobial activity of essential oil of *Chaerophyllum villosum* is found to be very effective with respect to standard used and 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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