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Antimicrobial activity of terpinen-4-ol rich chemotype of Himalayan cypress (*Cupressus torulosa*) essential oil

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The present study evaluated the antimicrobial potential of *Cupressus torulosa* needle essential oil (CTEO) against selected bacterial and fungal strains using the disc diffusion method. In the present investigation, CTEO exhibited concentration-dependent antimicrobial activity against all tested bacterial strains. At 500 µg/mL, the highest activity was observed against *Streptomyces griseus* (34±1 mm), followed by *Salmonella typhimurium* (30±1 mm), *Micrococcus luteus* (28±1 mm), and *Staphylococcus aureus* (23±2 mm). Among the fungal strains, CTEO showed moderate activity against *Aspergillus fumigatus* but was inactive against *Candida albicans*. The findings suggest that CTEO possesses promising antibacterial potential, particularly against Gram-positive bacteria, and may serve as a natural source of antimicrobial agents.

Keywords: *Cupressus torulosa*, Essential oil, Antimicrobial activity, Terpinen-4-ol, Antibacterial activity, Antifungal activity

INTRODUCTION

The rapid emergence of antimicrobial resistance has become a serious global health concern and has reduced the effectiveness of many conventional antimicrobial drugs. This situation has increased the demand for alternative antimicrobial agents from natural sources. In recent years, essential oils have attracted considerable attention because of their broad-spectrum antimicrobial activity and their comparatively lower risk of inducing resistance due to the presence of multiple bioactive compounds[1]. Monoterpenes and oxygenated monoterpenes present in essential oils are known to disrupt microbial cell membranes, alter permeability, and interfere with important metabolic processes of microorganisms. Several essential oils obtained from aromatic plants have shown promising antimicrobial activity against human pathogens. Essential oils from *Origanum vulgare*[2], *Thymus vulgaris*[3], *Syzygium aromaticum*[4] and *Cupressus arizonica*[5] have been widely reported for their antibacterial and antifungal properties and are increasingly being explored for pharmaceutical, cosmetic, food preservation, and healthcare applications.

Cupressus torulosa D. Don, commonly known as Himalayan cypress or Surai, is an evergreen conifer distributed throughout the Himalayan region of India. Different parts of the plant have been traditionally used for the treatment of rheumatism, cough, cold, inflammation, wounds, and microbial infections[6,7]. Previous studies on *C. torulosa* needle essential oil (CTEO) have mainly focused on its chemical composition, antioxidant activity, anti-inflammatory potential[7], and aroma profile[8]. The essential oil has been reported to be rich in monoterpenes such as terpinen-4-ol, sabinene, limonene, α-pinene, and δ-3-carene, compounds which are already known for their antimicrobial properties[9].

Despite earlier reports on the antimicrobial properties of CTEO[7,10], significant variations in chemical composition

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have been reported among oils obtained from different geographical regions and chemotypes. Previous studies mainly described α-pinene-, sabinene-, or δ-3-carene-rich oils[7,10,11], whereas the present CTEO was characterized by a terpinen-4-ol-rich profile. Since antimicrobial activity of essential oils is strongly influenced by their chemical composition, evaluation of biologically distinct chemotypes is scientifically important. In addition, limited information is available regarding the activity of terpinen-4-ol-rich CTEO against clinically and environmentally important microorganisms such as *Streptomyces griseus, Micrococcus luteus*, and *Salmonella typhimurium*. Therefore, the present study aimed to evaluate the antimicrobial potential of a terpinen-4-ol-rich chemotype of CTEO and relate its activity to its unique phytochemical profile.

Materials and Methods

Chemicals and reagents

Dimethyl sulphoxide (DMSO), dichloromethane, and anhydrous Na_2SO_4 were procured from Merck. Nutrient agar, nutrient broth, potato dextrose agar, potato dextrose broth, fluconazole (10 µg/disc), ciprofloxacin (10 µg/disc) and ampicillin (10 µg/disc) were obtained from Oxoid. Whatman No. 1 filter paper discs (6 mm) were used for antimicrobial screening.

Essential oil

The needles from *C. torulosa* trees were collected (~2 kg) from the Botanical Garden (30°20'30.4" N, 78°0'4.2" E; 693 meters) within the premises of the Forest Research Institute, Dehradun, India. Since the trees were part of the botanical garden and were clearly marked and identified, there was no need to submit a specimen for identification purposes. Fresh needles of *C. torulosa* (250 g) were subjected to hydro-distillation using a Clevenger-type apparatus for 4 h. The resulting aqueous distillate was collected, and the CTEO was separated from the distillate. The CTEO was then dried over anhydrous Na_2SO_4 to remove residual moisture and was finally stored in sealed vials at 4°C until further analysis. The chemical composition of CTEO has been reported previously[5] and was found to be rich in terpinen-4-ol (39.38%), totarol (5.50%), sabinene (4.37%), and sempervirol (4.08%). In continuation of our earlier work, the present study was undertaken to evaluate the antimicrobial potential of CTEO.

Microbial strains

The antimicrobial activity of CTEO was evaluated against four bacterial strains and two fungal strains obtained from the American Type Culture Collection (ATCC). The bacterial strains included *Staphylococcus aureus* (ATCC-700698), *Micrococcus luteus* (ATCC-10240), *Salmonella typhimurium* (ATCC-13311), and *Streptomyces griseus* (ATCC-10137). The fungal strains used in the study were *Aspergillus fumigatus* (ATCC-204305) and *Candida albicans* (ATCC-10231).

Disc diffusion assay

The antimicrobial activity of CTEO was evaluated by the disc diffusion method[12]. Bacterial cultures were grown overnight in nutrient broth, while fungal cultures were maintained in potato dextrose broth at 37°C. The microbial suspension was adjusted turbidometrically at 600 nm to obtain a final concentration of approximately 10^5–10^6 CFU/mL. CTEO was dissolved in DMSO to prepare concentrations of 50, 100, 250, and 500 µg/mL. For antimicrobial screening, 10 µL of the sample solution was loaded onto sterile Whatman No. 1 filter paper discs (6 mm diameter). The discs were carefully placed on nutrient agar or potato dextrose agar plates previously inoculated with the respective microbial cultures. The plates were then incubated at 37°C for 24 h. Antimicrobial activity was determined by measuring the diameter of the zone of inhibition around each disc and expressed in millimetres (mm). All experiments were carried out in triplicate. Fluconazole (10 µg/disc) was used as used as standard drug for antifungal activity, while ciprofloxacin (10 µg/disc) and ampicillin (10 µg/disc) were used as standard drugs for antibacterial activity.

Results and Discussion

CTEO inhibited all tested microbes with zones of inhibition increasing with concentration from 50 to 500 µg/mL (Table 1). At 500 µg/mL, the largest zones were observed for *S. griseus* (34±1 mm), followed by *S. typhimurium* (30±1 mm), *M. luteus* (28±1 mm), and *S. aureus* (23±2 mm). Among the bacterial controls, ciprofloxacin (10 µg/disc) produced zones ranging from 21±1 to 38±1 mm, while ampicillin (10 µg/disc) gave 27–32 mm, indicating that at the highest concentration CTEO approached or exceeded the activity of these reference antibiotics against some strains, especially *S. griseus* and *M. luteus*. For fungi, CTEO was completely inactive against *C. albicans* at all tested concentrations, whereas it showed moderate activity against *A. fumigatus*, with inhibition zones of 15±1 and 19±1 mm at 250 and 500 µg/mL, respectively. In comparison, fluconazole (10 µg/disc) exhibited a 31±1 mm zone against *C. albicans* and 37±1 mm against *A. fumigatus*, indicating that CTEO was less potent than the azole drug but still displayed relevant fungistatic effects on *A. fumigatus*.

Previous work on *C. torulosa* needle oil has also reported significant antimicrobial activity against both bacterial and fungal strains, supporting the present findings. In that study, CTEO rich in α-pinene and δ-3-carene showed marked inhibition of *Bacillus subtilis, Pseudomonas alcaligenes,*

Table 1. Antimicrobial activity of *Cupressus torulosa* needles essential oil

S. no	Strain name	Essential oil concentration (µg/mL)				Ciprofloxacin 10 µg/mL	Ampicillin 10 µg/mL	Fluconazole 10 µg/mL
		50	100	250	500			
		Zone of Inhibition (in mm)						
1	*M. luteus*	12±1	20±1	23±2	28±1	21±1	32±1	-
2	*S. griseus*	14±2	21±2	24±1	34±1	25±1	29±1	-
3	*S. typhimurium*	18±1	20±2	23±1	30±1	30±1	27±1	-
4	*S. aureus*	16±2	18±1	21±2	23±2	38±1	32±1	-
5	*C. albicans*	Inactive	Inactive	Inactive	Inactive	-	-	31±1
6	*A. fumigatus*	Inactive	Inactive	15±1	19±1	-	-	37±1

Results are presented as mean±SD of three observations, -: not tested

Micrococcus luteus and *Bacillus cereus*, with inhibition zones generally higher against Gram-positive bacteria[10], similar to the stronger responses observed here for *M. luteus, S. griseus* and *S. aureus*.

Essential oils from other *Cupressus* species show a comparable activity pattern. The leaf oil of *C. macrocarpa* displayed strong antibacterial effects against *S. aureus* and *Proteus vulgaris* with very low MIC values (0.01 *v/v*)[13], indicating high potency against Gram-positive cocci, in line with the pronounced response of *S. aureus* to CTEO in the present study. Likewise, extracts and essential oils of *C. sempervirens* have been reported to be most active against *S. aureus*, with inhibition zones in the 7–12.3 mm range and low MIC values[14], again confirming that *Cupressus* essential oils tend to be particularly effective against Gram-positive bacteria. Together, these reports indicate that the strong inhibition of *M. luteus* and *S. aureus* by CTEO observed here is consistent with the broader antimicrobial profile of the genus.

The moderate antifungal effect of CTEO against *A. fumigatus* observed in the present study is also supported by previous reports. Bhandari *et al*.[7] reported significant antifungal activity of CTEO against several pathogenic fungi, including *Aspergillus terreus, Candida* spp., *Trichophyton rubrum, T. mentagrophytes, Microsporum canis*, and *Penicillium* species. The study demonstrated maximum inhibition against *T. rubrum* and *T. mentagrophytes*, with inhibition zones of 16 mm for the undiluted oil and a MIC of 0.5 µL/mL against both dermatophytes. The authors also observed antifungal activity against *Candida* species, although the activity decreased upon dilution of the oil. Their CTEO was rich in α-pinene (31.99%), sabinene (19.23%), and limonene (9.06%), suggesting that monoterpene-rich chemotypes of *C. torulosa* possess considerable antifungal potential. In comparison, the present study found moderate inhibition of *A. fumigatus* but complete inactivity against *C. albicans*. These differences may arise from variation in the chemical profile of the essential oil, since the present CTEO was dominated by terpinen-4-ol and totarol rather than α-pinene-rich chemotypes reported previously. Differences in fungal strains, assay conditions, and oil concentration may also influence antifungal response. Nevertheless, both studies consistently demonstrate that CTEO possesses a broader antifungal potential, particularly against filamentous fungi and dermatophytes, supporting its possible application in the development of natural antifungal formulations.

The moderate antifungal effect of CTEO on *A. fumigatus* also agrees with the documented antifungal potential of other *Cupressus* oils. Essential oil from *C. macrocarpa* has been shown to possess notable antifungal activity against several filamentous fungi, although with lower potency than standard fungicides[12], a trend similar to the smaller zones of CTEO compared with fluconazole in the present work. However, the complete lack of activity against *C. albicans* contrasts with some reports where *Cupressus* oils showed at least modest inhibition of yeasts, suggesting that the antifungal spectrum of CTEO is species-dependent and may be influenced by chemotype or test conditions.

The antimicrobial effects observed can be rationalized by the chemical profile of CTEO, which is dominated by terpinen-4-ol, totarol, sabinene and related monoterpenes. Terpinen-4-ol is well documented as a potent antibacterial and antibiofilm agent against *S. aureus*[15]. Totarol, a phenolic diterpene commonly reported in conifers, also possesses strong anti-staphylococcal activity and has been shown to inhibit bacterial respiration and membrane function[16], which may further enhance the activity of terpinen-4-ol-rich oils against *S. aureus* and other Gram-positive species. Monoterpene hydrocarbons such as sabinene, α-pinene and δ-3-carene, which are abundant in *Cupressus* oils, are known to increase membrane permeability and cause leakage of cellular contents, thereby synergizing with oxygenated monoterpenes and phenolics[17]. The high responsiveness of Gram-positive strains and *A. fumigatus* to CTEO in this study is thus likely due to combined, possibly synergistic effects of these major constituents on cell membranes and wall-associated targets.

Conclusions

The present study demonstrated that terpinen-4-ol-rich chemotype of *C. torulosa* needle essential oil possesses promising antimicrobial activity against selected bacterial strains and moderate antifungal activity against *A. fumigatus*. The oil showed particularly strong activity against *S.s griseus*, *M. luteus*, and *S. aureus*, indicating better effectiveness against Gram-positive bacteria. The antimicrobial potential of CTEO is likely associated with the presence of terpinen-4-ol, totarol, sabinene, and other monoterpenes acting synergistically. Although the oil was inactive against *C. albicans*, the overall findings support the traditional use of *C. torulosa* and highlight its potential as a natural antimicrobial agent. Further studies on MIC determination, mechanism of action, and formulation development may help in exploring its possible pharmaceutical and preservative applications.

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Conflict of interests

The author(s) declare no competing interest.

Author's contribution statement

KC: Methodology, Validation, Writing - review & editing; PB: Conceptualization, Data curation, Investigation, Methodology, Writing - original draft.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work the author(s) used ChatGPT 5.2 in order to improve language. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

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