Original Article

In vitro Antimicrobial Activity of the Essential Oil of *Anaphalis contorta* Hook f.

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Abstract

The essential oil was isolated from the aerial parts of *Anaphalis contorta* Hook. f. (Asteraceae). The antimicrobial activity of the essential oil of *A. contorta* was screened by using well diffusion method, against human pathogenic bacteria and fungi at different concentrations (0.25 µg/ml, 0.125 µg/ml and 0.062 µg/ml). The oil was found to be active against the microorganism *Staphylococcus aureus* and *Microsporum canis* at a concentration of 0.125 µg/ml, while *Trichophyton rubrum* was more susceptible at 0.062 µg/ml of the essential oil of *A. contorta*.

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Key words: *Anaphalis contorta* Hook. f., Essential oil, Antimicrobial activity, Well diffusion.

Introduction

The antimicrobial properties of essential oils have been recognized long ago and they have been scientifically established [1]. Various medicinal plants have been used for years in day to day life to treat diseases all over the world. The use of traditional medicine and medicinal plants in developing countries, to meet some of their primary health care needs, has been widely documented [2]. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives [3]. These compounds protect the plant from microbial infection and deterioration [4].

The family Compositae (Asteraceae) is chemically characterized by synthesis and accumulation of many classes of natural products. The important classes of sesquiterpenes, sesquiterpene lactones have a wide distribution in the family Asteraceae [5]. *Anaphalis contorta* Hook. f. is an erect herb. The stem is branched from the base, branches are often decumbent. The leaves are linear, crowded, shortly lobed at the base, margins recurved sometimes. The heads are in dense terminal corymbs and in involucral bracts are broad, obtuse, erect in flower, spreading in fruit; outer ones often pale purple. *A. contorta* is widely distributed in temperate region from Kashmir to Sikkim and Afghanistan to South West China at a height of 1500-4500 m. [6,7]. The fresh leaves of this plant and some other *Anaphalis* species are bruised and applied to the cut wounds under a rag bandage [8]. When taken before meals the leaves stimulate the appetite and as a result, it is administered to convalescents for its sedative and tonic properties [9]. In an earlier study the main components were reported to be as caryophyllene oxide (20.3%), α–cadinol (8.4 %), caryophyllene alcohol (8.1%) γ–muurolene (6.4%), 14- hydroxyl-9-epi –β-caryophyllene (5.6%), α-curcumene (5.4%) and β-bisabolene (5.4%) [10]. This communication presents *in vitro* antimicrobial activity of the essential oil of *A. contorta*.

Materials and Methods

Plant material

Fresh aerial parts of *A. contorta* were collected in July 2005 from the pine forest near Nainital. The botanical identification of the plant specimen was done at Botany Department, Kumaun University, Nainital and confirmed by Forest Research Institute, Dehradun where a Herbarium specimen has been deposited (Voucher No. 28/100991).

Isolation of oil

The fresh aerial parts (1 kg) were steam distilled using copper still fitted with spiral glass condensers. The distillate was saturated with sodium chloride and the oil extracted with hexane and dichloromethane. The organic phase was dried

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Table 1. In vitro antimicrobial activity of the essential oil of A. contorta

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Essential oil</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>G (+)</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus (MTCC 737)</td>
<td>16</td>
</tr>
<tr>
<td>G (-)</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa (MTCC 414)</td>
<td>26</td>
</tr>
<tr>
<td>Escherichia coli (MTCC 443)</td>
<td>16</td>
</tr>
<tr>
<td>Salmonella typhi (MTCC 531)</td>
<td>20</td>
</tr>
<tr>
<td>Proteus vulgaris (MTCC 426)</td>
<td>24</td>
</tr>
<tr>
<td>Fungi</td>
<td></td>
</tr>
<tr>
<td>Microsporum canis (MTCC 2820)</td>
<td>31</td>
</tr>
<tr>
<td>Trichophyton rubrum (MTCC 296)</td>
<td>17</td>
</tr>
<tr>
<td>Candida albicans (MTCC 183)</td>
<td>36</td>
</tr>
</tbody>
</table>

\(^a\) Dilution of essential oil in 95 % ethanol, V/V: applied dose: 15 µl

\(^b\) Standard: applied dose: 15 µl

Diameter of well = 8 mm

over anhydrous sodium sulphate and the solvent was distilled off in a thin film rotary vacuum evaporator at temperature range 25–30°C. The yield of oil obtained was 0.2%.

Microbial strains
Microbial strains namely *Staphylococcus aureus* (MTCC 737) (Gram-positive bacteria), *Pseudomonas aeruginosa* (MTCC 414), *Escherichia coli* (MTCC 443), *Salmonella typhi* (MTCC 531), *Proteus vulgaris* (MTCC 426) (Gram-negative bacteria), *Microsporum canis* (MTCC 2820), *Trichophyton rubrum* (MTCC 296) and *Candida albicans* (MTCC 183) (Fungi) were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India.

Antimicrobial assay

Media preparation

Bacterial media
Nutrient agar (NA) was used for the screening of antibacterial activity of Gram-positive and Gram-negative bacteria. NA was weighed as per instructions provided by the manufacturer and dissolved in distilled water. After proper plugging, it was autoclaved at 120°C and 15 lbs for 20 minutes. Autoclaved nutrient agar when cooled at 45°C was poured into sterilized petri dishes containing nearly 20 ml agar medium under aseptic condition and kept undisturbed as such till solidify. After solidification, these petri plates were incubated at 37°C±1°C overnight for sterility testing.

Fungal media
Preparations of potato dextrose agar (PDA), sabouraud’s agar (SA) and yeast extract potato dextrose agar (YEPDA) media were used as per instruction provided by the manufacturer, for different fungal strains viz., *Micrococcus canis, Trichophyton rubrum* and *Candida albicans*, respectively. After proper plugging, the media were autoclaved at 120°C and 15 lbs for 20 minutes. Autoclaved PDA, SA and YEPD media when cooled at 45°C was poured into sterilized petri plates containing nearly 20 ml growth media under aseptic condition till solidified. After solidification these petri plates were incubated for sterility testing. The PDA plates incubated at 25°C±1°C while SA and YEPD plates at 30°C±1°C for ten days, seven days and 48 hours, respectively.

Antibacterial and antifungal activities of the essential oil
The essential oil of 0.25 µg/ml, 0.125 µg/ml and 0.062 µg/ml were prepared with some modification in 95% ethanol [11-13] and tested against human pathogens *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Proteus vulgaris, Microsporum canis, Trichophyton rubrum* and *Candida albicans*. The activity was carried out by well diffusion method.

Well diffusion method
Antibacterial and antifungal activities of the essential oil of *A. contorta* were tested using well diffusion method [14]. The autoclaved media was poured in the sterilized petri plates. These plates were dried for a period of 20 minutes under aseptic condition before its use. Freshly grown cultures of the tested bacteria and fungi in their media were streaked over the plates using a platinum wire inoculation loop. On sterile media plates, well of 8.0 mm diameter were punched with the help of a sterile gel cutter. Wells were sealed with the molten media to prevent the escape of essential oil through bottom. In the well of separate petri plates 15 µl of different concentrations (0.25µg/ml, 0.125µg/ml and 0.062µg/ml) of
essential oil were delivered. The positive control were used gentamicin 1% (w/v) (Fulford (India) Limited, Hyderabad) and fluconazole 1% (w/v) (Lark Laboratories (India) Ltd., New Delhi) for antibacterial and antifungal activity, respectively. The plates were incubated at 37°C ± 1°C for 24 hours for Gram-positive and Gram-negative bacteria, while 25°C ± 1°C, 30°C ± 1°C and 30°C ± 1°C for Microsporum canis, Trichophyton rubrum and Candida albicans fungi for ten days, seven days and 48 hours respectively. The plates were observed for the zone clearance around the wells. The zones of inhibition were calculated by measuring the diameter of the inhibition zone around the well in millimeter including the well diameter. The readings were taken in three different replicates and the average values were tabulated (Table 1).

Results and discussion

The present study was designed to evaluate the qualitative antimicrobial activity of the essential oil of the aerial parts of A. contorta. The results of antimicrobial activity of the essential oil using well diffusion assay are summarized in Table 1. The antimicrobial activity of the essential oil of A. contorta showed significant activity against the tested microorganisms at three different concentrations (0.25 µg/ml, 0.125 µg/ml and 0.062 µg/ml). The oil was found to be more activity against the microorganism Staphylococcus aureus (Gram-positive) followed by Pseudomonas aeruginosa (Gram-negative) bacteria and Microsporum canis (fungi) at a concentration of 0.125 µg/ml, while Trichophyton rubrum was more susceptible at 0.062 µg/ml of the essential oil of A. contorta. The pure essential oil of A. contorta showed remarkable antifungal property.

Conclusion

In vitro antimicrobial activity of the essential oil of A. contorta showed significant activity against tested human pathogens. The benefit of local application of the fresh leaves of A. contorta as an antiseptic to cuts/wounds could be attributed to their antimicrobial activity as observed in the present study.

References


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