THE ANTI-TRICHOPHYTON TERRESTRE ACTIVITY OF THE ESSENTIAL OIL OF LEPTOSPERMUM SCOPARIUM (MANUKA OIL)

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Abstract
The inhibitory activity of manuka oil against Trichophyton terrestre was evaluated in vitro by determining the MIC and in the inhibition of radial growth of mycelia. The MIC obtained for manuka oil against Trichophyton terrestre is 0.25% v/v which achieved a 100% inhibition in the radial growth of fungus mycelia.

Key words: Leptospermum scoparium, Trichophyton terrestre, manuka oil, antifungal agents, essential oils.

INTRODUCTION
The essential oils of aromatic plants have long been used in embalming to prevent bacterial growth and avoid decay, a practise generally associated with the Ancient Egyptians (5) . Many essential oils exhibit antimicrobial activity, including Tea tree oil (derived from the Australian native plant Melaleuca alternifolia), and have been recognised for their potential in the treatment of MRSA-related infection as well as an alternative for the treatment of mupirocin-resistant MRSA (2). A range of mechanisms of actions by which essential oils can inhibit microorganisms have been proposed. Of particular importance is hydrophobicity, which causes the lipid bilayer to detach from the cell membrane, thereby leading to leakage of whole cell contents. Essential oils may also destroy one, or more, of the bacteria’s vital enzyme systems (5). Manuka oil from the plant Leptospermum scoparium is being investigated as an antimicrobial agent; its activity being based on its high content of triketones (4, 3), which inhibits the growth of methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant enterococci (VRE) and multi-drug-resistant tuberculosis (MDR-TB) (9). In the present paper an attempt has been made to assessed the anti-Trichophyton terrestre potential of Manuka oil, the MIC determinant and the percentage of mycelia growth inhibition were determined.

MATERIALS AND METHODS
The Manuka oil used was a commercially produced by Phytomed Medicinal Herbs, Auckland, New Zealand.

The fungus Trichophyton terrestre (IMI 277732) was obtained from the CAB, Kew, where the Manuka oil used was a commercially produced by Phytomed Medicinal Herbs, Auckland, New Zealand.

The Trichophyton terrestre inoculum was prepared as follows; a standard-sized inoculum of T. terrestre was prepared from 7- to 14-day old cultures grown on PDA at 25°C. Mature colonies were covered with approximately 5 ml of sterile PBS (pH 7.4), PBS was then gently rubbed over the surface with a sterile spreader. The resulting mixture of conidia and hyphal fragments was drawn off with a pipette and transferred to sterile tubes. Heavy particles of the suspension were allowed to settle for 10 to 15 min at room temperature, and the upper homogeneous suspension was used for further testing. The optical densities of the suspensions were read at 530 nm and adjusted to 0.15 to 0.17 to yield 0.6 × 106 to 1.4 × 106 spores/ml of strains. The suspensions containing conidia and hyphal...
fragments were further diluted to obtain the final desired inoculum size of approximately 0.4 x 10^4 to 5 x 10^4 spores/ml.

In order to determine the MIC, a standardized culture was mixed with various concentrations (0.008–0.25 % v/v) of manuka oil then 15 µl of this mixture was placed in PDA media plate and incubated at 25°C for 7 days (7). The agar dilution method was used to determine the anti-Trichophyton terrestris activity of Manuka oil. Trichophyton terrestris was inoculated onto PDA (Potato Dextrose Agar) plates and incubated at 25°C for 7-10 days to obtain young, actively growing cultures consisting of mycelia and conidia. Sterilised diluted Manuka oil with 0.5 Tween 80 was incorporated into PDA sterilised pre-poured medium to give different final concentrations (0.25%, 0.125 and 0.063%). A mycelial disc, 8 mm in diameter, cut from the periphery of the 7-10-day-old cultures, was then aseptically inoculated onto the medium. The inoculated plates were then incubated at 25°C and the colony diameter measured and recorded after 10 days. The percentage of mycelial inhibition was calculated as follows: % mycelial inhibition = [(dc−dt)/dc] × 100; dc= colony diameter in the control, dt = colony diameter in treatment, three replicate plates were used for each treatment.

RESULTS AND DISCUSSION
Trichophyton terrestris is keratinophilic fungus, which causes dermatophytoses (Bokhari, 2009), i.e. superficial fungal infections of skin, hair, nail, or keratinised tissue in humans and animals (7).

As shown in Figure 1, the minimum inhibitory concentration of manuka oil towards Trichophyton terrestris is 0.25% v/v. The results shown in Figure 2 show that mycelial growth was significantly inhibited by manuka oil, 47.1±1.0 % of the radial growth of mycelia was inhibited at concentration 0.063 % v/v, 76.2 ± 3.7 % of the radial growth of mycelia was inhibited at concentration 0.123 % v/v and 100% of the radial growth of mycelia was inhibited at concentration 0.25 % v/v, i.e. these results confirm the previous findings showing that MIC is 0.25% v/v.
Figure 2. Percent inhibition of mycelial growth of *Trichophyton terrestre* in the presence of several concentrations of manuka oil. Percentage of mycelial growth inhibition respective to the control (no oil added). Means of three replicates ±SD.

The observed significant antifungal activity of manuka oil against *Trichophyton terrestre* could indicate its activity in general against dermatophytes. The addition of manuka oil to hair oils used for hair dressings in many developing countries, where dermatophytoses are common (8), e.g. India (6), could help reduce the incidence and distribution of hair–related.

REFERENCES