ANTIFUNGAL ACTIVITY OF LEATHER TREATED WITH *Anethum graveolens* AND *Melaleuca alternifolia* ESSENTIAL OILS AGAINST *Trichophyton interdigitale*

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ABSTRACT. *Trichophyton interdigitale* is a common fungus causing onychomycosis of the nail in humans. Plants are a rich source of bioactive compounds with antifungal properties used in various compositions in pharmaceuticals, cosmetics or various industrial products. In the present paper, the antifungal activity of two kinds of leathers was tested: ecologically processed sheepskin leather lining (Eco) and sheepskin leather lining processed with basic chromium salts (Cr), treated with *Anethum graveolens* and *Melaleuca alternifolia* essential oils. Dill (*Anethum graveolens*) essential oil rich in o-cymene (30.71%) and α -phellandrene (23.21%) and tea tree (*Melaleuca alternifolia*) essential oil rich in terpinene-4-ol (23.06%) were used. The results obtained in this study show that the essential oil of *Anethum graveolens* and *Melaleuca alternifolia* had a high antifungal effect against *Trichophyton interdigitale*. Tea tree and dill essential oils offer a much safer alternative protection against fungi as compared to synthetic compounds with adverse reactions to body and environment. The results of this study may have potential for use in developing applications for cosmetics, pharmaceuticals, obtaining leathers and textiles with selective bioproperties.

KEY WORDS: *Anethum graveolens* and *Melaleuca alternifolia* essential oil, antifungal activity, *Trichophyton interdigitale*, sheepskin leather lining

ACTIVITATEA ANTIFUNGICĂ A PIEILOR TRATATE CU ULEIURI ESENȚIALE DE *Anethum graveolens* ȘI *Melaleuca alternifolia* ÎMPOTRIVA *Trichophyton interdigitale*

REZUMAT. *Trichophyton interdigitale* este un fung comun care provoacă onicomicoza unghiei la om. Plantele sunt o sursă bogată de compuși bioactivi cu proprietăți antifungice utilizate în diferite compoziții în produse farmaceutice, produse cosmetice sau diverse produse industriale. În lucrarea de față s-a testat activitatea antifungică pe două tipuri de piele: căptușeala din piele de oaie procesată ecologic (piele Eco) și căptușeala din piele de oaie procesată cu săruri bazice de crom (piele Cr) tratate cu uleiuri esențiale de *Anethum graveolens* și *Melaleuca alternifolia*. S-a folosit ulei esențial de mărar (*Anethum graveolens*) bogat în o-cimol (30,71%) și α-felandren (23,21%) și ulei esențial de arbore de ceai (*Melaleuca alternifolia*) bogat în terpinen-4-ol (23,06%). Rezultatele obținute în acest studiu arată că uleiurile esențiale de *Anethum graveolens* și *Melaleuca alternifolia* au avut un efect antifungic ridicat împotriva *Trichophyton interdigitale*. Uleiurile esențiale de arbore de ceai și mărar oferă o alternativă de protecție mult mai sigură împotriva ciupercilor, comparativ cu compușii sintetici, care prezintă reacții adverse pentru corp și mediu. Rezultatele acestui studiu pot avea potențial de utilizare în dezvoltarea aplicațiilor în produse cosmetice, farmaceutice, obținerea de piei și textile cu bioproprietăți selective.

CUVINTE CHEIE: ulei esențial de *Anethum graveolens* și *Melaleuca alternifolia*, activitate antifungică, *Trichophyton interdigitale*, piei ovine pentru căptușeli

L'ACTIVITÉ ANTIFONGIQUE DES CUIRS TRAITÉS AVEC DES HUILES ESSENTIELLES D'*Anethum graveolens* ET *Melaleuca alternifolia* CONTRE *Trichophyton interdigitale*

RÉSUMÉ. *Trichophyton interdigitale* est un champignon commun provoquant l'onychomycose des ongles chez l'homme. Les plantes sont une riche source de composés bioactifs aux propriétés antifongiques utilisés dans diverses compositions pharmaceutiques, cosmétiques ou divers produits industriels. Dans cet article, on a testé l'activité antifongique de deux types de cuirs : la doublure en cuir de mouton traitée écologiquement (Eco) et la doublure en cuir de mouton traitée avec des sels basiques de chrome (Cr), traités avec des huiles essentielles d'*Anethum graveolens* et *Melaleuca alternifolia*. L'huile essentielle d'aneth (*Anethum graveolens*) riche en o-cymène (30,71%) et α -phellandrène (23,21%) et l'huile essentielle d'arbre à thé (*Melaleuca alternifolia*) riche en terpinène-4-ol (23,06%) ont été utilisées. Les résultats obtenus dans cette étude montrent que l'huile essentielle d'*Anethum graveolens* et *Melaleuca alternifolia* ont eu un effet antifongique élevé contre *Trichophyton interdigitale*. Les huiles essentielles d'arbre à thé et d'aneth offrent une protection alternative beaucoup plus sûre contre les champignons par rapport aux composés synthétiques ayant des réactions indésirables au corps et à l'environnement. Les résultats de cette étude peuvent avoir un potentiel d'utilisation dans le développement d'applications dans les cosmétiques, les produits pharmaceutiques, l'obtention de cuirs et de textiles à propriétés biologiques sélectives.

MOTS CLÉS : huile essentielle d'*Anethum graveolens* et *Melaleuca alternifolia*, activité antifongique, *Trichophyton interdigitale*, peau de mouton pour doublures

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INTRODUCTION

Plants are a rich source of bioactive compounds with antifungal properties used in various compositions in pharmaceuticals, cosmetics or various industrial products [1]. Medicinal plants have been used to treat various affections for thousands of years, because they are vast and diverse compositions of natural compounds that can have important selective bioproperties. The most important of these compounds are alkaloids, tannins, flavonoids, terpenoids, saponins and phenolic compounds. Researchers, pharmacists, doctors are interested in these compounds due to their bioactivity and low toxicity [2]. Many compounds were isolated from plants that could be used for the development of new compositions to inhibit growth of fungal pathogens with possibly new mechanisms of action [3]. Extensive studies have already been reported for the use of essential oils from plants as biocides in leather processing [4-9] and in the protection of footwear against the growth of microorganisms which are mainly responsible for the appearance of fungal infections on the nails [4, 10-13]. Considering the side effects of antifungal drugs obtained by chemical synthesis, remedies based on natural compounds are a safe choice for treating fungal infections. They have no side effects as have other medicines. Essential oils are a natural remedy to prevent and treat fungal infections. If used in optimal proportions, essential oils with antifungal properties are a natural way to cure fungal diseases of the foot such as athlete's foot, or treat other demanding areas of the foot [4]. The use of essential oils in biological control against fungi is mainly due to their richness in antifungal substances [14-18]. Numerous studies have established the efficacy of *Anethum graveolens* essential oil from the *Apiaceae* family [1] and *Melaleuca alternifolia* essential oil from the *Myrtaceae* family [19] against fungi [20-22]. Many members from these families are economically useful for medicines, cosmetics and various commercial uses [23]. Previous studies on the essential oils of *Apiaceae* and *Myrtaceae* have shown that these plants have a wide range of biological activities, in particular antifungal and antimicrobial potential. The chemical nature of the main ingredients was directly related to this activity [24]. Several previous studies have focused on chemical composition and antifungal effect of *Anethum graveolens* and *Melaleuca alternifolia* essential oils. Thus, their rich content in monoterpenes, gives an antifungal, antioxidant and antimicrobial activity. These properties promise their use as a natural antifungal in various pharmaceutical, cosmetic, industrial applications [25, 26]. There is much interest to find antimicrobial and antifungal agents to treat the shoe lining leather. For example, Koizhaiganova *et al*. studied the leather treated with silver doped hydroxyapatite. Good antibacterial properties against *S. aureus*, *B. subtilis*, *E. faecalis* as Gram positive bacteria, *E. coli*, *S. typhimurium*, *K. pneumoniae* and *P. aeruginosa* as Gram negative bacteria and the yeast *C. albicans* were reported with the increasing concentration of Ag [27]. In our previous paper, we presented the antifungal and antibacterial effects of geranium, pine, and rosemary essential oils on the cotton and leather linings with good results [28]. This fungus causes onychomycosis (also known as *tinea unguium*, a fungal infection on the nail) and *tinea pedis* (skin infection on the feet causes by fungus) in humans [30-32].

The aim of this paper is to test the antifungal effect of the essential oil extracted from dill (*Anethum graveolens*) and tea tree (*Melaleuca alternifolia*) against *Trichophyton interdigitale* on sheepskin lining leather used in the production of footwear, to avoid the appearance of nail fungus or bad smell of shoes by using natural antifungal agents.

EXPERIMENTAL

Materials and Methods

Materials

Anethum graveolens essential oil (EO) was acquired from S.C. Herbavit S.R.L. Oradea, Romania. *Melaleuca alternifolia* essential oil (EO) was acquired from S.C. Solaris Plant Radix S.R.L. Bucharest, Romania and Borron SE from Triderma SRL Bucharest. Sheepskin leather lining was processed at the National Research and Development Institute for Textiles and Leather – Division: Leather and Footwear Research Institute (INCDTP-ICPI), Bucharest.

Methods

Sheepskin Lining Leather

Sheepskin lining leather obtained by ecological processing without basic chromium salts (lining leather Eco) and sheepskin lining leather obtained by processing with basic chromium salts (lining leather Cr) were used. The samples of sheepskin lining leather were treated by immersion in 200% aqueous float with 5% essential oil and 5% Borron SE, relative to the weight of the lining materials, at 25°C for 30 minutes with stirring and then free dried at 25°C.

GC-MS Analysis

Chemical composition of essential oils was determined by Gas chromatography-Mass Spectrometry (GC-MS) with Agilent 6890 N and the majority constituent compounds were identified [29].

ATR-FTIR Analysis

The chemical structures of investigated leather samples were analyzed by use of Fourier Transform Infrared Spectroscopy-Attenuated Total Reflectance FT-IR/ATR spectrometer JASCO FT-IR 4200. The spectra were accumulated from 16 scans in a range of 4000-400 cm^{-1} scale, with a scan speed of 2.2 KHz and a resolution of 4 cm^{-1}, single-beam, with ATR accessory with diamond sensor.

Antifungal Activity Assessment

The antifungal activity was evaluated using *Trichophyton interdigitale* fungus. Figure 1 shows the culture of 7-day-old *Trichophyton interdigitale* grown on Sabouraud dextrose agar culture medium and analyzed using an optical microscope. The tests were performed in accordance with ASTM D4576-86:2016 – Standard Test Method for Mold Growth Resistance of Wet Blue and Wet White. In each Petri dish a Dextrose Agar Sabouraud culture medium was poured. Treated samples and control samples were placed in Petri dishes in the center of the surface of the culture medium, and then the culture medium was seeded in 3 points around the sample with *Trichophyton interdigitale* fungus, as an equilateral triangle. Petri dishes were placed in thermo-hygrostat (Memmert) at the temperature of 30°C and were analyzed after 3, 7, 14 and 21 days.

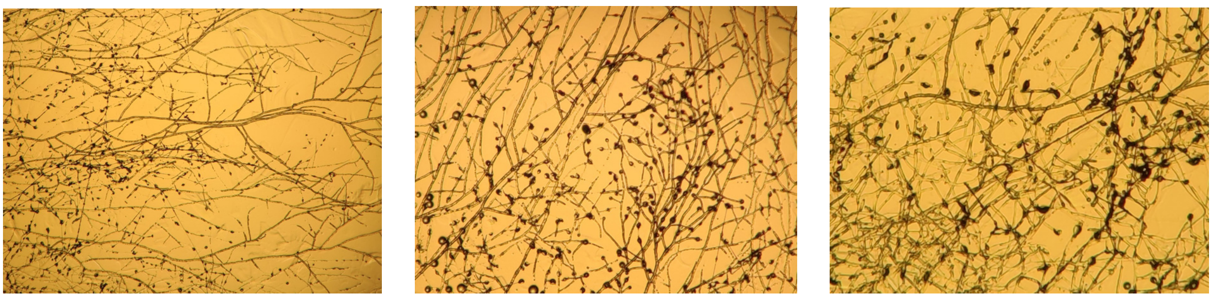


Figure 1. *Trichophyton interdigitale* fungus, optical microscopy images, 40x

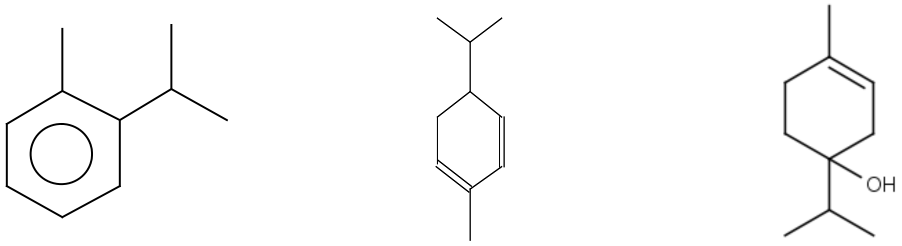
Optical Microscopy Investigation

Optical microscopy images were captured using a Leica stereomicroscope S8AP0 model with optical fiber cold light source, L2, with three levels of intensity, and 40X magnification.

RESULTS AND DISCUSSIONS

GC-MS Analysis of Essential Oils

The major compounds identified in *Anethum graveolens* essential oil were o-cymene with an area percentage of 30.71%, and α-phellandrene with an area percentage of 23.21%; other compounds had lower area percentages [1, 29]. The GC-MS analysis in the case of *Melaleuca alternifolia* essential oil showed that the major compound was terpinene-4-ol with an area percentage of 23.06% [14] (Figure 2). The chemical composition of tea tree essential oil consists largely of cyclic monoterpenes of which about 50% are oxygenated and about 50% are hydrocarbons [20].

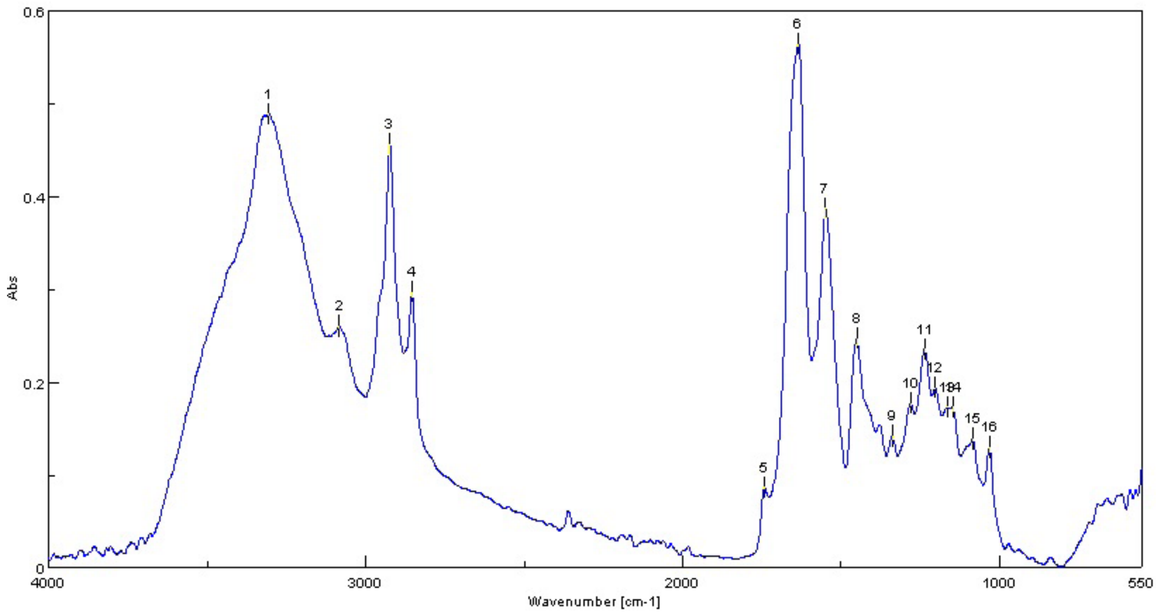


o-cimole α-felandren terpinene-4-ol

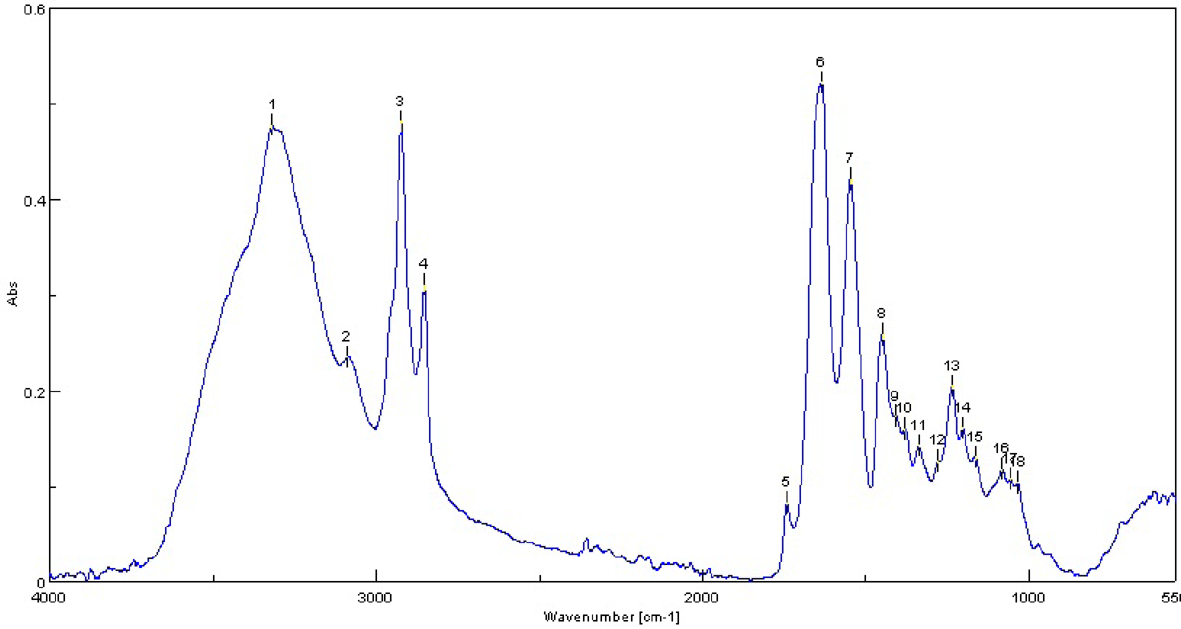
Figure 2. The main components of dill and tea tree essential oils

ATR-FTIR Analysis of Leathers

Mid-infrared spectra for un-treated leather lining - Eco control and leather lining - Cr control and leather samples treated with dill or tea tree essential oil are shown in Figures 3, 4 and 5. According to FTIR spectra from Figure 3, the characteristic bands of sheepskin leather lining - Eco control (a) and sheepskin leather lining - Cr control (b) are observed. Vibration bands at 1449 cm^{-1}, 2853 cm^{-1} and 3086 cm^{-1} are attributed to hydrogen bonds of associated functional groups C-H and those at 1082 cm^{-1} and 3318 cm^{-1} are attributed to hydrogen bonds of associated functional groups C-O [27]. Bands in the range of 3318-3307 cm^{-1} are related to O-H stretching. Peaks in the ranges of 1636-1633 cm^{-1} (–C=C stretch), 1548-1547 cm^{-1} and at 1236 cm^{-1} are attributed to I, II and III amide bands, respectively. Peaks of the C-O band were observed at 1032 cm^{-1} in the case of sheepskin leather lining - Eco control, 1085-1082 cm^{-1} and 1166-1655 cm^{-1} in all samples.



(a)



(b)

Figure 3. ATR-FTIR spectra for (a) sheepskin leather lining - Eco control, and (b) sheepskin leather lining - Cr control

The chemical changes occurring in the functional groups in the leather lining treated with tea tree essential oils are shown in Figure 4 compared to spectra for tea tree essential oil.

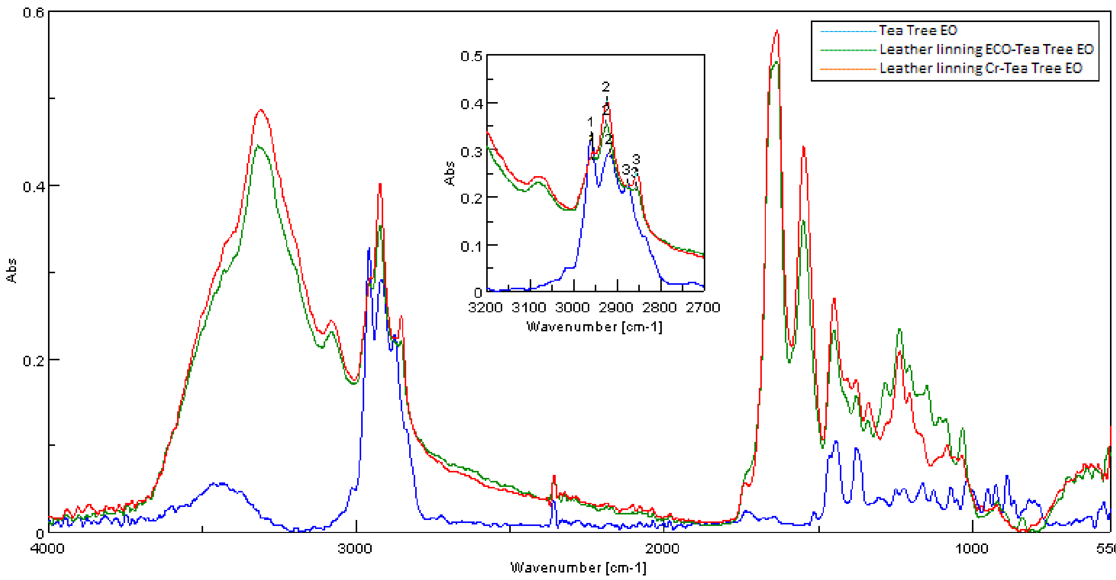


Figure 4. ATR-FTIR spectra of tea tree EO, leather lining - Eco and leather lining - Cr treated with tea tree EO

The treatment of sheepskin lining - Eco and sheepskin lining - Cr, respectively with tea tree essential oil leads to the evidence of absorption bands at 2958-2956 cm^{-1} (C–H stretch), 1449-1448 cm^{-1} (C–H bending) specific for this EO [33]. The absorption band at 2919 cm^{-1} (-CH_2- as well as from the asymmetric -$CH(CH_3)$ stretching vibration [34]) is shifted to 2925-2924 cm^{-1} in the case of treated leather lining samples. The band at 2877 cm^{-1} (-$CH(CH_2)$-, symmetric and asymmetric stretching vibrations) identified at tea tree spectrum [34] is found in the range of 2856-2855 cm^{-1} in the spectra of leather samples treated with tea tree EO. The bands at 3318-3307 cm^{-1}, 1548-1547 cm^{-1} found in control samples are also observed at the treated samples with tea tree essential oil. The band at 1441 cm^{-1} observed in the case of tea tree EO due the -CH_2- and -CH_3 scissoring vibration [34] is shifted in the range of 1449-1449 cm^{-1} for treated leather samples.

Figure 5 shows comparative spectra for dill EO, leather lining - Eco and leather lining - Cr treated with dill essential oil.

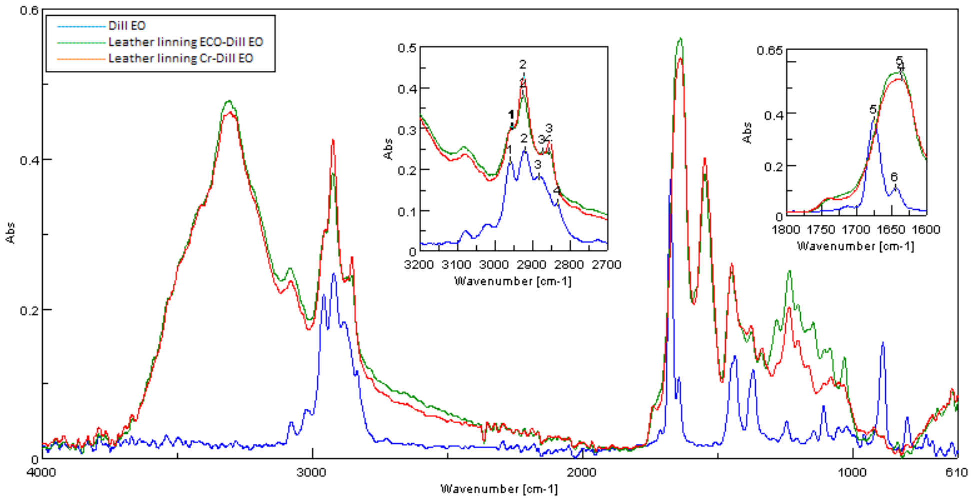


Figure 5. ATR-FTIR spectra of dill EO, leather lining - Eco treated with dill EO and leather lining - Cr treated with dill EO

The FTIR analysis of treated lining leather samples with dill essential oil is accompanied by the shifting of wave number from 2953 cm^{-1} and 2922 cm^{-1} (C–H stretch) assigned to EO to 2957-2955 cm^{-1} and 2925-2924 cm^{-1}. The characteristic band of EO from 2884 cm^{-1} assigned to CH3 stretching is moved to lower wave number (2874 cm^{-1}) in the case of treated sheepskin lining Eco leather.

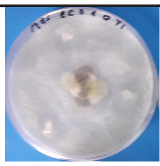
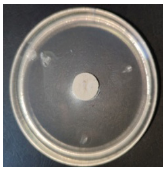
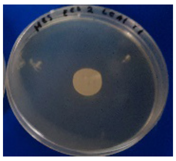
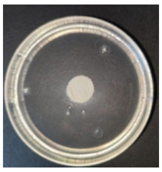
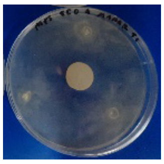
The bands at 3318 cm^{-1} and 3307 cm^{-1} (O-H stretching) from sheepskin lining - Cr spectrum and sheepskin lining - Eco spectrum are found in the treated samples with dill EO. The bands at 1644 cm^{-1} (–C=C stretch) and 1675 cm^{-1} (α-β unsaturated C = O stretch) [33] from dill EO are shifted to 1637-1637 cm^{-1} for treated samples.

The spectra presented in Figures 4-5 confirm the presence of tea tree or dill essential oils in the sheepskin lining samples.

Antifungal Activity against Trichophyton interdigitale

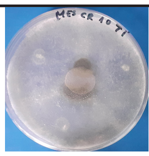
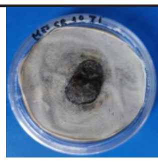
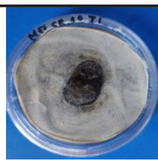
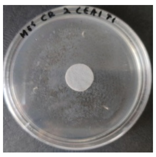
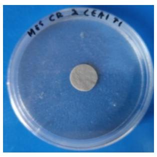
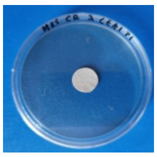
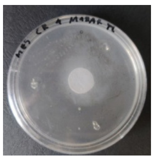
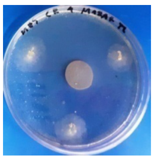
Samples of sheepskin leather lining - Eco control and treated with tea tree and dill essential oil was incubating at 30°C and the antifungal activity towards the grown of *Trichophyton interdigitale* was evaluated after 3, 7, 14, and 21 days (Table 1). Antifungal activity against *Trichophyton interdigitale* was ranked with indicators between 0-5, where 0 means that no microorganisms grew on the sample surface, 1 shows growth between 10-20%, 2 between 30-45%, 3 between 50-75%, 4 between 80-90% and 5 shows 100% growth of microorganisms on the evaluated sample surface. Control sheepskin leather lining - Eco samples show growth of microorganisms after 3 days on 90% of leather surface and it increases at 100% after 7 days. Sheepskin leather lining - Eco samples treated with tea tree essential oil show no growth of microorganisms after 3 days, 7 days and 14 days respectively, remaining without growth of *Trichophyton interdigitale* fungus on leather surface and on culture environment. After 21 days their growth covers 80% of the leather sample surface. Sheepskin leather lining - Eco samples treated with dill essential oil show no growth of microorganisms after 3 days and 7 days, respectively. After 14 days the microorganism growth was 10% on the surface of leather and it did not evolve after 21 days.

Table 1: Sheepskin lining leathers - Eco treated with tea tree and dill essential oil tested against *Trichophyton interdigitale* fungus as compared with control samples

Sample	3 days	7 days	14 days	21 days
Control sheepskin leather lining - Eco				
Grading	4	5	5	5
Sheepskin leather lining - Eco with tea tree EO				
Grading	0	0	0	4
Sheepskin leather lining - Eco with dill EO				
Grading	0	0	1	1

Also, samples of sheepskin leather lining - Cr control and treated with tea tree and dill essential oil, respectively, were incubated at 30°C for 3, 7, 14, and 21 days, respectively. After each test period, the antifungal activity was evaluated against *Trichophyton interdigitale* (Table 2). Control sheepskin leather lining - Eco samples show growth of microorganisms after 3 days on 20% from leather surface and increases at 100% after 7 days. Sheepskin leather lining - Cr samples treated with tea tree essential oil show no growth of microorganisms on the leather surface after 21 days of exposure. Sheepskin leather lining - Cr samples treated with dill essential oil show no growth of microorganisms after 3 days and 7 days, respectively. After 14 days, the microorganism growth was 100% on the surface of leather.

Table 2: Sheepskin lining leathers - Cr treated with tea tree and dill essential oil tested against *Trichophyton interdigitale* fungus as compared with control samples

Sample	3 days	7 days	14 days	21 days
Control sheepskin leather lining - Cr				
Grading	2	5	5	5
Sheepskin leather lining - Cr with tea tree EO				
Grading	0	0	0	0
Sheepskin leather lining - Cr with dill EO				
Grading	0	0	5	5

In the case of treatment of leather - Eco samples with tea tree EO, a good resistance against *Trichophyton interdigitale* microorganism with zero growth after 14 days, and no microorganism growth on leather with Cr sample were recorded throughout the experiment. Tea tree essential oil compounds affected cell membranes in microorganisms and induced cell membrane rupture [23]. Tea tree oil has a broad spectrum of antifungal and antimicrobial activity [35] which can be mainly attributed to the major compound, terpinen-4-ol [36, 37], through an action of penetrating the cell wall and cell membrane structures that stops the development of microorganisms [25].

Leather samples treated with dill essential oil has improved/better antifungal activity in the case of sheepskin leather lining Eco with zero growth of microorganism up to 7 days and an increase up to 10% surface coverage after 14 days. Sheepskin leather lining - Cr has also no growth of *Trichophyton interdigitale* up to 7 days and shows 100% increase after 14 days. In another study, it was reported that the dill seed essential oil (1.00 µL/mL) completely inhibited the growth of *S. sclerotiorum* fungus after 4 days of incubation due to the carvone and limonene content [22]. A completely inhibition of the growth of *Trichophyton interdigitale* species during 28 days on the sheepskin leather for lining finished with synthetic film was observed in the case of treatment with thyme essential oil [4]. The mechanism of the dill essential oil on the fungal inhibition could be explained by the low molecular weight and highly lipophilic compounds (terpene) that disrupt the fungus membrane [38]. In the case of tea tree essential oil, the fungal activity was assigned to the terpene-4-ol that exhibits antimycotic activities by disrupting cell walls, membranes and cytoplasm, which led to compromising fungal life processes [39].

A wide variety of essential oils have antifungal and antimicrobial properties and in many cases this activity is due to the presence of active monoterpenic constituents [40-42]. Several studies have also shown that monoterpenes exert a membrane dam with effects of cell aging and stopping the development of microorganisms [25, 43]. Essential oils used in optimal proportions have selective bioactive properties and no side effects [44]. The antifungal activity of tea tree and dill essential oils against *Trichophyton interdigitale* makes it possible to develop antifungal compositions based on natural compounds for footwear, gloves and other specific products [44].

CONCLUSIONS

The results obtained in this study show that the essential oils of *Anethum graveolens* and *Melaleuca alternifolia* had different antifungal effects against *Trichophyton interdigitale* depending on the tested period and type of leather tanning. The experiments showed that the tea tree essential oil totally inhibited the growth of *Trichophyton interdigitale* fungus on the sheepskin leather lining - Cr up to 21 days of exposure and on the sheepskin leather lining - Eco up to 14 days of exposure. The dill essential oil shown to enhance the growth inhibition of the *Trichophyton interdigitale* fungus up to 7 days. The results are promising for treatment of lining leather with essential oils having a great importance for foot health and hygiene. Also, tea tree and dill essential oils could offer a much safer alternative protection against fungus as compared to synthetic compounds with adverse reactions to body and environment. The results of this study may have potential for use in application development in cosmetics, pharmaceuticals, obtaining leathers and textiles with selective bioproperties.

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