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Antifungal Activity of Finished Chromium Tanned Leather Containing Thyme and Tea Tree Essential Oils

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Abstract

Leather products provide an ideal environment for microbial growth. In order to prevent the appearance and spread of various types of microorganisms, it is treated with selected biocides and even essential oils. The aim of this paper was to investigate the antifungal activity of selected essential oils applied to finished chromium tanned leather which is intended for leather fancy products. Antifungal activity was evaluated according to the PN-EN 14119: 2005 standard. Four finished chromium tanned leathers were tested, each of which was soaked in the following essential oils: tea tree essential oil at a concentration of 1, 2 and 5% and thyme essential oil at concentration of 1, 2 and 5%. The results obtained indicated that leather finishing with thyme essential oil showed the antimicrobial activity against *Aspergillus niger, Chaetomium globosum* and *Candida albicans strains*. The application of 5% thyme essential oil to leather samples inhibited the growth of all strains tested. Using tea tree essential oil on leather samples showed a antimicrobial effect only against *Candida albicans*. The application of essential oils in the leather finishing process may be an alternative to biocides used in the tanning industry.

Keywords

essential oils, antifungal activity, leather, microorganisms.

1. Introduction

Leather products, especially those often and intensively used, are an ideal environment for microbial growth. The leather protection against microbes at different stages of the tanning process does not make it completely resistant to microflora. The availability of various sources of nutrients stimulates spores to germinate, and then supports the growth of hyphae [1]. Also, a sufficiently high temperature and moisture content enhances bacterial and fungal colonisation by different microbes, such as *Bacillus, Corynebacterium, Clostridium, Staphylococcus, Penicillium, Aspergillus, Paecilomyces, Candida* and *Cryptococcus*. These microorganisms quickly colonise on the surface, as well as on clothing and footwear. Most of them are not dangerous to human health but can be responsible for the destruction of materials from which the inner parts of the products are made. The mechanism of the deterioration of leather and leather products by microbes is primarily related to enzymatic decomposition of the material. This leads to leather embrittlement, loss of elasticity, a decrease in collagen molecular weight, an unpleasant smell being felt, and spots appearing on its surface [2]. Discolorations and spotting on leather resulting from the presence of destructive microorganisms can be grey-white, violet, red, grey or even black [3, 4].

Finished leather, which is intended for the production of different leather products, should be characterised by increased microbiological resistance, consisting in a reduction in colonising microorganisms during storage and use. In order to prevent the appearance and development of various types of microorganisms and to prevent damage to leather, it is treated with certain biocides. Antimicrobial agents (biocides) used in the leather industry can be classified as quaternary ammonium compounds, isothiazoles, halogenated organic compounds, such as 2-bromo-2-nitro-propane-1,3-diol, isothiazoles and halogenated organic compounds containing heterocycles, such as derivatives of benzothiazole [1]. Unfortunately, biocides currently used in the leather industry are generally harmful to human health and the environment, and their use has been or shall be restricted or even banned. It affects consumers who are in contact with the products, penetrating through the clothes onto the consumer's skin, causing painful skin, dermatitis, including itching, irritation and redness. Therefore, the use of biocides for the protection of materials should be controlled within the scope of the dose applied and intended use of the final product [1]. Changes in consumers' preferences toward more natural products than synthetic ones stimulate also the use of natural products in the leather tanning process. In addition, numerous recent studies have reported the biological activity of essential oils: they exhibit antibacterial, fungicidal, antioxidant, and antiradical activities [5].

Essential oils are aroma products extracted from plants mainly by steam distillation. In terms of chemistry, volatile oils are complex mixtures of aliphatic and aromatic hydrocarbons, aldehydes, alcohols, esters and other constituents [6]. Depending on the type and amounts of compounds contained, essential oils show various properties, e.g. they inhibit the growth of infectious microbes, prevent fungal growth, accelerate wound healing and mitigate inflammations. Essential oils are widely used as components of drugs, biologically active

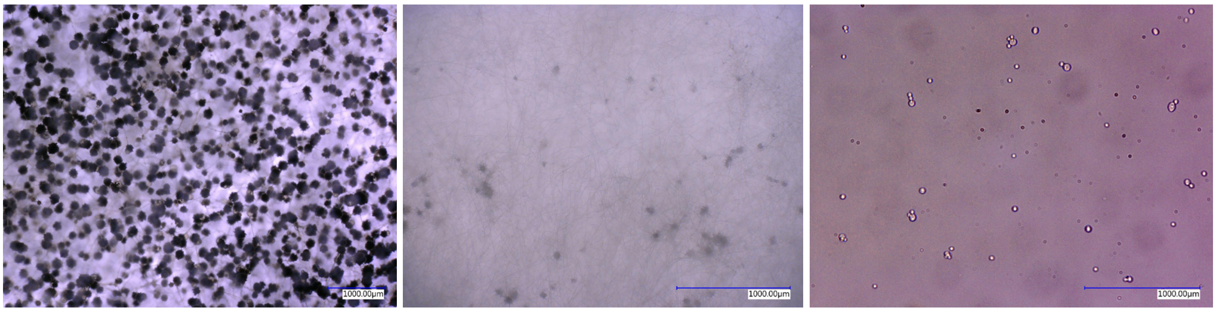

Fig. 1. Example photo of the microorganisms tested: Aspergillus niger, Chaetomiuum globosum, Candida albicans (KEYENCE VHX 95OF)

additives and dietary supplements, as well as in aromatherapy and the food and cosmetics industries [5, 7]. In addition, there have been attempts worldwide to use essential oils as natural bioactive substances for protecting leathers in the tanning industry [1, 2, 5]. Širvaitytė et al. (2012) investigated the possibility of using essential oils of *Thymus vulgaris* as an alternative preservative for chromium tanned leather. During tests it was found that the antibacterial activity of essential oils of thyme depends on their chemical composition, mainly on the phenolic compounds. The most important phenolic compounds in thyme oil are thymol and carvacrol. The authors of this study found that if essential oils are used as a preservative for chromium leather, their amount should not be less than 3% of the wet-blue mass. Smaller amounts (0.05% and 1.0%) showed no inhibition zone, although leather samples remained resistant to the bacteria selected [5]. Also, the results of Bayramoĝlu et al.'s (2006) study showed that three essential oils of oregano had much stronger bactericidal activity than that of commercial bactericide and fennel oil. These findings suggested that oregano essential oils could be used as bactericidal agents in the leather industry [2]. Falkiewicz-Dulik observed that tea tree oil preparation showed a good antibacterial effect with regard to *S. aureus* and *E. coli*, as well as biocidal activity against yeast-like fungi *C. albicans* and filamentous fungi with large inhibition zones of mould growth *A. brasiliensis* and dermatophyte *T. mentagrophytes* [1].

The aim of this work was to investigate the antifungal activity of thyme and tea tree oils applied to finished chromium leather intended for leather products, especially for leather accessories. The work included the selection of essential oils, determination of doses, the application of selected preparation to different leathers, and verification of antifungal activity.

2. Materials and methods

2.1. Essential oils and oil components

Two ready-made commercial essential oils (tea tree and thyme oils) were selected for the tests. Tea tree and thyme essential oils were obtained from CHDL SA (INDIA). They are 100% pure essential oils extracted by steam distillation, intended for the production of perfumes. Thyme oil is a colorless, reddish or yellow liquid with a strong aromatic thyme-like odour. The main ingredients of the oil are thymol (up to 55%), cymene (15–28%), linalool (4–6.5%) and carvacrol (1–4%). Tea tree essential oil is also a complex mixture of hydrocarbons and terpenes, consisting of approximately 100 components. All other chemicals were obtained from Pol-aura (Poland) unless otherwise specified. The following concentrations of essential oils were selected for the research: 1, 2 and 5%. These are the minimum concentrations ensuring acceptable organoleptic characteristics of the leather tested.

2.2. Leather samples protected with essential oils

Four types of finished chromium tanned leather were selected for the tests: I - black grain cowhide with a thickness of 1.30 mm, II - black grain cowhide with a thickness of 2.30 mm, III - brown full-grain cowhide with a thickness of 1.65 mm, and IV - purple cow nubuck with a thickness of 2.10 mm. Each of the four leathers tested was soaked in essential oils: tea tree oil at concentrations of 1, 2 and 5%, and thyme oil at concentrations of 1, 2 and 5%. The symbols of samples treated with thyme and tea tree essential oils are presented in ***Table 1***. The markings of the leather samples are described according to the following scheme: I, II, III, IV- leather number, 1, 2, 5 - concentration of the essential oil used, t and h - type of oil used (t- thyme oil, h-tea tree oil), and KON- leather sample without antimicrobial treatment. The essential oil solutions were applied to the leather with a sponge roller. Before the test, the leathers prepared in this way were air-dried and air-conditioned for at least 24 h according to the PN-EN ISO 2419:2012 standard under standard climatic conditions: relative humidity (50±5%) and temperature (23±2°C).

2.3. Microorganisms

The effectiveness of the essential oils selected (tea tree and thyme essential oils) was tested for three selected fungal strains: *Aspergillus niger* (ATCC 16404), *Chaetomiuum globosum* (ŁOCK 0476) and *Candida albicans* (ATCC 10231) (***Figure 1***). An antifungal activity test carried out in accordance with the PN-EN 14119:2005 standard. *Testing of textiles - Evaluation of the action of microfungi. Method B2* was applied [8]. These moulds came from the Pure Culture Collection at the Institute of Fermentation Technology and Microbiology LOCK (Lodz University of Technology, Poland).

Description of leather samples tested	Code	Leather number
Reference leather without an antimicrobial finish	I KON	I
Leather treated with 1% thyme essential oil	I 1t	I
Leather treated with 2% thyme essential oil	I 2t	I
Leather treated with 5% thyme essential oil	I 5t	I
Leather treated with 1% tea tree essential oil	I 1h	I
Leather treated with 2% tea tree essential oil	I 2h	I
Leather treated with 5% tea tree essential oil	I 5h	I
Reference leather without an antimicrobial finish	II KON	II
Leather treated with 1% thyme essential oil	II 1t	II
Leather treated with 2% thyme essential oil	II 2t	II
Leather treated with 5% thyme essential oil	II 5t	II
Leather treated with 1% tea tree essential oil	II 1h	II
Leather treated with 2% tea tree essential oil	II 2h	II
Leather treated with 5% tea tree essential oil	II 5h	II
Reference leather without an antimicrobial finish	III KON	III
Leather treated with 1% thyme essential oil	III 1t	III
Leather treated with 2% thyme essential oil	III 2t	III
Leather treated with 5% thyme essential oil	III 5t	III
Leather treated with 1% tea tree essential oil	III 1h	III
Leather treated with 2% tea tree essential oil	III 2h	III
Leather treated with 5% tea tree essential oil	III 5h	III
Reference leather without an antimicrobial finish	IV KON	IV
Leather treated with 1% thyme essential oil	IV 1t	IV
Leather treated with 2% thyme essential oil	IV 2t	IV
Leather treated with 5% thyme essential oil	IV 5t	IV
Leather treated with 1% tea tree essential oil	IV 1h	IV
Leather treated with 2% tea tree essential oil	IV 2h	IV
Leather treated with 5% tea tree essential oil	IV 5h	IV

Table 1. Description of leather samples tested

Growth [-]	Evaluation of fungal growth in agar medium
0	No visible growth assessed under the microscope (magnification 50x),
1	No visible growth without magnifying devices, clearly visible under the microscope.
2	Visible increase without magnifying devices, covering up to 25% of the examination surface.
3	Visible increase without magnifying devices, covering up to 50% of the examination surface.
4	Significant increase, covering more than 50% of the surface tested.
5	Heavy growth, covering the surface tested.

Table 2. Grading scale for visible antifungal effects

2.4. Assessment of antifungal activity

Evaluation of resistance to fungi and determination of the antifungal effect were conducted according to the PN-EN 14119:2005 standard [8]. Rectangles measuring 2.5 cm x 8 cm were cut from each leather sample and then trimmed to a width of 2 cm. The tests were carried out in triplicate for each trial. The working specimens were exposed to the standard fungal test spore mixture on a complete agar medium. Test samples prepared in this way were incubated for 2 weeks. During and after the cultivation, the plates were observed with the unaided eye and under a stereoscopic microscope. The fungal growth was evaluated on the basis of observations, according to the scale described in ***Table 2*** [8].

3. Results and discussion

Results of the microbiological tests of the leathers selected are presented in ***Tables 3-6***. *Aspergillus niger* ATCC 16404, *Chaetomium globosum* ŁOCK 0476 and *Candida albicans* ATCC 10231 growth was observed as white mycelium on the surface of the agar medium. In the case of leather I, a relationship was observed where the higher the concentration of thyme oil, the stronger the antifungal activity (***Table 3***). The results obtained indicate that the I 5t sample treated with thyme oil shows strong antimicrobial activity against strains *A. niger, Ch. globosum* and *C. albicans.* No visible growth was observed (magnification 50x) under the microscope, which means 0 for all strains tested. On the other hand, samples I 1h, I 2h and I 5h, treated with tea tree oil, had no inhibition effect against the strains. In this case, growth over the entire surface of all samples was observed. Therefore, the fungus growth on the all samples was rated at 5, which means heavy growth, covering the surface tested. For leather specimens finished without the use of essential oils (KON I), strong growth of all microbes tested around and on the leather was observed.

Very similar results were obtained for leather II (***Table 4***). In the case of thyme oil, the fungus growth was limited when a higher oil concentration was used. Microbial growth for II 5t was rated at 4, 2 and 0 for *A. niger, Ch. Globosum* and *C. albicans*, respectively. It means that no growth of *Candida albicans* was observed on the leather finished with thyme oil at a concentration of 5%. The use of tea tree oil again had no effect on microbial growth. The growth of the strains tested on leather II was rated at 5 in most cases, which means heavy growth, covering the surface tested. Results of the application of essential oils to leather IV are presented in ***Table 6***.

For the samples finished with thyme oil, the following relationship was obtained: the higher the concentration of thyme oil, the stronger the antifungal activity against the strain tested. In the case of *A. niger*, the growth was rated at 5, 4, 0 for thyme oil concentrations of 1%, 2% and 5%, respectively. The growth of *Ch. globosum* was rated at 2, 0, 0 for thyme oil concentrations of 1%, 2% and 5%, respectively. No *C. albicans* growth was observed on the test leather treated with thyme oil. Tea tree oil at concentrations of 2% and 5% showed effectiveness against *C. albicans* yeast only. The lower antifungal effect of tea tree oil has again been proven.

Microorganisms	Evaluation of fungal growth in agar medium [-]						
	Leather unfinished/finished with essential oils						
	I KON	I 1t	I 2t	I 5t	I 1h	I 2h	I 5h
Aspergillus niger	5	5	5	0	5	5	5
Chaetomium globosum	5	5	3	0	5	5	5
Candida albicans	5	5	2	0	5	5	5

Table 3. Evaluation of the antifungal activity of leather samples I against strains tested

Microorganisms	Evaluation of fungal growth in agar medium [-]						
	Leather unfinished/finished with essential oils						
	II KON	II 1t	II 2t	II 5t	II 1h	II 2h	II 5h
Aspergillus niger	5	5	5	4	5	5	5
Chaetomium globosum	5	4	4	2	5	5	5
Candida albicans	5	4	4	0	2	5	5

Table 4. Evaluation of the antifungal activity of leather samples II against strains tested

Microorganisms	Evaluation of fungal growth in agar medium [-]						
	Leather unfinished/finished with essential oils						
	III KON	III 1t	III 2t	III 5t	III 1h	III 2h	III 5h
Aspergillus niger	5	2	0	0	5	5	4
Chaetomium globosum	2	0	0	0	2	2	2
Candida albicans	0	0	0	0	0	0	0

Table 5. Evaluation of the antifungal activity of leather samples III against strains tested

The results for leather III were slightly different (***Table 5***). No growth of *C. albicans* was observed on any of the samples tested. The application of 2% and 5% thyme oil to the leather completely inhibited the growth of all the strains tested. Exceptionally, in this case, tea tree oil showed antifungal activity. At each concentration of this essential oil, the growth of *Ch. globosum* was rated 2, while the growth of *C. albicans* was 0. Interestingly, the III KON sample also showed antifungal activity against two strains of fungi. In this case, the growth of *Ch. globosum* was rated 2, while that of *C. albicans* was 0. This proves that leather III without antimicrobial finishing most likely had antimicrobial properties. This may be due to elevated chromium values or the presence of dye that is toxic to microorganisms. According to other studies [9, 10], some dyes, e.g. natural ones, show antimicrobial activities. Also, metal complex formazan dye demonstrated a significant antimicrobial propensity in solution and on leather by exhibiting a percentage reduction in bacterial growth [11]. Moreover, the presence of chromium in tanned leather may have an effect on antimicrobial properties. Taking that into account, the composition of leather III should be carefully examined in further studies. However, at this moment it can be concluded that *Aspergillus niger* is a very durable and resistant fungus to various physico-chemical and biological factors. Thanks to these properties, even with the possible presence of toxic substances in leather III, the growth of *A. niger* was rated 5. In turn, *Candida albicans* is a microorganism characterised by a relatively low resistance to toxic components. Therefore, in each case described in this work, *C. albicans* was the most sensitive strain among the microorganisms tested, which was especially visible in the case of leather III (***Table 5***).

According to the results obtained in the present work, thyme essential oil has decidedly stronger antifungal activity than tea tree essential oil. Moreover, the increased antimicrobial activity of thyme essential oil was observed with an increase in the concentration of this oil applied to the leather. The leather treated with 5% thyme essential oils inhibited the growth of all strains tested: *A. niger, Ch. Globosum* and *C. albicans.* The strong properties of thyme oil were also confirmed in other research [12, 13]. The study of Chirila et al. (2017) showed that *Thymus vulgaris* oil exhibited high antifungal activity against *Candida albicans* and *Aspergillus niger*. Thyme oil could be the best inhibitor of fungi due to the high thymol and carvacrol content [12]. In addition, results obtained in our work showed that tea tree oil was effective only against *C. albicans* and only when it was applied to leather samples III and IV. The antifungal activity of tea tree oil against *Candida* sp. was confirmed in another study [14]. However, leather III without an antimicrobial finish also showed some antimicrobial properties, which should be analysed in further research. In conclusion, the use of thyme essential oil at a suitable concentration can effectively prevent the growth of fungi on leather materials.

Microorganisms	Evaluation of fungal growth in agar medium [-]						
	Leather unfinished/finished with essential oils						
	IV KON	IV 1t	IV 2t	IV 5t	IV 1h	IV 2h	IV 5h
Aspergillus niger	5	5	4	0	5	5	5
Chaetomium globosum	5	2	0	0	5	5	5
Candida albicans	5	0	0	0	5	0	0

Table 6. Evaluation of the antifungal activity of leather samples IV against strains tested

4. Conclusions

- As the concentration of thyme essential oil applied to the leather was increased, the antimicrobial activity of this essential oil also increased. The application of 5% thyme essential oil to leather samples inhibited the growth of all strains tested.
- No fungistatic effect against *Aspergillus niger* ATCC 6275 and *Chaetomium globosum* ŁOCK 0476 was observed for leather with tea tree essential oil finishing. However, it slightly inhibited the growth of the yeast *Candida albicans* ATCC 10231.
- The III KON sample without antimicrobial finishing showed antifungal activity. It may be caused by the presence of additional substances in the leather, e.g. dyes or chromium, which may be toxic to microorganisms.
- The use of a natural bioactive substance such as thyme essential oil for the modification of leather in the leather finishing process may be an alternative to biocides used in the tanning industry, as well as which it can improve the hygienic properties of leather products.

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