An Examination of Antimicrobial Activity of Lining Leathers Fatliquored With Essential Oils

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Abstract

To prevent losses caused by microbiological decay of leathers during technological processing, biocidal and biostatic chemical agents are used. They are introduced into the material structure at various stages of the tanning process. It has been shown that some of these agents are not neutral to human health and to the environment. Thus, substitutes for chemical preparations of appropriate efficiency in control of bacteria and fungi, but being safe to humans and the environment are sought. Suitability has been adequately confirmed by research performed worldwide on essential oils.

In this paper antimicrobial resistance of lining leathers protected with essential oils extracted from plants *Cinnamomum verum, Eucalyptus globulus, Origanum vulgare, Leptospermum scoparium* and *Thymus vulgaris* is investigated. They were introduced into the leather at the fatliquoring stage at 5% per weight of leather. Antimicrobial activity of samples oiled with essential oils and controls (without these oils) was evaluated against bacteria *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and yeasts *Candida albicans* after 1 and 6 months. The growth inhibition zone around leather samples and microorganism growth, or lack of growth, within the sample-medium contact zone was determined. Investigations have confirmed the efficiency of three of five proposed oils: oregano, cinnamon and thyme. The strongest and most long-lasting antimicrobial activity was observed for leathers preserved with oregano oil. Insufficient effect was found for leathers oiled with manuka and eucalyptus oils.

Introduction

Hide - animal skin used as a raw material in the tanning industry - is a culture medium for microorganisms, i.e. bacteria and fungi. Activity of these microorganisms can affect both raw pelts during the tanning processes and storage as well as leather products during use (e.g. footwear).[1] The presence of leather decomposing microorganisms at each stage mentioned above is connected with material loss. In addition some fungal species found on leathers may also have harmful effects on human skin and cause diseases; this is especially important for leather products being in direct/close contact with the human body.

To protect leathers against microbiological decay during processing, biocides, i.e. chemical agents of antimicrobial activity, are used. In recent years it has been shown that some of these substances are harmful to human health and the environment and therefore they are completely withdrawn from use or their use is significantly restricted.[2,3,4] In addition, a number of biocides currently available do not provide long-lasting protection against microorganisms activity.[5] When seeking human and environmentally friendly biocide substitutes, attention has been drawn to essential oils having a number of valuable features (for example, antiseptic, antifungal and antioxidant activities[6]) and they are used in various industries: food, pharmaceutical and cosmetic.[7-11] Scientific research to evaluate antimicrobial activity of essential oils introduced into leather at different tanning stages has been conducted in Turkey and Lithuania. Oils were used during the soaking process (oil extracted from *Origanum onites* and other species of the genus

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Origanum, oil from the species *Foeniculum vulgare*),[12] pickling (oils from *Origanum minutiflorum, Laurus nobilis*, *Foeniculum vulgare* and *Schinus molle*),[13,14] tanning (oil from *Origanum minutiflorum*)[14] and fatliquoring (oils from *Eucaliptus globulus* and *Lavsonia officinalis*).[15] In experiments various oil concentrations were used and its effect on many bacterial and fungal species causing leather deterioration was investigated. When considering the possibility of using antimicrobial essential oil properties to protect raw materials in the tanning industry and in finished products, scent preferences seem to be an important aspect. The experiments carried out by the authors for selected essential oils have indicated that both women and men show the highest preference to leather specimens subjected to fatliquoring with an addition of oils from *Origanum vulgare* and *Cinnamomum verum*, while the least preferred one was that treated with oil from *Leptospermum scoparium*.[16]

Essential oils are volatile substances of plant origin. They have an intensive smell and are generated during secondary metabolism in plants. They are derived, for example from leaves, flowers, bark and roots. Those derived from different parts of the same plant may have different odors and features. Essential oils perform many substantial functions in the world of plants, for example they may scare insects away or protect plants against harmful effects of fungi and bacteria. Some of them attract animals that pollinate their host plants. Extracting essential oils is a complicated and expensive process. There are several methods to derive them; they include for example steam distillation, extraction, expression, and enfleurage.[6,17,18]

In this paper the results of examination aimed at the determination of antimicrobial activity of lining leathers oiled with five selected essential oils: cinnamon, eucalyptus, oregano, manuka and thyme against the three reference bacterial species (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa)* and one fungal species (*Candida albicans*) as microorganisms found on leathers.[15,19,20]

Experimental

Materials

Leather

Cowhides with black and white hair coverage and 12-16 kg in weight were used. These raw leathers were subjected to standard chrome tanning processes. Wet blue hides were split to a thickness of 1.4-1.6 mm. Hides for testing purposes were prepared at the company PPHU "TECHNO SKÓR" (Poland).

Essential Oils

In the experiment antimicrobial effects of essential oils derived from the following plant species were analyzed: *Cinnamomum verum* (cinnamon oil), *Eucalyptus globulus* (eucalyptus oil), *Origanum vulgare* (oregano oil), *Leptospermum scoparium* (manuka oil) and *Thymus vulgaris* (thyme oil). Oils for testing purposes were obtained at the Laboratorium Biologii Przemysłowej i Eksperymentalnej PWSZ im. St. Pigonia w Krośnie (Poland) – *Industrial and Experimental Biology Laboratory at PWSZ in Krosno.*

Methods

Preparing Leather for Testing

From the purchased "wet-blue" leather 18 rectangular pieces of 15x25 cm in size were cut. Three weighed leather pieces were placed in Wacker drum device. The following operations were carried out consecutively:

1. soaking,
2. retanning I,
3. rinsing,
4. retanning II,
5. fixation,
6. rinsing.

During the retanning II process the leather was fatliquored with standard fats with the addition of individual essential oils: cinnamon, eucalyptus, oregano, manuka and thyme at amount of 5% based on leather weight. According to the above scheme leather was also prepared by fatliquoring without essential oils (control sample).

After the cycle lasting about 8 hours the leathers were allowed to drain by hanging for 24 hours. Then, the leathers were laid down for 10 days to remove excess moisture. Afterwards, the test leathers were placed and stored in paper envelopes.

Microbiological Examination

Antimicrobial effect of lining leathers finished with essential oils derived from *Cinnamomum verum*, *Eucalyptus globulus*, *Origanum vulgare, Leptospermum scoparium* and *Thymus vulgaris* was examined according to the test standard Textile & fabrics – Determination of antibacterial activity – Agar diffusion plate test (ISO 20645:2006),[21] The following two bacterial species suggested by the standard were used during testing:

- *Staphylococcus aureus* (ATCC 25923) (Gram-positive),
- *Escherichia coli* (ATCC 25922) (Gram-negative),

and additionally the bacterial species*:*

- *Pseudomonas aeruginosa* (ATCC 27853) (Gram-negative),

and one species of fungus:

- *Candida albicans* (ATCC 10231).

These organisms have been selected due to their harmful activity on animal leathers or potential pathogenic effect on humans.[15,19]

From the prepared rectangular leather pieces circular test specimens of 25±5mm in diameter were cut. For leathers fatliquored by using essential oils for each oil type the following numbers of specimens were prepared:

- 12 – with 3 repetitions for each microorganism species,
- 1 – microbiological condition (contamination) of leather fatliquored with essential oil.

Analogously the specimens of leathers fatliquored by using no essential oils were prepared. The following media were used during testing:

- Tryptic Soy Agar TSA (for bacterial culture),
- Sabouraud-4% glucose agar SGA (for fungal culture).

From 24-hour bacterial cultures the suspensions of reference strain were prepared in sterile 0.9% NaCl saline and brought to 0.5 McFarland optical density (1.5×10^8 cfu*/ml) based on densitometric measurements. From yeast culture, suspensions of 1 McFarland optical density (3.0×10^8 cfu/ml) were prepared. These suspensions were used at an amount of 0.1 ml to inoculate Petri dishes. The fatliquored leather discs were placed centrally in Petri dishes with the grain side facing upward.

Microbiological contamination may also occur in the manufacturing process. To determine if the leathers are contaminated with microorganisms able to deteriorate leathers or create a hazard to users, specimen's fatliquored with essential oils were also placed in sterile media (TSA or SGA).

Cultures were incubated at 37±1°C for 18-24 hours for bacteria, and 48 hours for yeasts *Candida albicans*. After incubation the presence of the zone of microbial growth inhibition surrounding the specimen and the occurrence of microbial growth on the agar and under the test specimen were evaluated. The same procedure was also used to test the control sample (i.e. leather fatliquored without essential oils).

TABLE I
Antibacterial effect of the antibacterial treatment

Inhibition zone (mm) Mean value	Growth[a)]	Description	Assessment
>1	none	inhibition zone exceeding 1mm, no growth[b)]	good effect
1-0	none	inhibition zone up to 1 mm, no growth[b)]	
0	none	no inhibition zone, no growth[c)]	
0	slight	no inhibition zone, only some restricted colonies, growth nearly totally suppressed[d)]	limit of efficacy
0	moderate	no inhibition zone, compare to the control growth reduced by half[e)]	insufficient effect
0	heavy	no inhibition zone, compared to the control no growth reduction or only slightly reduced growth	

[a)]The growth of bacteria in the nutrient medium under the specimen.
[b)]The extent of the inhibition shall only partly be taken into account. A large inhibition zone may indicate certain reserves of active substances or a weak fixation of a product on the substrate.
[c)]The absence of bacterial growth, even without inhibition zone, may be regarded as a good effect, as the formation of such inhibition zone may have been prevented by a low diffusibility of the active substance.
[d)]"As good as no growth" indicates the limits of efficacy.
[e)]Reduced density of bacterial growth means either the number of colonies or the colony diameter.

The ability of leathers finished with essential oils to resist microorganism growth was examined after one month and six months after fatliquoring. Antimicrobial activity of oiled leathers was assessed by using a rating scale set forth in ISO 20645:2006 (Table I).

Results and Discussion

Tables II and III present the results of research on antimicrobial activity of leathers fatliquored without or with an essential oil at amount of 5% by weight after 1- and 6-month storage. For specimens prepared without using essential oils no typical growth inhibition zone around leather was observed after 1- and 6-month storage for each of microbes under investigation. After 1-month storage under the specimen a moderate effect on the growth of bacteria *Escherichia coli*, and a weak effect on *Staphylococcus aureus, Pseudomonas aeruginosa* and the fungus *Candida albicans*. This effect was similar after 6-month storage, but differences in growth intensity under specimens may result from close adhesion of the leather to the substrate surface, thus reducing its growth.

TABLE II
Evaluation of microbial growth/antimicrobial effect of leathers fatliquored without and with oil with essential oils added (at concentration of 5% per leather weight) after 1-month storage

Microorganisms	Growth inhibition zones around specimens [mm] Evaluation of microbial growth on the scale					
	leather fatliquored					
	without oils	by using				
		oregano oil	thyme oil	cinnamon oil	eucalyptus oil	manuka oil
Staphylococcus aureus	0	9	3	3-4	-[1]	2
	0 poor growth under the specimen	>1 no growth under the specimen	>1 no growth under the specimen	>1 no growth under the specimen	0[2] no growth under the specimen	>1 no growth under the specimen
Escherichia coli	0	8	2	2	-[1]	-[1]
	0 moderate growth under the specimen	>1 no growth under the specimen	>1 no growth under the specimen	>1 no growth under the specimen	0[2] no growth under the specimen	0[2] no growth under the specimen
Pseudomonas aeruginosa	0	0	0	0	-[1]	-[1]
	0 poor growth under the specimen	0 moderate growth under the specimen	0 poor growth under the specimen	0 moderate growth under the specimen	0[2] no growth under the specimen	0[2] no growth under the specimen
Candida albicans	0	14	6	9	-[1]	-[1]
	0 poor growth under the specimen	>1 no growth under the specimen	>1 no growth under the specimen	>1 no growth under the specimen	0[2] no growth under the specimen	0[2] no growth under the specimen

[1]lack of growth inhibition zone
[2]strong specimen adhesion

The highest antimicrobial activity against microbes under examination was found for leathers treated with the addition of oregano oil. After 1-month storage the growth inhibition zones of size ranging from about 8-9 mm (for *Escherichia coli, Staphylococcus aureus*) up to 14 mm (for *Candida albicans*) and no growth under specimens was observed. In the six-month experiment the growth inhibition zone for *Staphylococcus aureus* was smaller (4 mm), but this is still considered as good antibacterial effect. Slightly weaker activity against *Candida albicans* (12-14 mm) was observed (Figure 1).

The size of the growth inhibition zone for dishes containing *Escherichia coli* after 6 months was almost identical to that of after one-month storage. Both for the mentioned oregano oil and other oils, for which better antimicrobial effect was observed after longer storage. The results obtained may be explained by unequal specimen adhesion to the medium due thickness and stiffness; this is included into the error of the method. No antimicrobial effect against *Pseudomonas aeruginosa* was observed for leathers with oregano oil both after 1- and 6-month storage (lack of the growth inhibition zone around the specimen

TABLE III

Evaluation of microbial growth/antimicrobial effect of leathers fatliquored without oil and with essential oils added (at concentration of 5% per leather weight) after 6-month storage

Microorganisms	Growth inhibition zones around specimens [mm] Evaluation of microbial growth on the scale					
	Leather fatliquored					
	without oils	by using				
		oregano oil	thyme oil	cinnamon oil	eucalyptus oil	manuka oil
Staphylococcus aureus	0	4	0-1	2	-[1]	-
	0[2] poor growth under the specimen	>1 no growth under the specimen	0-1 no growth under the specimen	>1 no growth under the specimen	0[2] no growth under the specimen	0 no growth under the specimen
Escherichia coli	0	9	3	6	-[1]	-[1]
	0 moderate growth under the specimen	>1 no growth under the specimen	>1 no growth under the specimen	>1 no growth under the specimen	0[2] no growth under the specimen	0[2] no growth under the specimen
Pseudomonas aeruginosa	0	0	0	0	-[1]	-[1]
	0 poor growth under the specimen	0 moderate growth under the specimen	0 poor growth under the specimen	0 moderate growth under the specimen	0[2] no growth under the specimen	0[2] no growth under the specimen
Candida albicans	0	12-14	5	11	-[1]	-[1]
	0 poor growth under the specimen	>1 no growth under the specimen	>1 no growth under the specimen	>1 no growth under the specimen	0[2] no growth under the specimen	0[2] no growth under the specimen

[1] lack of growth inhibition zone
[2] strong specimen adhesion

and the average growth). This Gram-negative bacteria causes blue-black skin lesion (ecthyma gangrenosum) and is especially resistant to any agents that inhibit its growth.[22]

The growth of bacteria *Staphylococcus aureus*, *Escherichia coli* and the fungus *Candida albicans* was inhibited due to thyme oil used during the leather fatliquoring process. For these microorganisms the growth inhibition zone of 3 mm, 2 mm and 6 mm, respectively was observed and no microbe growth under specimens was recorded after one-month storage. The experiment repeated after six months indicated a slightly lowered efficiency in inhibiting the growth of *Staphylococcus aureus* and *Candida albicans* (Figure 2). There were comparable effects of antimicrobial activity against bacteria *Escherichia coli* after one- and six-month storage. No growth was observed under specimens. Thyme oil proved to be insufficient for leather protection against *Pseudomonas aeruginosa*. A weak bacterial growth under leather specimens in shorter and longer periods of time was visible.

The addition of cinnamon oil during the fatliquoring process allowed a good antimicrobial effect to be achieved for lining leathers. The growth inhibition of 9 mm in size was observed for the fungus *Candida albicans* after one-month storage, while this zone extended by 2 mm and reached 11 mm after six months (Figure 3). Its weaker activity against *Staphylococcus aureus* (the growth inhibition zone of 3-4 mm after one month and 2 mm after six months) was observed. For *Escherichia coli* antimicrobial effect was intensified and the growth inhibition zone increased from 2 mm after one month to 6 mm after six month-storage.

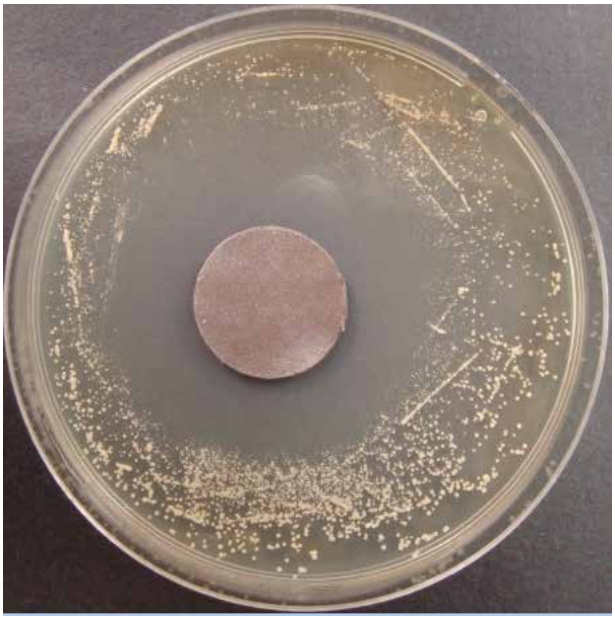

Figure 1. Leather fatliquored with oregano oil, view from the grain side - growth inhibition zone for the yeast Candida albicans (after 6-month storage).

No microorganism growth under specimen disks was observed. The leathers protected with cinnamon oil were not resistant to *Pseudomonas aeruginosa*. During this study a moderate bacterial growth under specimens was recorded after one month and six months after fatliquoring. The leathers protected with oregano, cinnamon and thyme oils showed a significant resistance to microbial activity (except for *Pseudomonas aeruginosa*).

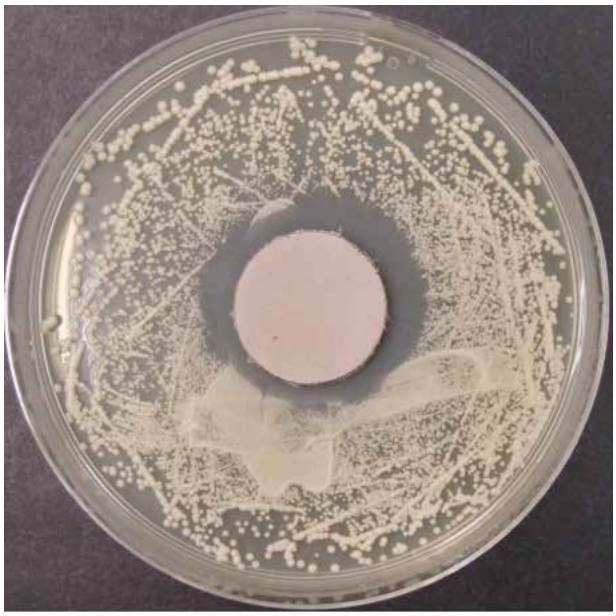

Figure 2. Leather fatliquored with thyme oil, view from the grain side - growth inhibition zone for the yeast Candida albicans. (after 6-month storage).

Figure 3. Leather fatliquored with cinnamon oil, view from the grain side - growth inhibition zone for the yeast Candida albicans. (after 6-month storage).

The results obtained for eucalyptus oil are hard to interpret. In the one-month examination no growth inhibition zone around leather specimens was observed. In dishes with *Staphylococcus aureus* no bacterial growth under specimens was observed. It can be explained as a good inhibition effect for oil that contains the active compounds with low diffusibility. For *Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* no growth under specimen was also visible, while strong growth was recorded at spots where specimens were poorly adhered to the substrate. Thus, it might be assumed that the lack of growth resulting from strong adhesion of the sample to the medium rather than antimicrobial effect. Very similar results were recorded for specimens stored for six months. For all microorganisms under investigation no growth inhibition zone around leather specimens was observed. Manuka oil introduced into leathers gave similar effects. After one-month storage the growth inhibition zone of 2 mm in size was observed for *Staphylococcus aureus* only. No inhibition zone was observed for *Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans*. No growth of bacteria mentioned above and fungus was observed, while for *Pseudomonas aeruginosa* and *Candida albicans* a microbial growth was observed at places where specimens poorly adhered to the substrate. Similar results were obtained for specimens treated with manuka oil after six-month storage. During these experiments no growth inhibition zone was observed for each microorganism. For *Staphylococcus aureus* no growth under specimen was found. However, for other microbes growth was visible at spots where leather specimens had poor contact with the substrate.

Conclusions

Leather preservation against deteriorating bacterial and fungal effects is an important concern in the tanning industry. Uncontrolled growth and multiplication of microbes lead to a decrease in value or total degradation of leather that is a valuable raw material, for example, in footwear and clothing industries. The study presented above confirmed an efficiency of selected essential oils as substances introduced during leather processing at fatliquoring stage in preventing microbial growth. The highest resistance to microbial growth was shown for the raw material into which oregano oil was added during the manufacturing process. Good effects of antibacterial treatment were also achieved for cinnamon oil. Both after one- and six-month treatment a strong effect of substances introduced into the material was observed. Lower, but still significant antimicrobial growth inhibiting activity in lining leather can be assigned to thyme leather. The oils mentioned above after being introduced into leathers showed antibacterial activity to *Staphylococcus aureus, Escherichia coli* and *Candida albicans*, while at the proposed concentration (5%) they did not reduce the growth and multiplication of bacteria *Pseudomonas aeruginosa*.

The results obtained in the experiment are not consistent - from the microbiological point of view - for leathers treated with eucalyptus oil. The specimens processed with an addition of manuka oil have satisfied antimicrobial effect for one case (*Staphylococcus aureus*), where only a small growth inhibition zone was observed.

Based on the research presented above and results obtained it may conclude that oregano, cinnamon and thyme essential oils implemented into lining leathers at amount of 5% by leather weight enable the most effective antimicrobial activity to be achieved.

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