TESTING OF MEDICAL SHEEP FUR WITH ANTIMICROBIAL PROPERTIES – PART 1

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ABSTRACT. The medical treatment of patients with rheumatic, joint and muscle diseases can be improved by alternative methods. The essential oils extracted from different plants have certain analgesic, anti-inflammatory, antiseptic, antibacterial, immunostimulating properties, etc. Products have been made based on essential oils with therapeutic properties (mint, cajeput, eucalyptus), which can be used to treat the surface of finished sheep fur for medical purposes. The sheep furs were tanned (without metals) with syntans based on phenolsulfonic acids and aromatic oxysulfones and treated with products based on essential oils with therapeutic properties, to be used to make medical fur articles (lumbar and cervical belts, knee pads, elbow pads, etc.). They can improve rheumatic, muscular and circulatory conditions, complementing the medical treatment of patients suffering from these conditions. The work presents the chemical, physical-mechanical and microbiological characterization of natural furs for medical use.

KEY WORDS: medical fur, essential oils, antimicrobial properties

TESTAREA BLĂNURILOR MEDICALE DE OAIE CU PROPRIETĂȚI ANTIMICROBIENE – PRIMA PARTE

REZUMAT. Tratamentul medical al pacienților cu afecțiuni reumatismale, articulare și musculare poate fi îmbunătățit prin metode alternative. Uleiurile esențiale extrase din diferite plante au anumite proprietăți analgezice, antiinflamatoare, antiseptice, antibacteriene, imunostimulante etc. S-au realizat produse pe bază de uleiuri esențiale cu proprietăți terapeutice (mentă, cajeput, eucalipt), care pot fi utilizate pentru tratarea suprafeței blănurilor de oaie finite în scopuri medicale. Blănurile de oaie au fost tăbăcite (fără metale) cu sintani pe bază de acizi fenolsulfonici și oxisulfone aromatice și tratate cu produse pe bază de uleiuri esențiale cu proprietăți terapeutice, pentru a fi utilizate la realizarea unor articole din blană de uz medical (centuri lombare și cervicale, genunchiere, cotiere etc.). Acestea pot ameliora afecțiunile reumatismale, musculare, circulatorii, completând tratamentul medical al pacienților care suferă de aceste afecțiuni. Lucrarea prezintă caracterizarea chimică, fizico-mecanică și microbiologică a blănurilor naturale de uz medical.

CUVINTE CHEIE: blănuri medicale, uleiuri esențiale, proprietăți antimicrobiene

TEST DES PEAUX DE MOUTON MÉDICALES AUX PROPRIÉTÉS ANTIMICROBIENNES – PREMIÈRE PARTIE

RÉSUMÉ. Le traitement médical des patients atteints de maladies rhumatismales, articulaires et musculaires peut être amélioré par des méthodes alternatives. Les huiles essentielles extraites de différentes plantes possèdent certaines propriétés analgésiques, anti-inflammatoires, antiseptiques, antibactériennes, immunostimulantes, etc. Des produits ont été élaborés à base d'huiles essentielles aux propriétés thérapeutiques (menthe, cajeput, eucalyptus), qui peuvent être utilisées pour traiter la surface de la peau de mouton finie à des fins médicales. Les peaux de moutons ont été tannées (sans métaux) avec des syntans à base d'acides phénolsulfoniques et d'oxysulfones aromatiques et traitées avec des produits à base d'huiles essentielles aux propriétés thérapeutiques, destinés à la confection d'articles médicaux en fourrure (ceintures lombaires et minerves, genouillères, coudières, etc.). Ils peuvent améliorer les affections rhumatismales, musculaires et circulatoires, en complément du traitement médical des patients souffrant de ces affections. L'article présente la caractérisation chimique, physico-mécanique et microbiologique des fourrures naturelles à usage médical.

MOTS CLÉS : fourrures médicales, huiles essentielles, propriétés antimicrobiennes

INTRODUCTION

The essential oils extracted from different plants have certain analgesic, anti-inflammatory, antiseptic, antibacterial, immunostimulating properties, etc. [1-5]. The antioxidant, antimicrobial, antifungal, flavoring properties demonstrated by the many studies conducted in recent years on the composition of essential oils make them important in areas such as the chemical, pharmaceutical, food and perfumery industries and medicine. Essential oils (EOs) are mixtures of aromatic, volatile, lipophilic biomolecules, extracted from regions of plants. They are formed of complex mixtures of hydrophobic molecules, which exhibit a broad spectrum of antimicrobial activity against bacteria, fungi, and viruses [6-11].

The medical treatment of patients with rheumatic, joint and muscle diseases can be improved by alternative methods. Ecological requirements as well as requirements related to fur assortment characteristics have led to the development of new fur processing technologies, such as:

- wet-white tanning of fur to eliminate or reduce the amount of complex salts of trivalent chromium;

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- tanning using chemically modified vegetable tannins or small molecular phenolic and polyphenolic compounds, including those derived from cellulose-making and refining techniques;

- sanitation of natural fur by binding some therapeutic species to the dermis and/or the hair.

In this research study, sheep furs were tanned (without metals) with syntans based on phenolsulfonic acids and aromatic oxysulfones and treated with products based on essential oils with therapeutic properties, to be used to make medical fur articles (lumbar and cervical belts, knee pads, elbow pads, etc.) [12-15]. The antibacterial properties of the sheepskins were subsequently evaluated by standardized methods [16-18]. Products have been made based on essential oils with therapeutic properties (mint, cajeput, eucalyptus), which can be used to treat the surface of finished sheep fur for medical purposes [19]. The medical fur articles can improve rheumatic, muscular and circulatory conditions, complementing the medical treatment of patients suffering from these conditions.

EXPERIMENTAL

Materials

- Sheepskins tanned with syntans based on phenolsulphonic acids and aromatic oxysulfones (I.N.C.D.T.P. – Division: Leather and Footwear Research Institute, Bucharest, Romania) [12, 15];

- Woolen sheepskins (Merinos) treated during fatliquoring operation with products based on essential oils (mint, cajeput, eucalyptus);

- Product P-M based on mint essential oil: dry substance – 20-21%, pH (10% solution) – 4-4.5, density – 0.900-0.920 g/cm^3;

- Product P-C based on cajeput essential oil: dry substance – 19-20%, pH (10% solution) – 4-4.5, density – 0.890-0.900 g/cm^3;

- Product P-E based on eucalyptus essential oil: dry substance – 18-19%, pH (10% solution) – 4-4.5, density – 0.880-0.890 g/cm^3 [19].

Methods

Chemical and Physico-Mechanical Tests

Chemical characteristics of products based on essential oils were determined according to the following standards: dry substance (%) – SR EN ISO 4684:2006; pH – SR-EN ISO 4098: 2006.

Chemical and mechanical characteristics of furs were determined according to the following standards: volatile matter % – SR EN ISO 4684:2012, extractable substances % – SR EN ISO 4048:2002, ash % – SR EN ISO 4047:2002, shrinkage temperatures (°C) – SR EN ISO 3380:2003, the longitudinal and transverse tensile strength – SR EN ISO 3376:2012.

Antibacterial Evaluation

The antibacterial properties of the sheepskins were evaluated by the inhibition zone diameter method according to DIN EN ISO 20645-2005 [16].

Staphylococcus aureus (ATCC 653) and *Escherichia coli* (ATCC 10536) were placed into 5 ml of medium and shaken for 24 h in a constant temperature shaker, then the bacterial solution was diluted to a concentration of 1 × 105 CFU/ml with phosphate-buffered saline (PBS) buffer. Then Luria-Bertani (LB) broth powder (10 g/l peptone, 5 g/l yeast extract powder, 10 g/l sodium chloride) was added to 950 ml distilled water, then adjusted to pH 7.0-7.2 with 0.1 mol/l NaOH solution after entirely dissolving and stirring all contents, and then made up to a volume of 1000 ml with distilled water. Agar powder (1.5 g per 100 ml of the medium) was added to the medium, and then autoclaved for 30 min after heating and dissolving. The medium solution (20 ml) was poured into a culture dish at a temperature of 45°C and UV-sterilized for 30 min to prepare an agar medium plate. The alloy sample was placed in the center of the plate and 500 µl of the bacterial suspension was evenly spread on the surface of the agar medium with a pipette. At least five times duplicates were measured for statistical analysis. The leather

specimens (2 cm diameter) are placed on the surface of the nutrient medium and then incubated at 37°C for 24 h.

Inhibition zones were calculated according to the formula given by [16]:

$$H = \frac{D-d}{2} \quad (1)$$

where H is the inhibition zone in mm, *D* is the total diameter of the specimen and inhibition zone in mm, and *d* is the diameter of the specimen in mm. When H is equal to or larger than 1 mm and there is no growth of bacteria, the antibacterial property is good; when H is equal to 0 mm and there are regions with some bacteria, the antibacterial property is limited; and when H is equal to 0 mm and there are regions with many bacteria, there is no antibacterial property.

Absorption Test

This test method evaluates the antibacterial activity of footwear products treated with antibacterial finish by making use of the method in which the test bacterial suspension is inoculated directly on to samples. In this study, we measured antibacterial properties of the prepared samples with the ISO 16187 Absorption test [17]. We placed the target sample (50 mm × 50 mm × 1 mm) on the Petri dish, added 0.4 mL of bacterial solution containing the target bacterial species (*S. aureus*, *E. coli*), and attached the film from the top. After a cultivation of 24 h at 35°C, we washed out the bacteria in a dedicated medium (SCDLP) and counted the number of colonies [18].

The antibacterial effect of the sample was determined by using the antibacterial activity value.

Calculation of Antibacterial Activity Ratio

The bacteriostatic activity ratio was obtained according to the following formula:

$$R = \frac{C_t - T_t}{C_t} X100\% \quad (2)$$

R is the antibacterial activity ratio;

C_t is the average number of colonies of two control samples after 24 h or the specified incubation period, expressed as CFU/ml;

T_t is the average number of colonies of two test samples after 24 h or the specified incubation period, expressed as CFU/ml.

Obtaining Ecologic Medical Sheepskins

Ecologic medical sheepskins were obtained using the products based on sulphated fatty alcohols, oils based on sulphated and sulphonated natural and synthetic fatty substances and syntans based on phenol sulphonic acids and aromatic oxysulphones [12]. Sheep fur was tanned (non-metallic tanning) and was treated with the product based on essential oils with therapeutic properties (mint, cajeput, eucalyptus).

Woolen sheep skins (Merinos) were treated during fatliquoring operation with 20-30g products based on essential oils (mint – P-M, or cajeput – P-C, or eucalyptus – P-E) /1000g fur tanned weight:

- P-M-1 – Sheep fur treated with 20g product P-M/1000g fur tanned weight;
- P-M-2 – Sheep fur treated with 30g product P-M/1000g fur tanned weight;
- P-C-1 – Sheep fur treated with 20g product P-C/1000g fur tanned weight;
- P-C-2 – Sheep fur treated with 30g product P-C/1000g fur tanned weight;
- P-E-1 – Sheep fur treated with 20g product P-E/1000g fur tanned weight;
- P-E-2 – Sheep fur treated with 30g product P-E/1000g fur tanned weight.

The products based on essential oils contain 55-60% essential oil (mint – P-M, or cajeput – P-C, or eucalyptus – P-E), 10-15% ethyl alcohol, 8-10% lauric alcohol ethoxylate with seven moles of ethylene oxide, 8-10% polyethylene glycol 400 (non-ionogenic) and deionized water [19].

RESULTS AND DISCUSSIONS

Characterization of Furs by Physical-Chemical and Physical-Mechanical Analyses

The values of the physical-chemical characteristics of the medical furs are comparable to the values set by the standards for sheep furskins intended for clothing (volatile dermal matter 10.40-12.70% and volatile wool matter 9.60-12.30%, extractable dermal substances 9.20-12.20% and wool extracts 0.50-0.80%, ash 3.20-3.80%, pH of aqueous extract, 4-4.5.

Values of shrinkage temperatures for medical sheep furskins are lower (75°C) than those of sheep furs processed with basic chromium salts (approx. 80°C).

The longitudinal tensile strength tests resulted in a value of 210-280 N, compared to the standard for the sheep furskins tanned with chromium salts for clothing, which are of min. 110 N, and the transverse tensile strength values are 180-220 N, compared to the values given in the standard for sheep furskins tanned with chromium salts for clothing, which are of min. 80 N.

Antibacterial Activity

The natural furs for medical use were tested for microbial activity against two bacteria strains, including *Escherichia coli* and *Staphylococcus aureus* according to SR EN ISO 20645/2005—Control of the antibacterial activity. The evaluation of the samples is based on the absence or presence of bacterial multiplication in the contact area between the inoculum and the sample and on the appearance of a possible inhibition zone around the samples (Figure 1).

Images of Petri plates after 24h incubation are shown in Figure 1 and assessment of antibacterial activity is shown in Table 1.

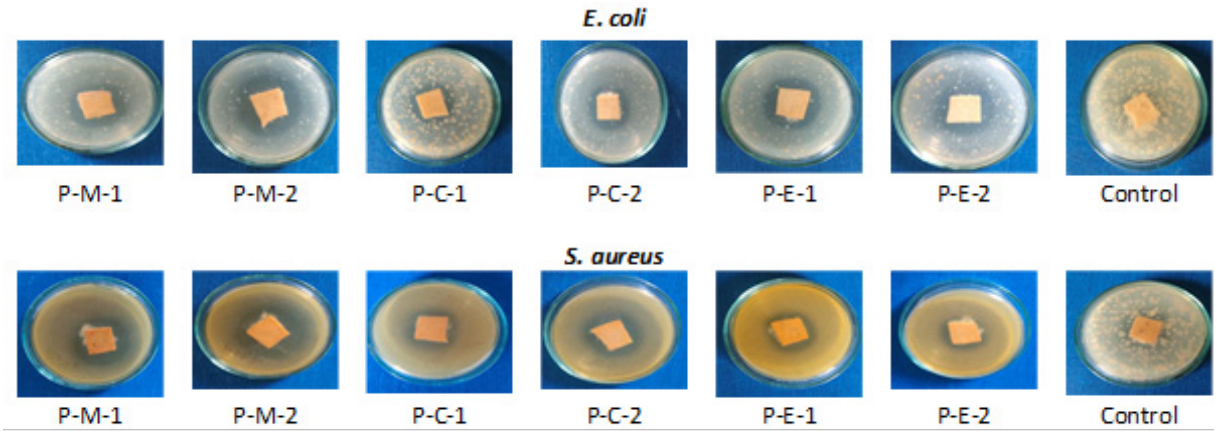

Figure 1. Images of Petri plates showing antibacterial effect after 24 h of incubation

The results summarized in Table 1 revealed that all samples treated with essential oil products presented microbiological activity and do not allow the development of aerobic germs for any of the bacteria tested.

Table 1: Evaluation of the antibacterial activity

	E. coli		*S. aureus*	
Code	Inhibition zone (mm)	Evaluation	Inhibition zone (mm)	Evaluation
P-M-1	+++++	Total inhibition zone	+++++	Total inhibition zone
P-M-2	+++++	Total inhibition zone	+++++	Total inhibition zone
P-C-1	+++++	Total inhibition zone	+++++	Total inhibition zone
P-C-2	+++++	Total inhibition zone	+++++	Total inhibition zone
P-E-1	+++++	Total inhibition zone	+++++	Total inhibition zone
P-E-2	+++++	Total inhibition zone	+++++	Total inhibition zone
Control	-	Unsatisfactory effect	-	Unsatisfactory effect

An insufficient effect was obtained for the control sample which did not present antimicrobial activity against the bacterial strains. The inhibition areas produced by all the formulations loaded with products based on essential oils showed diameters ranging between 5 and 11 mm when tested against *Staphylococcus aureus* and 2 and 6 mm against *Escherichia coli* after 24 h of incubation.

Absorption Test

Antibacterial performance of the functionalised natural furs for medical use was assessed quantitatively using a test method as described in the Absorption test [17]. Growth reduction rate R (%) was calculated and summarised in Tables 2 and 3. The formula describes the difference in the number of viable bacteria on the test samples compared to the control samples after incubation. The number of viable bacteria post incubation on the control samples should increase compared to time zero. If the test samples have antibacterial activity, the number of viable bacteria attached on the surface after incubation should be fewer than the number on the control.

Table 2: Growth reduction rate (R %) of the natural fur samples after 24 h contact time for *Staphylococcus aureus* ATCC 6538

Sample	Result	R%	Log_{10} red.
Inoculum concentration	T_0=1x10^{5}CFU/mL		
P-M-1	T_0=1x10^{5}CFU/mL T_{24}=10 UFC/mL	99.99%	4.00
P-M-2	T_0=1x10^{5}CFU/mL T_{24}=7 UFC/mL	99.99%	4.15
P-C-1	T_0=1x10^{5}CFU/mL T_{24}=0 UFC/mL	100%	5.00
P-C-2	T_0=1x10^{5}CFU/mL T_{24}=0 UFC/mL	100%	5.00
P-E-1	T_0=1x10^{5}CFU/mL T_{24}=0 UFC/mL	100%	5.00
P-E-2	T_0=1x10^{5}CFU/mL T_{24}=0UFC/mL	100%	5.00
Control	T_0=1x10^{5}CFU/mL T_{24}=4.5x10^{4}UFC/mL	55.00%	0.35

Table 3: Growth reduction rate (R %) of the natural fur samples after 24 h contact time for *Escherichia coli* ATCC 10536

Sample	Result	R%	Log_{10} red.
Inoculum concentration	T_0=1x10^{5}CFU/mL		
P-M-1	T_0=1x10^{5}CFU/mL T_{24}=6 UFC/mL	99.99%	4.22
P-M-2	T_0=1x10^{5}CFU/mL T_{24}=3 UFC/mL	100%	4.52
P-C-1	T_0=1x10^{5}CFU/mL T_{24}=0 UFC/mL	100%	5.00
P-C-2	T_0=1x10^{5}CFU/mL T_{24}=0 UFC/mL	100%	5.00
P-E-1	T_0=1x10^{5}CFU/mL T_{24}=0 UFC/mL	100%	5.00
P-E-2	T_0=1x10^{5}CFU/mL T_{24}=2UFC/mL	100%	4.70
Control	T_0=1x10^{5}CFU/mL T_{24}=5.5x10^{4}UFC/mL	45.00%	0.26

The results of antimicrobial tests against gram-negative, gram-positive are presented in Tables 2 and 3 and showed efficiency above 99-100% in all cases. As can be seen, natural furs without the addition of essential oils showed low antibacterial activities against the two bacterial species tested, with "R" values of 55.00 and 45.00 for *S. aureus* and *E. coli*, respectively.

Characterisation of Obtained Fur Assortments for Medical Use

The prepared products with therapeutic properties (analgesic, anti-inflammatory and relaxing) can be used for treatment of medical furs. Menthol, the ingredient in the composition of peppermint oil, stimulates receptors signalling the cold sensation and inhibits receptors reacting to pain stimuli, temporarily relieving muscle pain. Eucalyptol, the ingredient in the composition of cajeput and eucalyptus oils, with analgesic and disinfectant properties, is effective in the treatment of patients suffering from rheumatism, lumbar radiculopathy and cervical spondylosis, stimulating blood circulation and relieving rheumatic and joint pain.

Mint oil contains 40.04% menthol, 23.78% l-menthone, 14.51% i-menthone, 4.24% menthyl acetate etc. Cajeput oil contains 57.02% eucalyptol, 2.93% alpha-linalool, 2.93% alpha-linalool, 2.77% caryophyllene etc. Eucalyptus oil contains 73.23% eucalyptol, 14.99% d-limonene, 4.10% o-cymene, 2.82% gamma-terpinene etc [13, 14].

The results of the antimicrobial tests highlighted a strong antibacterial character of the sheep fur samples tested, having a "satisfying effect", because no bacterial multiplication was observed [16]. Sheep fur samples, treated with materials based on essential oils (mint, cajeput, eucalyptus) do not allow the development of aerobic germs for the tested bacteria, namely, *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). Untreated control materials have not shown microbial reduction.

The product based on essential oils can be used to treat the sheep furskins (free of

metals) for medical purposes and improve the quality of natural fur and fur articles (lumbar and cervical belts, knee pads, elbow pads etc.) used to prevent, relieve and treat rheumatic, muscular, circulatory disorders, complementing the medical treatment of patients suffering from these conditions, keeping the fur-covered area warm. Treatment with these products can be repeated at certain time intervals, on the fur surface or fur articles.

CONCLUSIONS

▪ Sheepskins were tanned with syntans based on phenolsulphonic acids and aromatic oxysulfones.

▪ The products based on essential oils (mint, cajeput, eucalyptus) with therapeutic properties (analgesic, anti-inflammatory and relaxing) can be used for treatment of medical furs.

▪ The results of the antimicrobial tests highlighted a strong antibacterial character of the sheep fur samples tested, having a "satisfying effect", because no bacterial multiplication was observed.

▪ Sheep fur samples, treated with materials based on essential oils (mint, cajeput, eucalyptus) do not allow the development of aerobic germs for the tested bacteria, namely, *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*).

▪ The product based on essential oils can be used to treat the sheep furskins (free of metals) for medical purposes and improve the quality of natural fur and fur articles (lumbar and cervical belts, knee pads, elbow pads etc.) used to prevent, relieve and treat rheumatic, muscular, circulatory disorders, complementing the medical treatment of patients suffering from these conditions, keeping the fur-covered area warm.

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