TESTING OF MEDICAL SHEEP FUR WITH ANTIMICROBIAL PROPERTIES – PART 2

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Received: 28.09.2022 Accepted: 09.12.2022 https://doi.org/10.24264/lfj.22.4.4

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ABSTRACT. There are a number of people with rheumatic, joint and muscle diseases, and the medical treatment of patients suffering from these diseases requires an improvement through alternative methods. Pain is the main symptom of rheumatic diseases and chronic pain affects the physical and mental condition, lowering quality of life and ability to work. Natural products derived from plants with antimicrobial, anti-inflammatory, antioxidant, and chemo-preventive properties have been used for many generations in traditional medicine. 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 (daphne, ginger, basil), which can be used to treat the surface of tanned sheep fur for medical purposes. Ecological requirements 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. 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, bootees, etc.). They can improve rheumatic, muscular and circulatory conditions, complementing the medical treatment of patients suffering from these conditions. Heat can relieve rheumatic pains (transmitted through the application of natural fur). 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 – A DOUA PARTE

REZUMAT. Există o serie de persoane cu afecțiuni reumatismale, articulare și musculare, iar tratamentul medical al pacienților care suferă de aceste boli necesită o îmbunătățire prin metode alternative. Durerea este principalul simptom al afecțiunilor reumatismale, iar durerea cronică afectează starea fizică și psihică și astfel scade calitatea vieții și capacitatea de muncă. Produsele naturale derivate din plante cu proprietăți antimicrobiene, antiinflamatorii, antioxidante și chimiopreventive au fost folosite de multe generații în medicina tradițională. 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 (dafin, ghimbir, busuioc), care pot fi utilizate pentru tratarea suprafeței blănurilor de oaie tăbăcite în scopuri medicale. Cerințele ecologice au dus la dezvoltarea unor noi tehnologii de prelucrare a blănurilor, cum ar fi tăbăcirea wet-white pentru a elimina sau reduce cantitatea de săruri complexe de crom trivalent. 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, botoși etc.). Acestea pot ameliora afecțiunile reumatismale, musculare, circulatorii, completând tratamentul medical al pacienților care suferă de aceste afecțiuni. Căldura poate ameliora durerile reumatice (transmise prin aplicarea blănii naturale). 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 – DEUXIÈME PARTIE

RÉSUMÉ. Il existe un nombre de personnes atteintes de maladies rhumatismales, articulaires et musculaires, et le traitement médical des patients souffrant de ces maladies nécessite une amélioration par des méthodes alternatives. La douleur est le principal symptôme des affections rhumatismales et la douleur chronique affecte l'état physique et mental et diminue ainsi la qualité de vie et la capacité de travailler. Les produits naturels issus de plantes aux propriétés antimicrobiennes, anti-inflammatoires, antioxydantes et chimiopréventives sont utilisés depuis de nombreuses générations en médecine traditionnelle. 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 (laurier, gingembre, basilic), qui peuvent être utilisées pour traiter la surface de la fourrure de mouton tannée à des fins médicales. Les exigences écologiques ont conduit au développement de nouvelles technologies de transformation de la fourrure, comme le tannage wet-white pour éliminer ou réduire la quantité de sels complexes de chrome trivalent. Les fourrures 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, botillons, etc.). Ils peuvent améliorer les affections rhumatismales, musculaires et circulatoires, en complément du traitement médical des patients souffrant de ces affections. La chaleur peut soulager les douleurs rhumatismales (transmises par l'utilisation de fourrure naturelle). L'article présente la caractérisation chimique, physico-mécanique et microbiologique des fourrures naturelles à usage médical.

MOTS CLÉS : fourrures médicales, huiles essentielles, propriétés antimicrobiennes

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INTRODUCTION

Ecological requirements as well as requirements related to fur assortment characteristics have led to the development of new fur processing technologies, such as, sanitation of natural fur by binding some therapeutic species of plants to the dermis and/or the hair. 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. Natural products derived from plants with antimicrobial, anti-inflammatory, antioxidant, and chemo-preventive properties have been used for many generations in traditional 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. Essential oils contain terpene compounds, which can be acyclical (hydrocarbons, alcohols, carbonyl compounds) and cyclical (hydrocarbons, alcohols and ethers, carbonyl compounds and esters) [6-11].

Eugenol (the principal component of daphne essential oil) is characterized by a high antimicrobial action against a variety of microorganisms.

Linalool and eucalyptol are the principal components of basil essential oil and play a major role in the anti-inflammatory activity provided by the essential oils containing them.

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, bootees etc.) [12-15]. The antibacterial properties of the sheepskins were evaluated by standardized methods [16-18]. Products have been made based on essential oils with therapeutic properties (daphne, ginger, basil), which can be used to treat the surface of tanned sheep fur for medical purposes [19].

The medical treatment of patients with rheumatic, joint and muscle diseases can be improved by alternative methods. Pain is the main symptom of rheumatic diseases and chronic pain affects the physical and mental condition and thus lowers the quality of life and ability to work. For these diseases are recommended herbal plant species with antiallergic, anti-inflammatory effects, etc. The medical fur articles can improve rheumatic, muscular and circulatory conditions, complementing the medical treatment of patients suffering from these conditions. Heat can relieve rheumatic pains (transmitted through the application of natural fur).

EXPERIMENTAL

Materials

- Sheepskins tanned with syntans based on phenolsulfonic acids and aromatic oxysulfones (I.N.C.D.T.P. – Division Leather and Footwear Research Institute Bucharest, Romania) [12, 15];
- Woolen Sheep skins (Merinos) treated during fatliquoring operation with products based on essential oils (daphne, ginger, basil);
- Product P-D based on daphne essential oil: dry substance – 18-19%, pH (10% solution) – 4-4.5, density – 0.880-0.890 g/cm^3;
- Product P-G based on ginger essential oil: dry substance – 20-21%, pH (10% solution) – 4-4.5, density – 0.920-0.930 g/cm^3;
- Product P-B based on basil essential oil: dry substance – 17-18%, pH (10% solution) – 4-4.5, density – 0.870-0.880 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 1×10^5 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]:

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

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 phenolsulfonic acids and aromatic oxysulfones [12]. Sheep fur was tanned (free of metals) and was treated with the product based on essential oils with therapeutic properties (daphne, ginger, basil).

Woolen sheep skins (Merinos) were treated during fatliquoring operation with 20-30g products based on essential oils (daphne – P-D, or ginger – P-G, or basil – P-B) /1000g fur tanned weight:

- P-D-1 – Sheep fur treated with 20g product P-D/1000g fur tanned weight;

- P-D-2 – Sheep fur treated with 30g product P-D/1000g fur tanned weight;
- P-G-1 – Sheep fur treated with 20g product P-G/1000g fur tanned weight;
- P-G-2 – Sheep fur treated with 30g product P-G/1000g fur tanned weight;
- P-B-1 – Sheep fur treated with 20g product P-B/1000g fur tanned weight;
- P-B-2 – Sheep fur treated with 30g product P-B/1000g fur tanned weight.

The products based on essential oils contain 55-60% essential oil (daphne – P-D, or ginger – P-G, or basil – P-B), 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, 20].

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 11.20-13.50% and volatile wool matter 9.10-11.80%, extractable dermal substances 10.30-14.40% and wool extracts 0.70-0.90%, ash 3.60-3.90%, 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 250-270 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 170-210N, 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

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

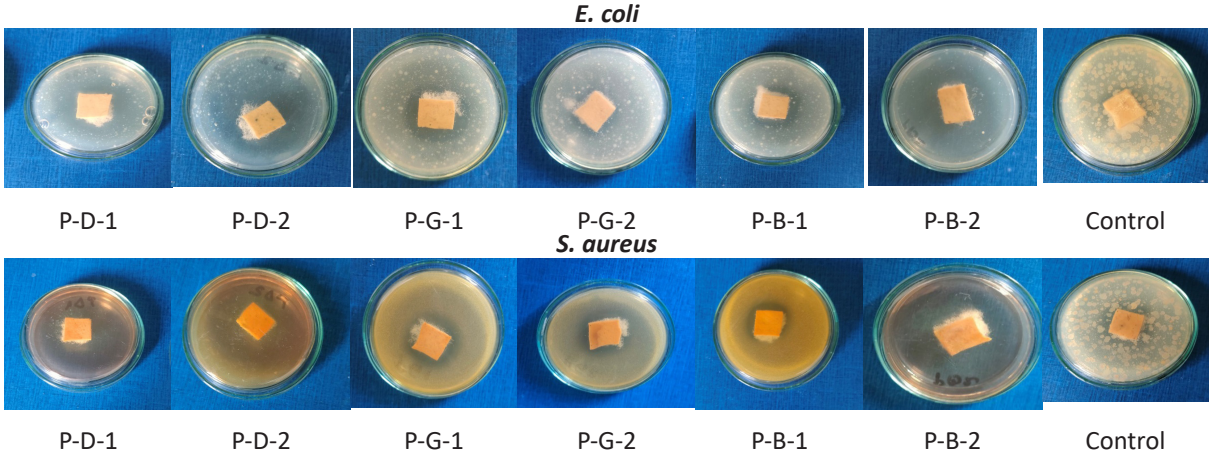

Figure 1. Images of Petri plates showing antibacterial effect after 24 h of incubation

The results of antimicrobial activity of natural fur samples against *Staphylococcus aureus* and *Escherichia coli* according to EN ISO 20645:2004 expressed as inhibition zone (H) are presented in Table 1.

Table 1: Evaluation of the antibacterial activity

	E. coli (ATCC 10536)		*S. aureus* (ATCC 6538)	
Code	Inhibition zone (mm)	Evaluation	Inhibition zone (mm)	Evaluation
P-D-1	11.5	Satisfactory effect	25	Satisfactory effect
P-D-2	20	Satisfactory effect	25	Satisfactory effect
P-G-1	5	Satisfactory effect	9	Satisfactory effect
P-G-2	2.5	Satisfactory effect	7	Satisfactory effect
P-B-1	3.5	Satisfactory effect	5	Satisfactory effect
P-B-2	25	Satisfactory effect	25	Satisfactory effect
Control	-	Unsatisfactory effect	-	Unsatisfactory effect

According to the standard, excellent antimicrobial protection is for H ≥ 1. The results of antimicrobial activity shown in Table 1 indicate that all antimicrobial treatments regardless of applied agents resulted in antimicrobial protection. Antiseptic treatments and essential oils resulted in a high degree of antimicrobial protection to both bacteria – Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. Nevertheless, taking into account the extremely high value of inhibition zone, essential oils are indispensable for antibacterial protection.

Absorption Test

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-D-1	T_0=1x10^{5}CFU/mL T_{24}=4 CFU/mL	100%	4.40
P-D-2	T_0=1x10^{5}CFU/mL T_{24}=0 CFU/mL	100%	5.00
P-G-1	T_0=1x10^{5}CFU/mL T_{24}=8 CFU/mL	99.99%	4.10
P-G-2	T_0=1x10^{5}CFU/mL T_{24}=5 CFU/mL	100%	4.30
P-B-1	T_0=1x10^{5}CFU/mL T_{24}=10 CFU/mL	99.99%	4.00
P-B-2	T_0=1x10^{5}CFU/ml T_{24}=7 CFU/mL	99.99%	4.15
Control	T_0=1x10^{5}CFU/mL T_{24}=4.5x10^4 CFU/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-D-1	T_0=1x10^{5}CFU/mL T_{24}=2 CFU/mL	100%	4.70
P-D-2	T_0=1x10^{5}CFU/mL T_{24}=4 CFU/mL	100%	4.40
P-G-1	T_0=1x10^{5}CFU/mL T_{24}=4 CFU/mL	100%	4.40
P-G-2	T_0=1x10^{5}CFU/mL T_{24}=1 CFU/mL	100%	4.90
P-B-1	T_0=1x10^{5}CFU/mL T_{24}=6 CFU/mL	99.99%	4.22
P-B-2	T_0=1x10^{5}CFU/mL T_{24}=3 CFU/mL	100%	4.52
Control	T_0=1x10^{5}CFU/mL T_{24}=5.5x10^{4}CFU/mL	45.00%	0.26

This antimicrobial activity assay was performed for both target bacteria and the results are expressed as mean values of three biological replicates. The antibacterial activity value was calculated according to ISO 16187 Absorption test. Using this method all the samples showed strong efficacy against both bacteria.

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. Eugenol is characterized by a high antimicrobial action against a variety of microorganisms. Eugenol, d-limonene, linalool, eucalyptol – the ingredients in the composition of daphne, basil and ginger oils, with analgesic and disinfectant properties, are effective in the treatment of patients suffering from rheumatism, lumbar radiculopathy and cervical spondylosis, stimulating blood circulation and relieving rheumatic and joint pain.

Daphne oil contains 46.95% eugenol, 43.37% d-limonene, 7.14% alpha terpinolene, 0.93% alpha terpinene etc. Ginger oil contains d-limonene 21.88%, camphene 21.47%, alpha-pinene 11.29%, cineole 10.46%, zingiberene 9.32% etc. Basil oil contains 65.88% linalool, 5.37% eucalyptol, 3.87% p-allyl anisole, 3.23% alpha-cadinene, 0.79% eugenol etc. [13, 14].

The results of the antimicrobial tests highlighted a strong antibacterial character of the sheep fur samples tested, having a "satisfactory effect", because no bacterial multiplication was observed [16]. Sheep fur samples treated with materials based on essential oils (daphne, ginger, basil) 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, bootees, 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 phenolsulfonic acids and aromatic oxysulfones.
- The products based essential oils (daphne, ginger, basil) 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 "satisfactory effect", because no bacterial multiplication was observed.
- Sheep fur samples, treated with materials based on essential oils (daphne, ginger, basil) do not allow the development of aerobic germs for the tested bacteria, namely, *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*).
- Samples P-D-1, P-D-2 and P-G-2 had maximum antimicrobial activity, 100%, against *S. aureus* and *E. coli*.
- Eugenol, d-limonene, linalool, eucalyptol the ingredients in the composition of daphne, basil and ginger 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.
- The product based 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, bootees 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, as heat can relieve rheumatic pains (transmitted through the application of natural fur).

Acknowledgements

This research was funded by the Romanian Ministry of Research and Digitalization, CCCDI - UEFISCDI, Nucleus Program, TEX-PEL-VISION 2022, 4N/8.02.2019, project number PN 19 17 01 02_CREATIV_PIEL, and PNCDI III - Program 1 – Development of the national RD system, Subprogram 1.2-Institutional Performance-RDI excellence funding projects, Contract no. 4PFE/ 2022.

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