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NATURAL LEATHER FOOTWEAR PROTECTION AGAINST FUNGI USING ESSENTIAL OILS

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Abstract: *Biocides used in the leather and footwear industry, based on beta-naphthol, benzothiazole and sulfone derivatives, etc. are toxic to humans and environment, some of these being prohibited by the directives in force.*
Many studies revealed utilization of essential oils for leather and leather objects protection against fungi. Fungi grow quickly under high humidity conditions and temperatures ranging between 25 and 30°C. Research aims the replacement of potentially toxic biocides with ecologic materials – essential oils extracted from plants. The biological activity of essential oils depends on their composition. Essential oils that contain substituted phenols (eugenol) exhibit strong antibacterial and antioxidant effects.The paper presents the possibility of using essential oil of laurel as alternative preservatives for skin treatment. Essential oil isolated from laurel (Laurus Nobilis) containing: eugenol – 46.95%, DL limonene – 43.37% and alpha terpinolene – 7.14%. Testing of essential oil of laurel was carried out monitoring the manner in which mold growth is influenced by the treatment applied to the sample through the resistance to mold in simulated contamination conditions. Following the study, it is concluded that the selected essential oil of laurel can be used as an antifungal agent in the field of natural leather footwear processing. The natural leathers for linings can be treated with new product based on essential oil with antifungal and antibacterial effect.

Key words: *natural leather footwear, essential oils, Gas Chromatography Mass Spectrometry (GC/MS), FT-IR spectrometry, Aspergillus niger*

1. INTRODUCTION

Both tanned and finished leather may be damaged by fungi from *Aspergillus flavus* and *Aspergillus niger*, *Trichoderma viride*, *Penicillium glaucom* and *Penicillium cyclopium*, and *Paecilomyces variotii* species which irreversibly damage leather through the enzymes (collagenases, lipases and proteases) they produce.

Many natural antimicrobial agents have been identified over the last decades, such as essential oils.

Essential oils from aromatic and medicinal plants have been known to possess potential as natural agents for leather preservation, including antibacterial and antifungal.

The essential oils have been qualified as natural biocides and offered as potential substitutes of synthetic biocides in specific steps of leather processing [1-3].

Many studies revealed utilization of essential oils as biocides for leather and leather objects protection against fungi (in leather manufacture and in footwear) [4-7].

2. EXPERIMENTAL

2.1 Materials

• The nappa bovine leathers, mineral tanned and wet finished by retanning, fatliquoring and dyeing (1.0-1.2 mm thick, dyed brown) (INCDTP – Division Leather and Footwear Research Institute Bucharest, Romania).

• Laurel essential oil, Laurus Nobilis (Adams, Romania) containing: eugenol – 46.95%, DL limonene – 43.37% and alpha terpinolene – 7.14%.

• Ethanol (Chemical Company, Germany), density – 0.789 g/cm^3 at 20^{0}C, boiling point – 78^{0}C, melting point –114^{0}C, water solubility – in any proportion;

• Biologic Material: *Aspergillus niger*.

2.2. Methods

• Gas Chromatography Mass Spectrometry (GC/MS) Analysis: Analysis of the essential oils carried out by using Agilent 7890 A GC System equipped with Agilent 5795 C MS, and HP-5 MS (0.25 mm x 30 m i.d., film thickness 0.25). The carried gas helium (99.9%) at a flow rate of 1 mL/ min; ionization energy was 70 eV. Mass range m/z 50-650 amu. Data acquisition was scan mode. MS transfer line temperature was 250 $_0$C, MS Ionization source temperature was 230 $_0$C, the injection port temperature was 250 $_0$C. The samples were injected with 250 split ratio. The injection volume was 1 µL. Oven temperature was programmed in the range of 50 to 250 $_0$C at 3$_0$C/ min. The structure of each compound was identified by comparison with their mass spectrum (Nist 05 and Wiley 7 library) [8].

• Attenuated Total Reflectance Fourier transform infrared spectroscopy (ATR-FTIR) measurements were run with a Jasco instrument (model 4200), in the following conditions: wavenumber range – 600-4000 cm$_{-1}$; data pitch – 0.964233 cm$_{-1}$; data points – 3610: aperture setting – 7.1 mm; scanning speed – 2 mm/s; number of scans – 30; resolution – 4 cm$_{-1}$; filter – 30 kHz; angle of incident radiation – 45$_o$ [9].

• Applying essential oil of laurel on leather samples was made by dropping 0.2, 0.4 and 0.6 mL oil and ethanol 1:1) on the surface of 2.0 x 2.0 cm2.

The samples treated with essential oil and untreated were placed in each Petri dish in the center of the surface of the culture medium, then the culture medium was seeded in 3 points around the sample, as an equilateral triangle. Petri dishes were placed in thermo-hygrostat at 30°C temperature and were analized after 7, 14, 21 and 28 days. *Aspergillus niger* strain development was assessed by ranking: 0 – absence of stems and a strong fungitoxic effect, 5 – an almost non-existent effect (the mold covers the entire surface of the specimen) [10].

• The goal was to monitor the influence of the treatment applied to the sample on mold growth through the mold resistance under simulated contamination, according to no.12697/A 9:2008 „Finished leathers. Mold resistance testing".

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

• Chemical characteristics of the uncoated leathers were determined according to the following standards: moisture (%) – SR EN ISO 4684:2006; the content extractables (%) – SR EN ISO 4648:2008; the content of chromium oxide (%) – SR EN ISO 5398:2008.

2.3. Identification of compounds in the composition of laurel essential oil

Laurel essential oil was analysed using GC-MS [8].

Chromatogram for laurel oil is shown in Figure 1, and identification of compounds in their composition is presented in table 1.

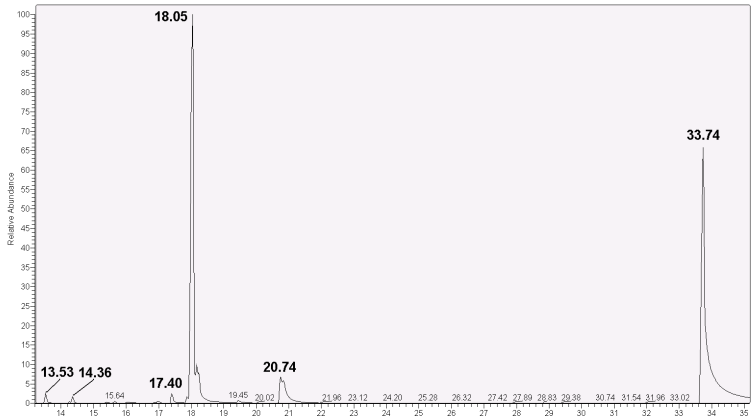


Fig.1. *Chromatogram of organic compounds in the laurel essential oil*

Table 1. *Identification of organic compounds in the laurel essential oil by GC-MS*

No.	RT	Amount, %	Compounds
1	13.53	0.88	Alpha Pinene
2	14.36	0.71	Camphene
3	17.4	0.93	Alpha Terpinene
4	18.05	43.37	DL Limonene
5	20.74	7.14	Alpha Terpinolene
6	33.74	46.95	Eugenol

The following compounds are found in the highest amount: eugenol – 46.95%, DL limonene – 43.37% and alpha terpinolene – 7.14%.

Laurel essential oil was analyzed using FT-IR. [9]

FT-IR (ATR) spectrum of laurel essential oil is shown in Figure 2.

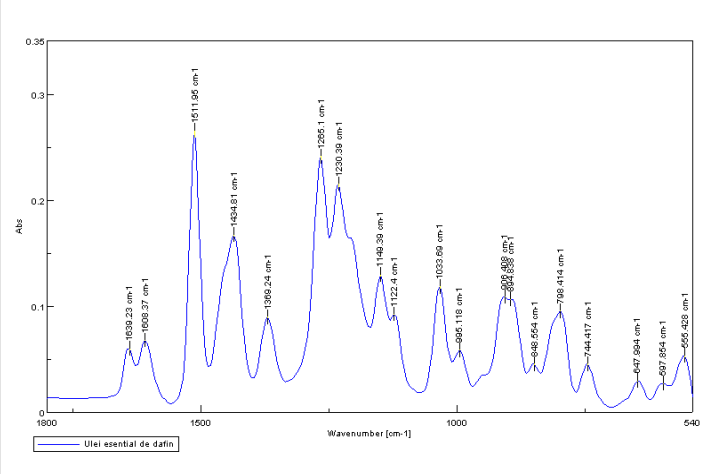


Fig. 2. *FT-IR spectrum of laurel essential oil*

The main bands of laurel oil are (Fig. 2): 1639 cm-¹ and 1608 cm-¹ – indicating the presence of C=O group from ester, 1511 cm-¹, 1434 cm-¹ and 1360 cm-¹ – assigned to the C-H group, 1265 cm-¹, 1230 cm-¹, 1149 cm-¹, 1033 cm-¹, 906 cm-¹ given by the C-O-C group from ether.

3. RESULTS

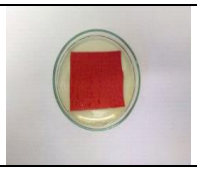
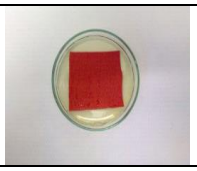
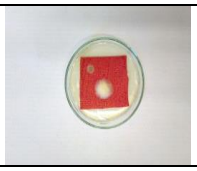
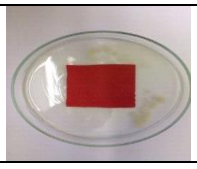
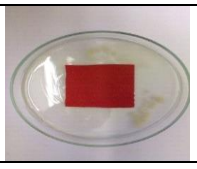
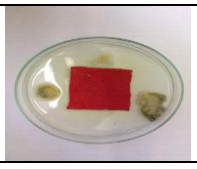
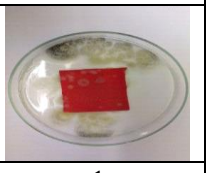
3.1. Biological characterisation of the leather samples

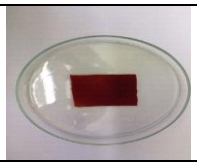
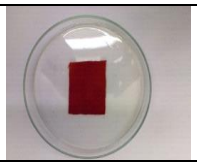
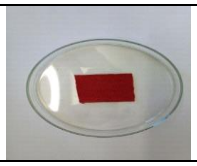
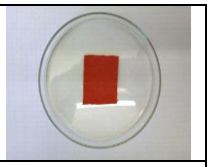
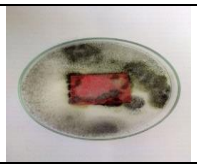
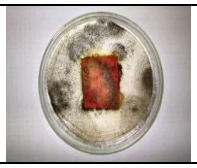
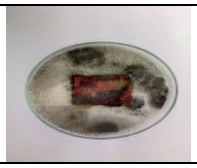
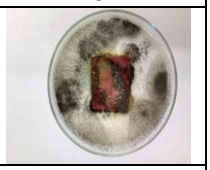
The samples treated with different amounts of laurel essential oil on the surface of unfinished leather, L1 (0.2 mL oil and eyhanol 1:1), L2 (0.4 mL oil and ethanol 1:1) and L3 (0.6 mL oil and ethanol 1:1), were inoculated with biological material – *Aspergillus niger* spores.

Mold development on leather specimens, and macroscopic images of samples treated with L1-L3, after 7, 14, 21 and 28 days from treatment, are presented in table 3.

The numbers under the images are the marks given according to the standard.

Table 3. *Macroscopic images of samples treated with laurel essential oil after 7, 14, 21, 28 days*

Sample/ day	7	14	21	28
L1				
	0	0	2	3
L2				
	0	0	0	1

L3				
	0	0	0	0
Control				
	4	5	5	5

The antifugal effect of the essential oil of laurel is higher as the amount of oil applied to the skin is higher.

The laurel essential oil used to treat nappa bovine leathers, improves their quality and resistance to fungi and bacteria.

The sample L1 does not develop fungi for 14 days – mark 0.

The sample L2 does not develop fungi for 21 days – mark 0.

The sample L3 does not develop fungi for 28 days – mark 0.

Leather Control sample untreated with the laurel essential oil develops fungi, as shown by marks ranging between 4 after 7 days and 5 after 14-28 days.

3.2. Chemical characterization of the leather samples

Chemical characteristics of the uncoated leathers used to obtain bovine hides into natural grain nappa determined in accordance with standards no. 1619:1994 (Table 4).

Table 4. *Chemical characteristics of bovine hides into natural grain nappa*

Sample/Characteristics	L1	L2	L3	Control	ST 1619:1994
Moisture, %	14.92	14.74	14.86	14.65	14-15
The content extractables, %	9.68	9.77	9.64	8.97	Max.10
The content of chromium oxide, %	5.45	5.86	5.78	5.40	Min.3.5

Chemical characteristics of the natural grain nappa bovine are within the limits specified in standard.

4. CONCLUSION

• Laurel essential oil containing: eugenol – 46.95%, DL limonene – 43.37% and alpha terpinolene – 7.14%.

• The laurel essential oil used to treat nappa bovine leathers, improves their quality and resistance to fungi and bacteria, reducing the surface defects of natural skin caused by fungi and bacteria.

• The selected essential oil of laurel can be used as an antifungal agent in the field of natural leather footwear processing.

• The natural leathers for linings can be treated with essential oil of laurel with antifungal and antibacterial effect.

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