THYME ESSENTIAL OIL AS NATURAL LEATHER PRESERVATIVE AGAINST FUNGI

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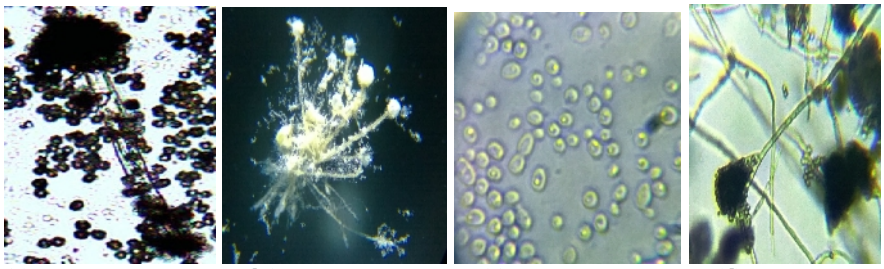
This work covers the chemical composition of essential oil isolated from thyme (*Thymus vulgaris)* analyzed using GC-MS and its antifungal activity. The main components of *Thymus vulgaris* oil were tymol (32.43%) and carvacrol (57.355%). The thyme oil was assayed for antifungal activity against *Candida albicans* and *Aspergillus niger* on sheepskins for footwear lining treated with thyme oil at a rate of 30g/1000g wet blue leather, during fatliquoring process. These pathogens are very contagious and result in severe damage of leather. Compounds were investigated by ASTM D4576–86 (1996) method against *Aspergillus niger* and by Standard SR EN ISO 20645:2004 against *Candida albicans*. The essential oil of thyme showed the best antifungal activity on sheepskins for footwear lining.

Keywords: lining leather, thyme essential oil, antifungal activity, fungi

INTRODUCTION

The hides, skins and leather can be easily contaminated with different species of bacteria and fungi. These microorganisms can damage the material or can be pathogenic for the user. Natural skins are very sensitive to the destructive action of fungi. Thus, once with raw skin can enter in tannery the diverse bacteria that may be pathogenic as *Staphylococcus, Bacillus, Pseudomonas, Enterobacter, Nocardiopsis* and other microorganisms decay. Also during processing and commercialization, particularly in transport over seas the wet blue or finished leather must ensure protection against the main species of fungi that can grow on the skin (Ţârlea *et al*., 2009; Chirilă *et al*., 2014). These microorganisms include the following species: *Aspergillus niger, Aspergillus flavus, Trichoderma viride, Penicillium glaucum, Penicillium cyclopium, Paecilomyces variotii, Candida albicans, Scopulariopsis brevicaulis* which develop on various leather items, degrading the material (Figure 1).



(a) (b) (c) (d)

Figure 1. *Aspergillus niger* (a), *Aspergillus flavus* (b), *Candida albicans* (c), *Penicillium* (d)

Biocides are used for the protection of leather, fur, footwear and leather objects against microorganisms (Deselnicu *et al.*, 2005; Deselnicu *et al.*, 2007). “Biocide” is a generic term that refers to both bactericidal (which are effective against bacteria) and fungicides (which are effective against fungi). They act as oxidizing agents or by

distorting or crosslinking proteins, through a number of mechanisms including inhibition of bacterial enzyme systems (Radwan *et al*., 2014).

Bactericides are used mainly at the beginning of leather processing during conservation and soaking phases.

Fungicides are commonly used from the pickling to the drying steps, because the pH conditions of these processes are ideal for mold growth.

Biocides are among chemicals potentially harmful to humans and the environment, therefore their production and marketing is regulated and monitored continuously by European directives and regulations of European Parliament and Council (Deselnicu, 2014; Deselnicu *et al*., 2014). There is no EU legislation in regulating specifically the content of chemicals in footwear and other leather products, but the use of chemicals is restricted by REACH (EC 1907/2006).

Directive 94/11/EC, also called the EU Directive on Labeling of Footwear is specifically linked to the European market harmonization of laws and administrative provisions of the Member States relating to labeling of the materials used in the main components of footwear for sale by consumers. In Romania, Directive 98/8/EC was adopted by HG 956/2005, complete with HG 545/2008 on the placing on the market of biocidal products.

The biocides used in the leather industry are classified as quaternary ammonium compounds, isothiazole, thiocarbamates and the like, such as sulfur-containing heterocycles, for example benzothiazole derivatives of 2-(tiocianatmetiltio)-1,3) benzothiazole and glutaraldehyde. Fungicides include phenol derivatives (ortho-phenylphenol), TCMTB, carbamates, etc. Halogenated organic compounds are also used, for example bronopol [2-bromo-2-nitropropane-1,3-diol]. Due to their toxicity and negative effects they have on the environment and human health it is necessary to replace these chemicals with natural, environmentally friendly and low toxicity compounds.

Many studies have been made that aimed at replacing potentially hazardous substances used in the processing of hides, skins and furs:

- Synthesis of new classes of biocides based on derivatives of 2-amino-benzothiazole-6-substituted with methyl, methoxy, chloro, nitro, modified chemically by sulphonation (Ţârlea *et al*., 2009a) and use in leather processing cattle (Ţârlea *et al*., 2009b), which have been shown to be effective against *Aspergillus niger* species, but not against the *Trichoderma viride* species;

- Synthesis of new classes of tanning agents to replace chromium in tanning hides and furs (Crudu *et al*., 2008a,b; Crudu *et al*., 2010; Crudu *et al*., 2012; Deselnicu *et al*., 2008; Pruneanu *et al*., 2010; Pruneanu *et al*., 2011; Albu *et al*., 2011).

Several studies have been conducted on the use of natural products derived from plants treating leather and fur:

- Bayramoglu *et al*. (2010) from Ege University in Turkey used *Origanum* species to extract essential oils from plants through steam distillation process. It has been found that utilization of the essential oil of *Origanum minutiflorum* during pickling has antifungal activity and that its effect is improved with increasing concentration. As a result of this study, it was found that the wet blue leather specimens that were treated with 1% *Origanum sp.* essential oil showed antibacterial effect to Gram-positive bacteria. *Origanum minutiflorum* essential oil had an antifungal effect on wet blue. The bacteria are more resistant to the essential oils than yeasts and moulds. A concentration of 21% relative to the fleet of the essential oils of oregano from three different species

and fennel oil was tested for their antimicrobial activity along with 7-25% of phenol and 4-chloro-3-methyl-phenol as a commercial bactericides commonly used in the leather industry. The results showed that the three essential oils of oregano had a much stronger bactericidal activity than commercial and may find use as antibacterial agents in the leather industry.

- Use of essential oils in finishing leather and fur (Niculescu *et al*., 2015a; 2015b);
- Use of essential oils to stop the growth of fungi (Stević *et al*., 2014).

The aim of this work is to test antifungal activity of essential oils extracted from thyme (*Thymus vulgaris*) against *Candida albicans* and *Aspergillus niger* on sheepskin leather for footwear lining.

MATERIALS AND METHODS

Materials

Essential oil isolated from thyme *(Thymus vulgaris)* was obtained by hydro distillation in a continuous extractor type Clevenger (Berechet, 2015).

Sheep skins for footwear linings treated during fatliquoring operation with thyme oil 30g/1000g wet blue.

Biologic material: *Candida albicans 10231* and *Aspergillus niger ATCC 6275*.

Methods

Chemical composition of essential oils was determined by Gas Chromatography-Mass Spectrometry (GC-MS) with AGILENT 6890 N.

Antifungal activity against *Candida albicans:* The tests were performed in accord with Standard SR EN ISO 20645:2004 - Textile fabrics – Determination of antibacterial activity - Agar diffusion plate test. In each Petri dish Dextrose Agar Sabouraud culture medium was poured. Both samples treated with thyme essential oil and control samples were placed in each Petri dish in the center of the surface of the culture medium, and then the culture medium was seeded in 3 points around the sample, as an equilateral triangle. There have been two parallel samples. Petri dishes were placed in thermo-hygrostat at 30°C temperature and were analyzed after 3, 7, 14 and 21 days.

Antifungal activity against *Aspergillus niger:* The tests were made following standard ASTM D4576–86 (1996) - Standard Test Method for Mold Growth Resistance of Wet Blue. Petri dishes were placed in thermo-hygrostat at 30°C temperature and were analyzed after 3, 7, 14, 21 and 28 days.

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.

RESULTS AND DISCUSSION

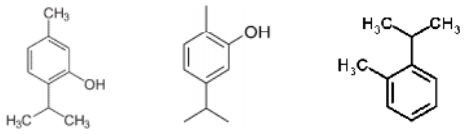
Chemical Composition of Essential Oils

Table 1 presents the components of thyme essential oil determined by GC/GC-MS chromatography.

Table 1. Main chemical compounds identified in *Thymus vulgaris* essential oil

No	Retention time, min	Compound	Formula	%	Kovats Indices, KI
1	23,060	Carvacrol (Antioxine)	$C_{10}H_{14}O$	57.355	$2{,}291x10^3$
2	12,940	Tymol	$C_{10}H_{16}$	32.430	$1{,}095x10^3$
3	11,240	o-Cymol	$C_{10}H_{14}$	3.993	$1{,}057x10^3$

Analysis allowed the identification of 16 components of which, carvacrol (57,355%) and Tymol (32,430%) and o-Cymol (3.993%) were detected as major constituents.



Tymol Carvacrol o-Cymol

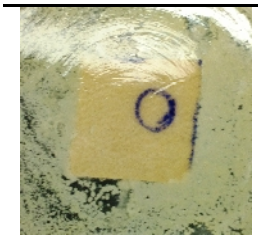
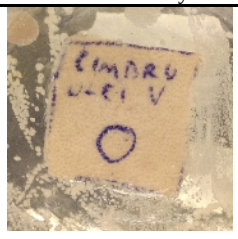
Figure 2. Main components of thyme essential oil

Thymol is a natural monoterpene phenol derivative of cymene, $C_{10}H_{14}O$, isomeric with carvacrol; Carvacrol, or cymophenol, $C_6H_3CH_3(OH)(C_3H_7)$, is a monoterpenoid phenol; o-cymol is *o*-Isopropyltoluol.

Antifungal Activity

Table 2 presents antifungal activity against *Candida albicans* after 3 days of incubation at 37°C of sheepskin leather treated with essential oil of thyme.

Table 2. Antifungal activity after 3 days of incubation at 37°C on sheepskin leather for lining treated with essential oil of thyme against *Candida albicans*

Control sample	Sheepskin leather for lining treated with essential oil of thyme
	
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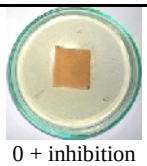
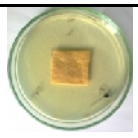
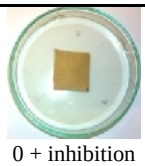
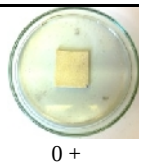
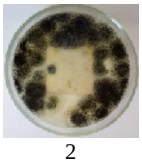
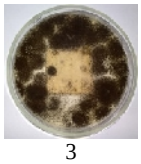
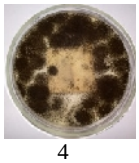
- : No growth, +: Mild growth, ++: Moderate growth, +++: Heavy growth

The results of tests made on sheepskins for footwear linings treated during fatliquoring operation with thyme oil 30g/1000g wet blue revealed that the number of *Candida albicans* colonies appeared on the culture medium is lower in treated leather with essential oil of thyme compared to the untreated control leather.

Table 3 presents antifungal activity of thyme essential oil against *Aspergillus niger* on sheepskin leather for footwear lining.

On the treated specimens with thyme essential oil no growth of *Aspergillus niger* can be observed anymore; thyme essential oil totally inhibited the grows of fungi.

Table 3. Antifungal activity of thyme essential oil against *Aspergillus niger* on sheepskin leather for lining

	3 days	7 days	14 days	21 days
Thyme essential oil				
Grade	0 + inhibition zone	0 + inhibition zone	0 + inhibition zone	0 + inhibition zone
Control				
Grade	2	3	3	4

CONCLUSION

The results obtained in this study show that *Thymus vulgaris* oil exhibited high antifungal activity against *Candida albicans* and *Aspergillus niger*. Thyme oil proved to be the best inhibitor of fungi conferred by high thymol and/or carvacrol content. This study suggests the possibility of using the thyme oil as natural leather preservative.

Acknowledgements

This study was funded by ANCSI in the framework of Nucleu Program INOVA-TEX-PEL, 2016-2017, project code PN 16 34 02 05, contract 26/14.03.

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