ANTIFUNGAL ACTIVITY OF THYME ESSENTIAL OIL ON WOOLEN SHEEPSKINS

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This work covers the chemical composition of thyme essential oil analyzed using GC-MS and its antifungal activity on woolen sheepskins. The main components of thyme oil were determined: carvacrol (57.355%), Tymol (32.430%) and o-Cymol (3.993%). The woolen sheepskins treated with thyme essential oil in a rate of 30g/1000g tanned furs were assayed for antifungal activity against *Candida albicans* and *Aspergillus niger*. These pathogens are very contagious and results in severe damage of leather/skins/furs. The woolen sheepskins treated with thyme essential oil 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 *Thymus vulgaris* displayed a moderate antifungal activity against *Candida albicans* and *Aspergillus niger* on woolen sheepskins.

Keywords: woolen sheepskin, thyme essential oils, antifungal activity, *Candida albicans*, *Aspergillus niger*

INTRODUCTION

Both in woolen sheepskins production and during use and storage, finished fur products may be damaged due to external factors and insects. These effects can be controlled or corrected by finishing and maintenance treatment of fur and fur products.

For protection against insects and fungi, insecticide and fungicide have to be applied very early. Several chemicals, so called fungicides and insecticides, have been used to prevent and kill fungi and insects in various environments. Although several synthetic preservatives have been used with good results, the disadvantages of using them are their potential negative effects on the environment, for instance toxicity, accumulation in soil and water and build-up of resistance in fungal and insect populations, limited their applications. In relation with that, the availability and legal acceptance of suitable preservatives differ from country to country (Deselnicu, D.C., 2014; Deselnicu, V. *et al*., 2014).

On the other hand, various different natural substances from plant extracts have been investigated in terms of fungal activities. The plants can act as the potent sources of biologically active substances which can be applied as preservative to protect woolen sheepskins and its products from biological deterioration (Niculescu *et al*., 2015).

Essential oils are liquid substances, with oily appearance, insoluble in water, soluble in alcohol and organic solvents, with the characteristic smell of volatile substances they contain. In terms of chemistry, volatile oils are complex mixtures of aliphatic and aromatic hydrocarbons, aldehydes, alcohols, esters and other constituents. Aromatic essences are extracted using three procedures: cold pressing, solvent extraction and water vapour distillation.

Volatile oils can be extracted from various parts of the plant, from flowers, seeds, leaves, stems, peels, roots, rhizomes, tubers, flower buds etc. Essential oils are very concentrated in active chemical elements and have various properties: they are antiseptic, antibacterial, immuno-stimulant, etc. (Constantinescu *et al.*, 2004; Stanescu *et al.*, 2004).

Due to the main disadvantages of the current preservative agents, the public demand has grown for more environmental friendly methods (Deselnicu *et al*., 2005; Deselnicu, V., *et al*., 2014). Therefore, it is important that more effective and less toxic naturally-occurring preservative agents with novel mechanisms of action be discovered and developed.

Many papers reported antifungal activity of Thyme, e.g.: effect of thyme on *Candida albicans* and moulds isolated from different sources (Radwan *et al*., 2014; Nzeako *et al*., 2006; Janssen *et al*., 1987); the use of essential oils for inhibition of fungi growth (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 woolen sheepskins and possibility for their application in the woolen sheep skins preservation. The research activity will be directed to discover a new, effective and environmentally-acceptable plant-derived preservative and the suitable preservative treatment method.

MATERIALS AND METHODS

Materials

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

Woolen Sheep skins treated during fatliquoring operation with 30g thyme oil /1000g fur tanned weight;

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 was poured Dextrose Agar Sabouraud culture medium. 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 a 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 Oil

Thyme essential oil was analysed by GC and GC/MS (Figure 1). Analysis allowed the identification of 16 components presented in Table 1 of which, carvacrol (57.355%), Tymol (32.430%) and o-Cymol (3.993%) were detected as major constituents (see Figure 2).

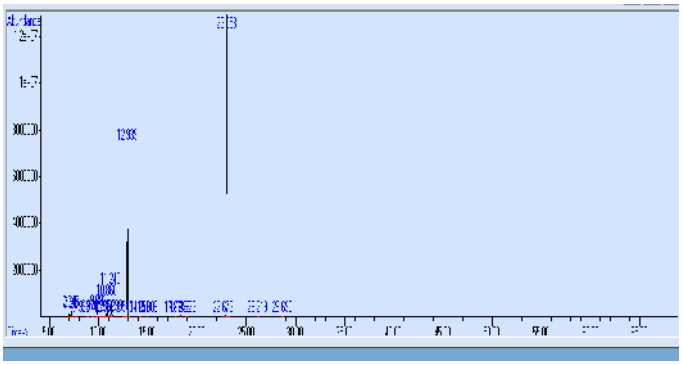
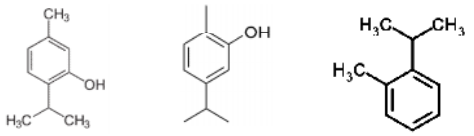


Figure 1. Thyme essential oil chromatogram

Table 1. Chemical compounds identified in *Thymus vulgaris* essential oil by GC-MS

No	Retention time, min	Compound	Formula	Area, %	Kovats Indices, KI
1	7,045	α-Phellandrene	$C_{10}H_{16}$	0,660	650,501
2	7,241	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl	$C_{10}H_{16}$	0,596	655,987
3	9,449	cis-2-Ethyl-2-hexen-1-ol	$C_8H_{16}O$	0,058	709,19
4	9,882	β-Pinene	$C_{10}H_{16}$	1,008	718,147
5	10,533	3-Carene	$C_{10}H_{16}$	0,044	$1,039x10^3$
6	10,860	Terpinolen	$C_{10}H_{16}$	2,319	$1,048x10^3$
7	11,240	o-Cymol	$C_{10}H_{14}$	3,993	$1,057x10^3$
8	11,400	Limonene	$C_{10}H_{16}$	0,318	$1,061x10^3$
9	12,542	2,7-Bis(spirocyclopropane) bicyclo[2.2.1]heptan-5-one	$C_{11}H_{14}O$	0,060	$1,086x10^3$
10	12,940	γ-Terpinen-	$C_{10}H_{16}$	32,430	$1,095x10^3$
11	17,774	3,4-Dimethyl-1H-pyrrole-2-carboxylic acid	$C_7H_9NO_2$	0,042	$1,18x10^3$
12	18,292	4-Terpineol	$C_{10}H_{18}O$	0,213	$1,578x10^3$
13	18,925	1,2,3,5,6,7-Hexahydro-inden-4-one	$C_9H_{12}O$	0,050	$1,591x10^3$
14	23,060	Carvacrol (Antioxine)	$C_{10}H_{14}O$	57,355	$2,291x10^3$
15	26,220	Neoclovene	$C_{15}H_{24}$	0,127	$2,595x10^3$
16	28,690	Seychellene	$C_{15}H_{24}$	0,156	$2,855x10^3$



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.

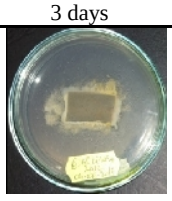
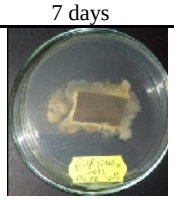
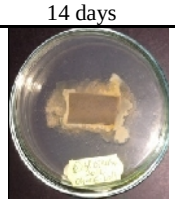
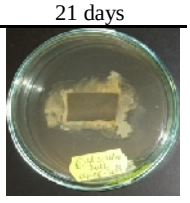
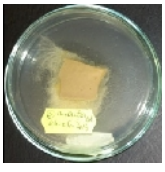
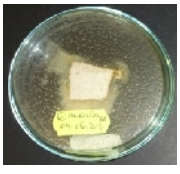
Antifungal Activity

The thyme essential oil was screened for its *in vitro* antifungal activity against *Candida albicans* 10231 and *Aspergillus niger* ATCC 6275.

Antifungal Activity against Candida albicans

During microbiological tests in the presence of *Candida albicans* it was observed that the first growth occurred only after 14 days in the control samples. *Candida albicans* formed pseudo-micelles (Table 2 and Figure 3). Samples treated with thyme essential oil completely inhibited the growth of *Candida albicans.*

Table 2. Antifungal activity of thyme essential oil against *Candida albicans*

	3 days	7 days	14 days	21 days
Thyme essential oil				
Control				

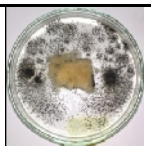
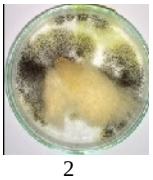
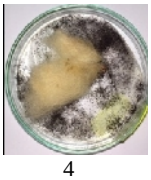
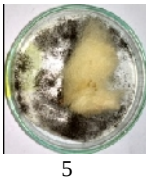
In conclusion, thyme essential oil may inhibit the growth of *Candida albicans* colonies for 14 days.

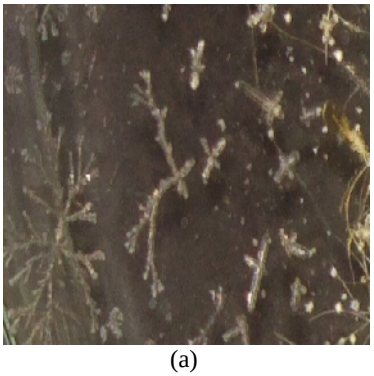
Antifungal Activity against Aspergillus niger

After 3 days, growth of *Aspergillus niger* occurred in Petri plates, but not on fur samples treated with thyme oil; there is an area of 5 mm inhibition around, which was kept till the end of the experiment (Table 3 and Figure 3b).

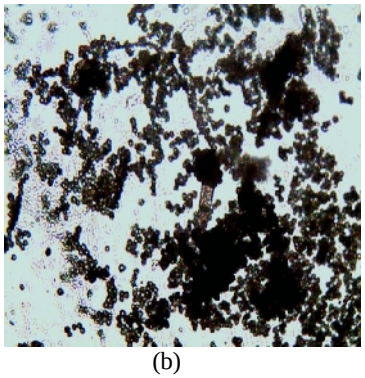
In conclusion, thyme oil has a mild inhibitory effect against *Aspergillus niger* on fur skins treated.

Table 3. Antifungal activity of thyme essential oil against *Aspergillus niger*

	3 days	7 days	14 days	21 days	28 days
Thyme essential oil					
Grade	0	0	0	0	0
Control					
Grade	2	3	4	5	5



(a)



(b)

Figure 3. *Candida albicans* filaments after 21 days (a), and *Aspergillus niger* colonies (b)

CONCLUSIONS

Thyme essential oil has high contents of cavacrol, thymol which displayed antifungal activity against yeasts and molds. The antifungal activity of thyme may be interpreted by reduction of ergo-sterol content: the major sterol content in fungal cell membrane (Pinto *et al.*, 2006).

In the field experiment, the active compound from thyme essential oil displayed potent activity against woolen sheepskins degrading fungi. The research results open the possibility to apply extract of *Thymus vulgaris* and their active compounds as natural preservatives. The extract and active compounds offer a safer application for human and

animals and are more environmentally friendly than chemical synthetic preservatives because they are easily degradable in nature during disposal.

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REFERENCES

Bayramoğlu, E.E., Gülümser, G. and Karaboz, I. (2008), "The Investigation of Antibacterial Activities of Some Essential Oils in Wet Blue Leather", *International Journal of Natural and Engineering Sciences*, 2 (1), 33-36.

Berechet, M.D. (2015), "Essential oils – Their Use in Practice and Research", Doctoral Thesis.

Constantinescu, D.G., Hatieganu, E. and Busuricu, F. (2004), *Medicinal Herbs Used in Therapeutics*, Medical Press, Bucharest.

Deselnicu, D.C. (2014), *European Policies for Products and their Relevance for the Footwear Sector* (RO), ISBN: 978-973-720-555-1 (245pg), Agir Press.

Deselnicu, D.C., Vasilescu, A.M., Purcarea, A.A. and Militaru, G. (2014), "Sustainable consumption and production in the footwear sector", *Leather and Footwear Journal*, 14(3), 159-180.

Deselnicu, V., Deselnicu, D.C., Vasilescu, A.M. and Militaru, G. (2014), "EU Policy for Sustainable Consumption and Production – EU Ecolebal for footwear", *Proceedings of the 5th International Conference on Advanced Materials and Systems* ICAMS 2014, 23-25 October 2014, Bucharest, 641-646.

Deselnicu, V., Maier, S.S., Deselnicu, O.C. and Florescu, M. (2005), "Impact of technological changes on increased health and comfort efficiency", *Proceedings of The 4th International Conference in Management of Technological Change*, Book 1, Chania, Greece, 87-92.

Janssen, A.M., Scheffer, J.C. and Baerheim Svendsen, A. (1987), "Antimicrobial Activity of Essential Oils: A 1976—1986 Literature Review. Aspects of the Test Methods", *Planta medica*, 395-398.

Nzeako, B.C., Al-Kharousi, Z.S.N. and Al-Mahrooqui, Z. (2006), "Antimicrobial Activities of Clove and Thyme Extracts", *Sultan Qaboos University Medical Journal*, 6(1), 33–39.

Pinto, E., Pina-Vaz, C., Salgueiro, L., Concalves, M.J., Costa-De-Oliveira, S. *et al*. (2006), "Antifungal activity of the essential oil of Thymus pulegioides on Candida, Aspergillus and dermatophyte species", *Journal of Medical Microbiology*, 55, 1367-1373, DOI: 10.1099/jmm.0.46443-0.

Radwan, I.A., Abed, A.H., Abeer, M.R., Ibrahim, R.A. and Abdallah, A.S. (2014), "Effect of thyme, clove and cinnamon essential oils on Candida albicans and moulds isolated from different sources", *American Journal of Animal and Veterinary Sciences*, 9(4), 303-314.

Stanescu, U., Miron, A., Hancianu, M. and Aprotosoaie, C. (2004), *Medicinal Herbs from A to Z*, Gr. T. Popa Press, Iasi.

Stević, T., Berić, T., Šavikin, K., Soković, M., Gođevac, D., Dimkić, I. and Stanković, S. (2014), "Antifungal activity of selected essential oils against fungi isolated from medicinal plant", *Industrial Crops and Products*, 55, 116–122.