ASPECTS REGARDING THE EFFECTIVENESS OF VOLATILE OILS OF THE TANACETUM VULGARE KIND IN THE CONSERVATION OF HERITAGE OBJECTS

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The paper refers to a composition with antifungal and antibacterial effect in order to test new materials for preserving heritage objects on collagen support with bactericidal/antifungal role, essential oils-based from Vetrice (*Tanacetum vulgare*, *Compositae* family), having antifungal and antibacterial properties. The biocidal effect of plant extracts is due to the presence of constituent agents, such as alcohols, ethers, phenols, aldehydes, ketones, which makes them extremely effective against a wide range of microbial strains. The essential oil was obtained by boiling the plants through continuous hydrodistillation with Clevenger refrigerant. The testing of the antimicrobial efficiency of the plant extracts was performed on two strains of pathogenic fungi, respectively *Trichophyton interdigitale* and *Epidermophyton floccosum*. The microbial inoculum was mixed with the plant extract from various sources (leaves, flowers and mixed flower-leaves). All extracts were tested in duplicate according to the specific test standards, and the results were expressed as a mean percentage and logarithmic reduction between the readings on the two Petri plates corresponding to each sample. To quantify the antimicrobial efficacy, the degree of microbial and logarithmic reduction of each sample was calculated, relative to the initial cell concentration. The results of antimicrobial tests showed a high antifungal character of the extracts obtained from flowers, leaves and mixed flower-leaves.

Keywords: preserving heritage objects, antifungal and antibacterial effect

INTRODUCTION

The paper refers to a study on the antifungal and antibacterial effect of *Tanacetum vulgare* essential oil in the curative and preventive conservation of heritage objects on collagen support.

Animal hide, but also tanned leather, due to its structure and chemical composition, which includes a large amount of proteins (collagen, elastin, albumin), mucopolysaccharides, water, fat and mineral salts is an excellent culture medium for the development of biological factors-microorganisms (bacteria, molds), which damage both the surface and the structure of the skin.

Since ancient times, essential oils have been widely used for bactericidal, fungicidal, insecticidal, medicinal and cosmetic applications. They are currently used in the pharmaceutical, sanitary, cosmetic, agricultural and food industries. Plant-derived essential oils have great potential as antimicrobial agents against a wide range of pathogens. The biocidal effect of plant extracts is due to the presence of constituent agents, such as alcohols, ethers, phenols, aldehydes, ketones, which makes them extremely effective against a wide range of microbial strains. Fast and sensitive detection of dermatophytes is very helpful because most superficial fungal infections are caused by this group of fungi (Kupsch *et al.*, 2016; Garg *et al.*, 2009). Their exact

identification is essential for choosing the most appropriate antimicrobial treatment and for tracking the sources of infection, thus helping to avoid reinfection (Jung *et al.*, 2009).

Various emollient and conservation compositions based on lanolin, hoof oil, beeswax and hexane are known, with cedar oil as an antifungal preservative [British Museum dressing]. Research is also known on the use of lavender oil to preserve and protect the atmosphere in archives and libraries that are effective against insects and molds. It is known that in libraries and warehouses, collagen-based objects are subject to the attack of mold and bacteria due to variable temperature conditions, degradation of skin structure, changes in acidity.

Tanacetum vulgare (family *Asteraceae*), popularly called Vetrice is a species native to Europe (up to one meter tall, strong chrysanthemum-like scent, specific, dark green, with beautiful intense yellow flowers), but widespread also in the spontaneous flora of Asia and North America. This plant has been known since ancient times as a therapeutic remedy, being cultivated in gardens, having uses in medicine (antibacterial, antiseptic, anti-inflammatory) but also in cosmetics, food (spice), obtaining perfumes, insecticides, preservatives, all these properties being obtained due to its composition rich in bioactive components such as polyphenols, flavonoids and tannins. The basic chemical composition of the volatile oil from *Tanacetum vulgare* is complex, depending on the morpho-anatomical part of the plant (leaves or flowers; the stem has no volatile oils), climate (temperature, amount of precipitation) of soil, altitude, etc. For the extraction process, the plant was separated into flowers and leaves, the extractions being performed separately for each of them with the help of a Clevenger extractor. There are numerous studies on the composition of essential oils extracted from *Tanacetum vulgare* and which highlight the potential of their use especially in medicine, pharmacy and cosmetics (Muresan, 2015; Boucher *et al.*, 2017; Kumar *et al.*, 2016; Ulukanli *et al.*, 2017; Marian *et al.*, 2013).

The effectiveness of the active principles extracted *Tanacetum vulgare* has been studied on *Epidermophyton* and *Trichophyton* which are fungi that cause skin diseases in animals and humans. Conventional methods for identifying these fungi are quick and simple, but they are qualitatively comparable to molecular methods (Grumbt *et al.*, 2013). Both strains were selected due to their enzymatic structure.

Trichophyton interdigitale is an anthropophilic fungus that is a common cause of *tinea pedis*, especially vesicular type, *tinea corporis*, and sometimes superficial nail invasion. *Trichophyton interdigitale* is also responsible for the condition called “athlete’s foot” that affects the skin between the toes (Wisselink *et al.*, 2011).

Epidermophyton floccosum is an anthropophilic dermatophyte with a worldwide distribution and one of the most common causes of dermatophytosis in healthy individuals. It infects the skin (*tinea corporis*, *tinea cruris*, *tinea pedis*) and nails (onychomycosis). Infection is limited to the *stratum corneum* of the epidermis because the microorganism does not have the ability to penetrate viable tissues of the immunocompetent host (Gueho *et al.*, 2015).

The use of conservation products aims to lubricate the fibers of tanned leather, with a role in reducing fragility and the tendency to break, preventing them from sticking to repeated drying. The products offer softness, suppleness and reduce water absorption. The role of the products used in the conservation of heritage leather is to surround the fibrillar elements of the tanned leather, so as to give it the softness and elasticity

necessary for a proper use, and also to offer protection in case of fungicidal and bactericidal attacks.

EXPERIMENTAL PART

Materials

In order to test new materials for the conservation of heritage objects on collagenic support with bactericidal / antifungal role, essential oils were obtained from Vetrice (*Tanacetum vulgare*, fam. *Compositae*), a perennial plant that sows itself, having antifungal and antibacterial properties. The chopped plant, which can be dried or green, has been extracted in a Clevenger refrigerant. Essential oils are mixtures of 5, 10 to tens and even a hundred compounds. In an essential oil each compound has specific biological properties and participates in the overall harmonization of the odor and specific activity (antimicrobial, antifungal, regenerative, antibacterial) of the mixture. Extracts of essential oils with various origins were tested, as follows: Flower extract, with F notations; Leaf extract, with L notations; Mixed flower and leaf extract, with M notations. Each extract was tested in 2 concentrations, the dilutions being made in paraffin oil, respectively: 1% and 3%;

Method

Testing of the extracts was performed in a 1: 1 ratio with the microbial inoculum, for a final solution volume of 1mL (500 μ L extract as such or diluted + 500 μ L microbial inoculum). Fresh cultures were obtained from each strain, by growing in nutritious Czapek-Dox broth, at 28 °C for 14 days, representing the stock culture. For testing, two decimal dilutions in paraffin oil (10^{-2}) were made from each culture and the cell concentration in the inoculum used was 9.8×10^3 UFC / mL (Colony Forming Units) for *Trichophyton interdigitale* (with Ti notations). and 8.92×10^3 UFC / mL for *Epidermophyton floccosum* (with Ef notations).

The initial cell concentration was previously determined by decimal dilutions (10^{-4}) in sterile deionized water, and from the last dilution, for each strain, 100 μ L were taken and spread on Czapek-Dox agarized nutrient medium. The counts on the plate were performed after 24 h of incubation, these being kept as a reference for the cellular developments in the control sample from the sample set (taken as the sample performed in paraffin oil). Thus, plates with cell density similar to that of dilution 10^{-4} were considered to have similar UFC values (9.8×10^3 UFC / mL-*Trichophyton interdigitale* and 8.92×10^3 UFC / mL-*Epidermophyton floccosum*). This method was chosen due to the very low dilution (10^{-2}) performed in the case of the inoculum that was tested on extracts, respectively of a high cellular concentration, which makes it extremely difficult to count filamentous fungal colonies in plates with abundant development.

The experiments on extracts were performed in Eppendorf tubes, previously sterilized at 121 °C, for 15 min. The extracts were not sterilized, in order to avoid the volatilization of their active principles. The microbial inoculum was mixed with the plant extract, from various origins (flowers, leaves and mixed flower leaves), both the microbial inoculum and the extract having constant volumes (500 μ L), only the concentration of extract and paraffin oil varying in these 500 μ L. The microbial inoculum solution was prepared in paraffin oil to rule out its possible antimicrobial activity on microbial cells.

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RESULTS AND DISCUSSIONS

All extracts were tested in duplicate according to the specific test standards, and the results were expressed as a mean percentage and logarithmic reduction between the readings on the two Petri plates corresponding to each sample. Plate counts were performed after 24 h incubation to detect colony-forming cell units. Pictures of the Petri dishes were taken after 48 h incubation. To quantify the antimicrobial efficacy, the degree of microbial and logarithmic reduction of each sample was calculated, relative to the initial cell concentration (Table 1).

Table 1. Antifungal test results

Sample	Petri Image	Mean 2 plates	*R%	**Log ₁₀ red.	Source
<i>Trichophyton interdigitale</i>					
M		(9800+9800)/2 M=9800 colonies => 9,8x10 ³ UFC/mL	-	-	Untreated Sample
1		(13+13)/2 M=130 colonies => 13x10 ¹ UFC/mL	95,55	1,88	1% FTi
2		(14+14)/2 M=140 colonies => 14x10 ¹ UFC/mL	95,5	1,85	1% LTi
3		(19+20)/2 M=1950 colonies => 19,5x10 ³ UFC/mL	92,3	1,7	1% MTi
4		(3+4)/2 M=35 colonies => 3,5x10 ¹ UFC/mL	99,65	1,45	3% FTi
5		(0+1)/2 M= 5colonii => 5UFC/mL	100	3,26	3% LTi
6		(1+1)/2 M=10 colonies => 1x10 ¹ UFC/mL	99,9	2,99	3% MTi

Sample	Petri Image	Mean 2 plates	*R%	**Log ₁₀ red.	Source
<i>Epidermophyton floccosum</i>					
M		(8920+8920)/2 M=8920 colonies => 8,92x10 ³ UFC/mL			Untreated Sample
7		(1+1)/2 M=10 colonies => 1x10 ¹ UFC/mL	99,9	2,95	1% FEf
8		(12+14)/2 M=130 colonies => 13x10 ² UFC/mL	95,5	1,84	1% LEf
9		(14+16)/2 M= colonies => 15x10 ² UFC/mL	95,55	1,78	1% MEf
10		(2+2)/2 M=2 colonies => 2x10 ¹ UFC/mL	99,77	2,65	3% FEf
11		(1+1)/2 M=10 colonies => 1x10 ¹ UFC/mL	99,9	2,95	3% LEf
12		(8+9)/2 M=125 colonies => 12,5x10 ¹ UFC/mL	99,85	1,86	3% MEf

* Antibacterial activity ratio (R) $R = \frac{C_t - T_t}{C_t} \times 100\%$; 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. The percentage reduction can be interpreted in logarithmic reduction, as follows: 90% reduction = 1log reduction (1,000,000 cells reduced to 100,000 represents 1log reduction); 99% reduction = 2log reduction (1,000,000 cells reduced to 10,000 represents 2log reduction); 99.9% reduction = 3log reduction (1,000,000 cells reduced to 1000 represents 3log reduction); 99.99% reduction = 4log reduction (1,000,000 cells reduced to 100 represents 4log reduction)

** Antibacterial activity value (A), $A = \lg C_t - \lg T_t$ where A - is the antibacterial activity value; $\lg C_t$ is the average number of colonies of the two control samples after incubation,

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expressed as CFU/ml; $\lg T_t$ is the average number of colonies of the two control samples immediately after incubation, expressed as CFU/ml.

CONCLUSIONS

The results of antimicrobial tests presented a strong antifungal character of the extracts obtained from flowers, leaves and mixed flower leaves. The extracts obtained from the leaf showed antimicrobial activity at a concentration 3% better than 1%, this being confirmed on both strains. At the same time, it is observed that the sample with 3% leaf extract (L) has the same influence depending on the strain tested, *Trichophyton interdigitale* and *Epidermophyton floccosum*. At the same time, it is highlighted that the entire antifungal character is due to the extracts, and not to the paraffin oil, which does not inhibit the degree of cell viability.

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