Natural Extracts for preventing Artefacts Biodeterioration

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Abstract: Cultural heritage artefacts (by organic nature – leather, wood, bones, ivory, antler, etc.- by inorganic nature, as stone, metals, glass or clay or composite organic/inorganic artefacts, such as some pigments or other combination of organic/inorganic materials) belong to the world heritage, and their welfare should concern everyone (archaeologists, museum curators or scientists from other areas).
Fungi are found all over in nature and can cause undesirable effects on both organic and inorganic materials. Once infected the artefacts, the fungus will grow using as food the substrate or even airborne particles (depending on the type of artefact). Biodegradation of the artefacts can often lead to irreversible effects. The use of antifungal natural extracts was always a viable alternative to the use of harmful chemicals (that often affects the rest of the environment or even human health). For example, nettle or olive leaf extracts are used since ancient times as antifungals. This paper presents the obtaining and characterization of natural extracts with antifungal potential. The plants used are native in Romania (*Allium ursinum* and *Ocimum basilicum*), and extraction methods used solvents as little toxic as possible (water, ethanol/water mixture, ethanol) and different extraction conditions. Extracts were characterized using analytical techniques (UV-Vis and FTIR spectroscopy, GC-MS) and in terms of antifungal potential (using the diluted inoculum technique). The results obtained are promising, allowing us to hope for a natural extracts based recipe for the removal and prevention of biodeterioration of the artefacts.

Keywords: Natural extracts, characterization, biodeterioration.

Introduction

The term *cultural heritage* may be defined as a complex set of material signs (artistic or symbolic) – inherited from the past generation to each culture and, in consequence, to the whole of humankind (JOKILEHTO 2005). As this legacy is unique and irreplaceable, the responsibility regarding its preservation is placed on the current generation.
Legally speaking, *the cultural heritage* was defined in Article I of the 1954 Hague Convention (“movable or immovable property of great importance to the cultural heritage of every people, such as monuments of architecture, art or history, whether religious or secular; archaeological sites; groups of buildings which, as a whole, are of historical or artistic interest; works of art; manuscripts, books and other objects of artistic, historical or archaeological interest; as well as scientific collections and important collections of books or archives or of reproductions of the property defined above”) (UNESCO 1954) and in Article I of the 1970 UNESCO Convention (“'Cultural property' means property which, on religious or secular grounds, is

specifically designated by each State as being of importance for archaeology, prehistory, history, literature, art or science") (UNESCO 1970).

At international level, the state of the cultural heritage sites is supervised by UNESCO. At this moment, the *World Heritage List* includes 962 properties, considered by the World Heritage Committee as having outstanding universal value. The list includes 745 cultural, 188 natural and 29 mixed properties in 157 States Parties (UNESCO 2013).

Biodeterioration can be generally defined as "a decrease in the economic value of materials caused by biological organisms" (STEPHAN et al. 2011). Previously, Hueck proposed a definition rapidly accepted by the scientific community at that time: "any undesirable change in the properties of a material caused by the vital activities of organisms" (HUECK 1965, HUECK 1968). Regardless its definition, biodeterioration can cause irreparable damages to the artefacts. Biological agents that produce deterioration are referred to as biodeteriogens (LOGNOLI et al. 2002), and these range from microorganisms like fungi to higher plants and to animals such as insects and rodents.

From all the biodeteriogens threatening the artefacts, the present paper aims to discuss the problem of fungal infestation, fungi being often associated with the biodeterioration of organic and inorganic artifacts (URZI & DE LEO 2010). Fungi can affect various artefacts, ranging from stone materials (KUMAR & KUMAR 1989) to paper (KEOPANNHA 2008), textiles (ARANYANAK 1995) and even walls ((FLORIAN 2002).

Most encountered fungal species when speaking of biodeterioration are belonging to *Aspergillus Sp*. and *Penicillium Sp*. The literature data provides valuable results regarding the use of natural extracts as fungicides, as most of the side-effects of chemical can thus be avoided (AFIFI 2012, SATISH et al. 2007).

The aim of the present study was to investigate the potential fungicide effect of some native plants (*Allium ursinum* and *Ocimum basilicum*), using some simulated artifacts (FIERASCU et al. 2013).

Experimental

Materials

Allium ursinum (ramsons) was harvested from nature, being part of spontaneous flora. *Ocimum basilicum* (basil) was harvested from a private garden. The ethanol used for extracts was analytic grade, purchased from Merck Co. (Darmstadt, Germany). The water used for all the experiments was bidistilled water, obtained in our laboratory. In order to avoid the possible damage to real artifacts, simulated artifacts were used, as previously reported (FIERASCU et al. 2013). The extracts used for the study were obtained from fresh plant, following three methods: a) 20 g of finely cut plant material mixed in 700 ml of water, under heating and vigorous stirring (method a); b) 20 g of finely cut plant material mixed in 700 ml 1:1 ethanol: water solution, under vigorous stirring at room temperature (method b); c) 20 g of finely cut plant material kept in 700 ml 1:1 ethanol: water solution at 80°C for 2 hours (method c). The extracts obtained were given the following encodings: extracts obtained by method a - *Allium ursinum* - extract 1, *Ocimum basilicum* - extract 4; extracts obtained by method b - *Allium ursinum* - extract 2, *Ocimum basilicum* - extract 5; extracts obtained by method c - *Allium ursinum* - extract 3, *Ocimum basilicum* - extract 6.

Methods

For the UV-Vis analyses, a Perkin Elmer, Lambda 25 spectrophotometer was used. The FTIR analyses were performed on a Spectrum GX (Perkin Elmer) spectrometer by KBr pellet method, on the frequency range 4000–400 cm^{-1} at 4 cm^{-1} resolution. To obtain a high signal/noise ratio 32 scans were accumulated for each sample. The GC-MS analyses were performed on a Clarus 500 (Perkin Elmer) chromatograph, under the following working conditions: Elite 5 column (length 60 m, inner diameter 0.32 mm), injector temperature 280°C, column temperature T1=50°C, t1=2 min, r1=5°C/min, T2=280 °C, t2=15 min; GC-MS interface temperature 250°C, GC-MS source temperature 200°C, GC-MS source: EI (electron impact), electron energy 70 eV. All the obtained results were processed using professional data analysis software (Origin 8.0). In order to determine the biological contaminants present on the surface of the materials we used the diluted inoculums technique. For this type of analysis, samples are collected and suspended in sterile distilled water. The samples are inoculated at the surface of a solid growth medium in Petri dishes; the liquid is dispersed evenly on the surface of the plate (using a Drigalski rod, through tilt/rotation motions of the plate). The plates are incubated at 28°C for several days. The culture media used was solid Sabouraud (SS) (produced by INCDMI Cantacuzino, Bucharest, Romania).

Results and discussions

Analytical characterization

When speaking of natural extracts with antifungal activity, the literature data assigns the main role as antifungal agents to the terpene-type compounds (α-pinene, cineole, squalene, sesquiterpenes, etc.), terpene alcohols and derivatives (linalool, terpinenol, linalyl acetate, etc.) (ABAD et al. 2007). Firstly, the presence of terpenes was confirmed, in different amounts, in all the samples, according to Liebermann-Burchard's Test, using acetic anhydride and sulphuric acid (Merck Co., Darmstadt, Germany) (MIKAIL 2010). The blue-green ring appearing attested the presence of terpenes, especially in extract 6.
The obtained extracts were characterized by FTIR (Fig. 1, 2), UV-VIS (Fig. 3, 4) and GC-MS (Fig. 5, 6).

The FTIR results suggest the presence of specific characteristic compounds for each plant: for ramsons (Fig. 1), S=O appeared at around 1080 cm^{-1} and S at around 1260 cm^{-1}. The peaks at 2983 cm^{-1} are due to the C–H stretching symmetric of $=CH_2$, while the one around 1470 cm^{-1} to δ C–H deformation of $=CH_2$ (RAJAM et al. 2012); the basil extracts presents bands at 2915, 2850, 1720, 1624, 1470, 1350, 1275,1093,1045, 730 cm^{-1}, consistent with available literature data (BABA et al. 2012).

As visible from the UV-VIS spectra presented above (Fig. 3 and 4), all the extracts exhibits high absorbance in the area 200-300 nm (where most terpenes present absorbance) (COHEN et al. 2011). The sulphur compounds present in the ramsons extracts presents high absorbance in the range 200-300 nm (ILIC et al. 2010).

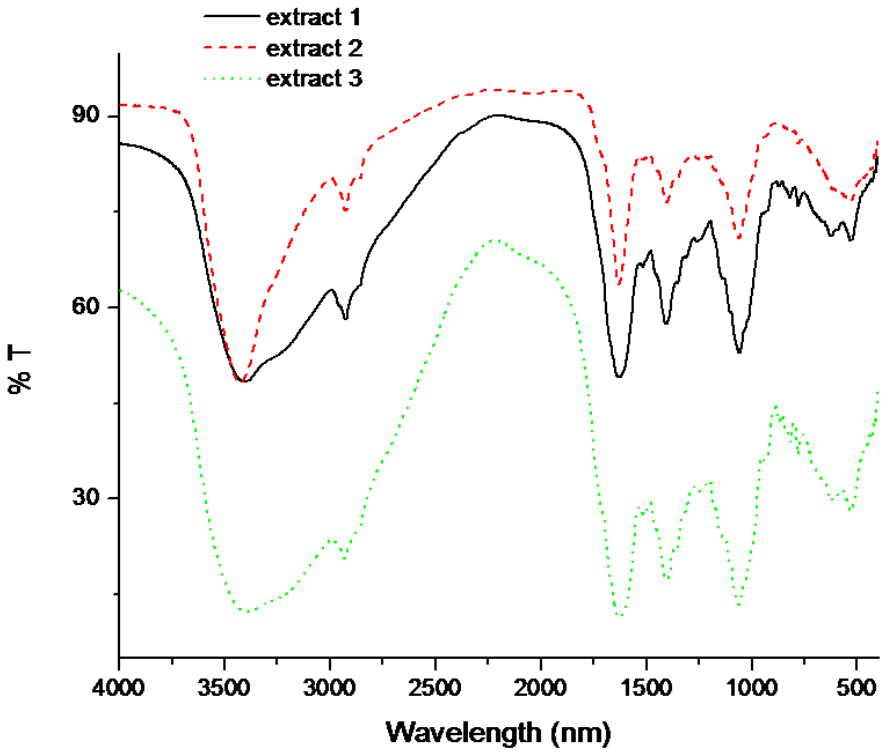

Fig. 1 – FTIR results for ramsons extracts

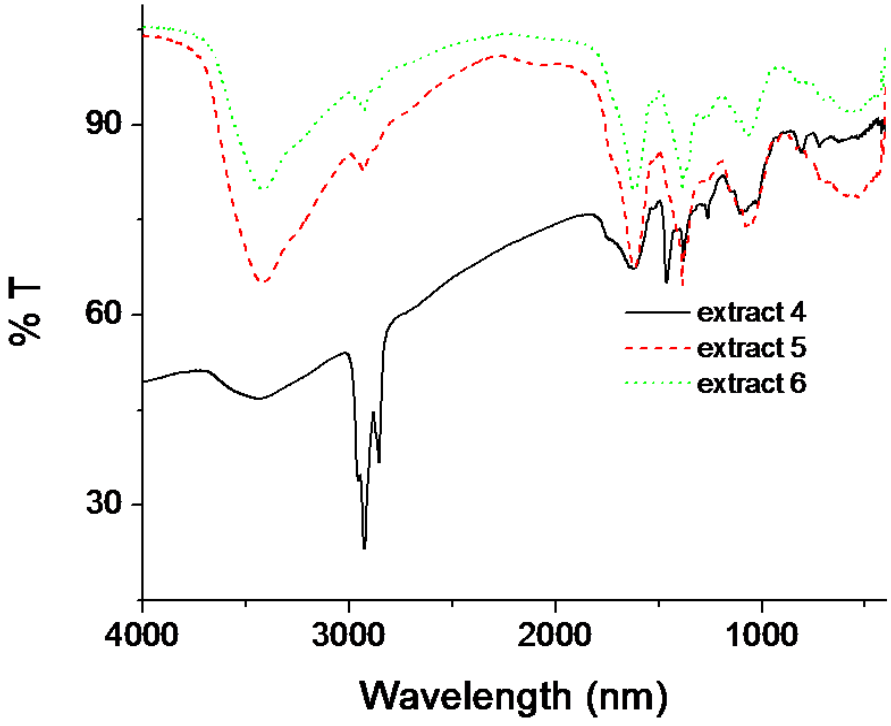

Fig. 2 – FTIR results for basil extracts

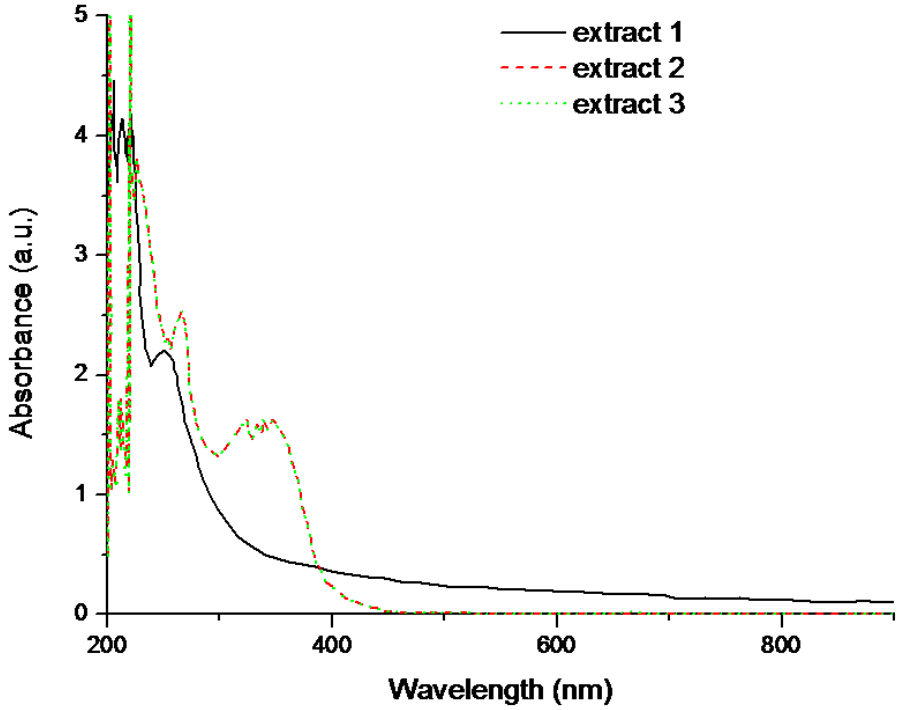

Fig. 3 – UV-VIS results for ramsons extracts

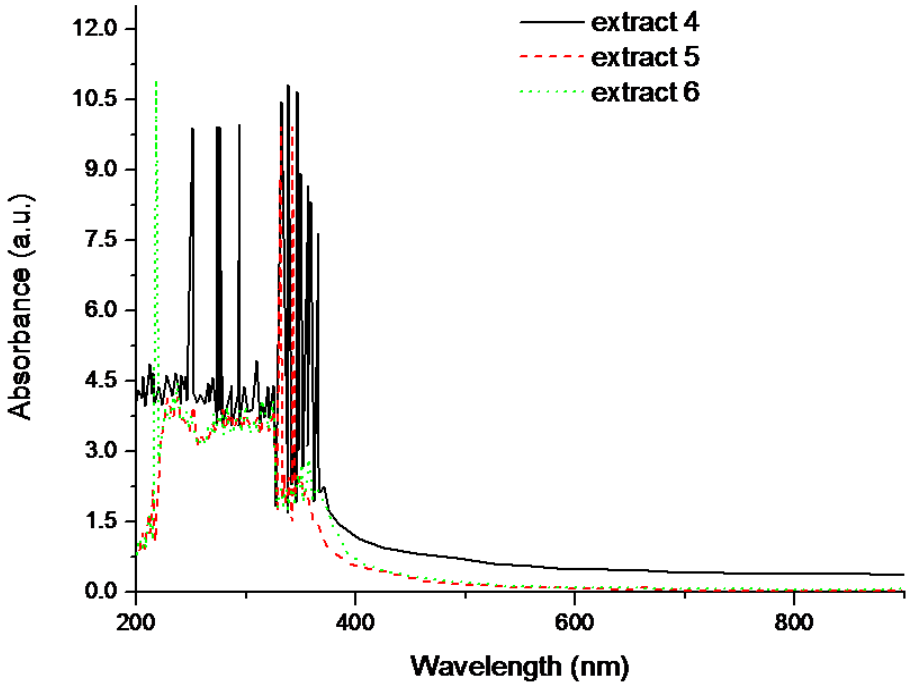

Fig. 4 – UV-VIS results for basil extracts

The GC-MS analyses (Fig. 5 and 6) revealed the presence in the ramsons extract (Fig. 5) of pyranone (18.668 min.), allyl methyl trisulphide (17.918), nonyl pentyl sulphide (24.830), dimethyl trisulphide (12.921), allyl trisulphide (22.604), prop-1-enyl dithiopropanonate (16.647), garlicin (16.187), diallyl disulphide (16.122) and furfural (9.599). For the basil extract, the analysis identified the presence of squalene (51.946), γ-

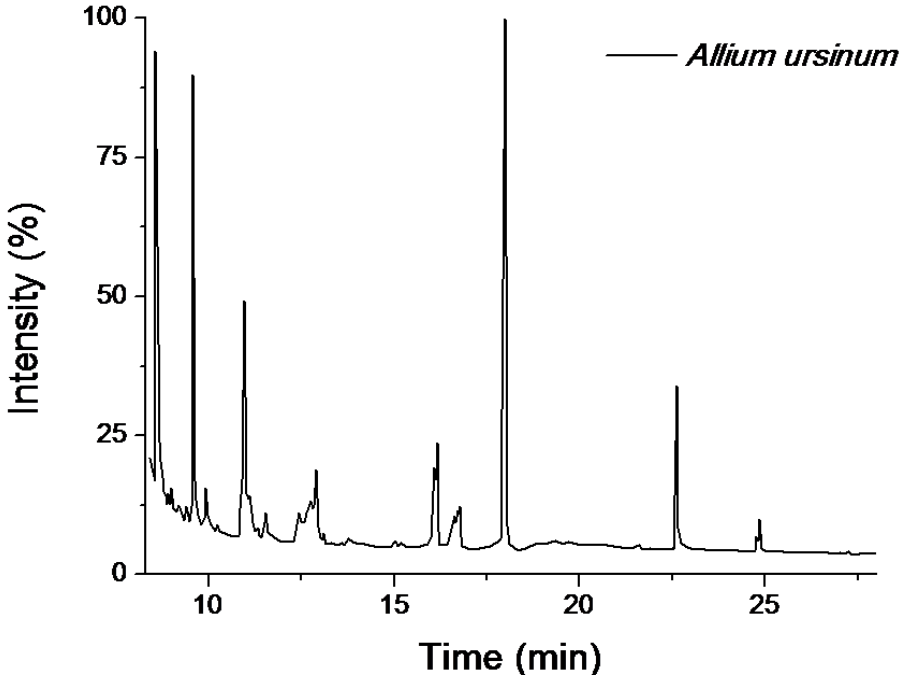

Fig. 5– GC-MS results for ramsons extract

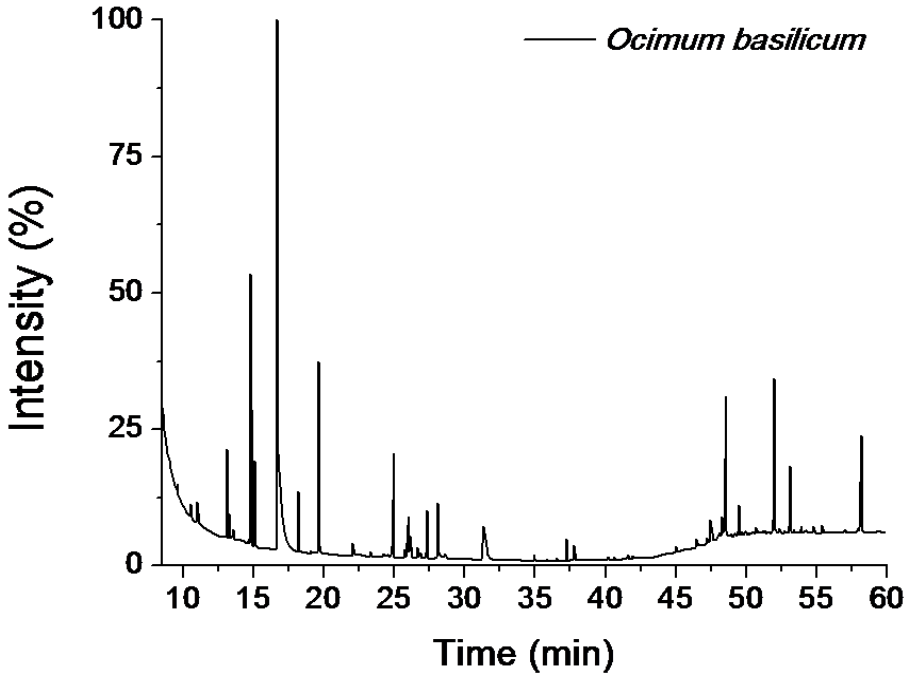

Fig. 6 – GC-MS results for basil extract

Cadinene (28.102), β-Cubebene (27.296), 2-Norpinene (26.011), levo-β-elemene (24.945), β-linalool (16.957), β-(E)-ocimene (15.047) and cineole (14.741) (Fig. 6).

Antifungal activity

The characterization of the obtained natural extracts in terms of antifungal action was realized using the diluted inoculums technique on culture media. The treatment was performed by pulverizing the simulated

artifacts samples with every extracts. After a period of 15 days, in which the samples were kept for 15 days in a dark and humid environment (favourable for the fungi growth) (FIERASCU et al. 2013), samples were collected in order to determine the efficiency of the treatment. The samples were inoculated at the surface of solid Sabouraud medium in Petri dishes. The plates were incubated at 28°C for seven days (except for blank sample, incubated for 120 h. and the sample treated with basil extract obtained by method c - extract 6 - incubated for 240 h.). All the experiments were carried out in triplicate. Representative results are presented in Fig. 7.

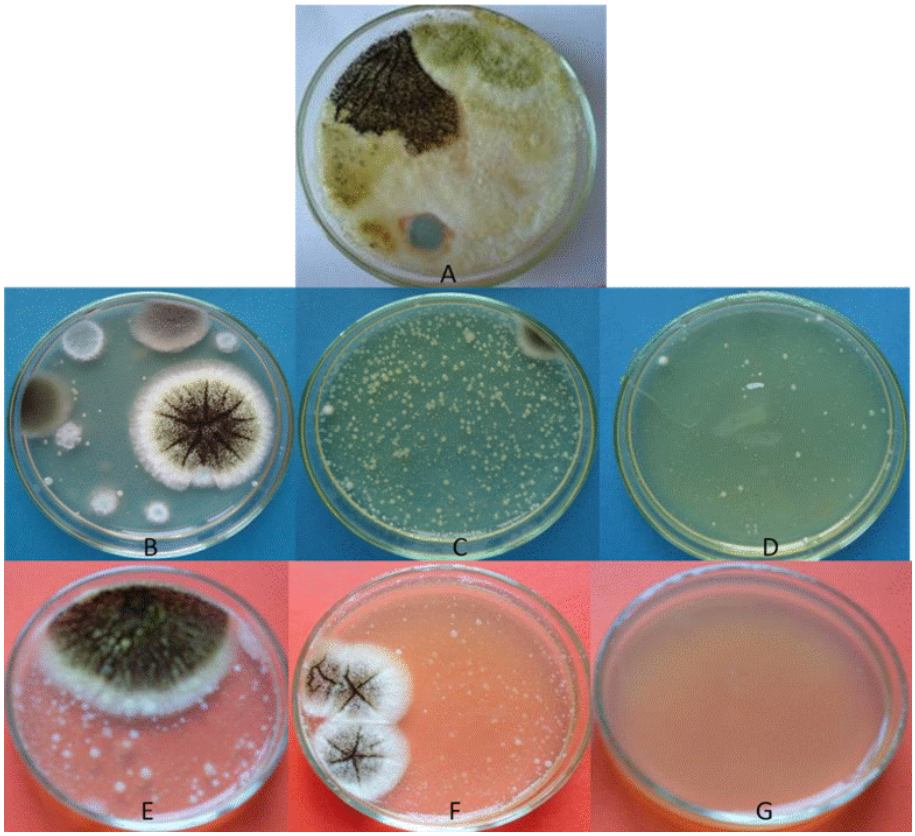

Fig. 7– Results for the fungal growth after treatment: a – control (blank sample), b – ramsons extract 1, c – ramsons extract 2, d – ramsons extract 3, e – basil extract 4, f – basil extract 5, g – basil extract 6

The incubation of the blank sample was stopped after 120 hours due to the extensive growth of fungal colonies. Sample G (sample after treatment with basil extract 6) was kept for 240 hours in order evaluate the time necessary for fungal colonies to appear. After that period, no colonies were identified.

From the results presented is visible the growth of significant fewer fungi colonies in all treated samples, as compared with blank sample. Even so, among the extracts, some can be distinguished as having very intense antifungal activity (extracts 3 and 6), some have a medium antifungal activity (extracts 2 and 5), while the rest (extracts 1 and 4) presents a weak antifungal activity.

From these results, some conclusions can be drawn:

- the aqueous extracts (method a) presents the weakest antifungal activity;
- the extracts obtained by method c presents the highest antifungal activity;
- the extracts obtained by method b presents a medium antifungal activity

Conclusions

The preservation of our cultural heritage is a task of utmost importance for the current generation. All the artifacts are subjected, in one way or another, to biodeterioration. As it is practically impossible to develop a universal method to prevent the damages caused by biodeteriogens, studies are conducted all over the world, driven by the need for new, less toxic and effective recipes. Among most aggressive and widespread biodeteriogens are found the fungi (*Aspergillus* being one of the most encountered fungi all over the world). The present paper describes the use of natural extracts for the protection of artifacts against biodeterioration. Native plants (*Allium ursinum* – ramsons and *Ocimum basilicum* - basil) were used and the extracts were obtained by following three possible routes, thus resulting in six extracts studied for their antifungal activity (using the diluted inoculums technique). The obtained extracts were analytically characterized (by FTIR, GC-MS and UV-Vis), even though the author did not aimed to fully elucidate their composition. After the analytical characterization of the extracts it is proved that the main role as antifungal agents is assigned to the terpene-type compounds (α-pinene, squalene, sesquiterpenes, etc.), terpene alcohols and derivatives (linalool, terpinenol, cineole, linalyl acetate, etc.)

The results obtained after treatment allows us to draw several conclusions regarding the possible use of some of the extracts: the extracts encoded 3 and 6 (obtained by method c) are the best candidates for the use as antifungal solutions; the extracts encoded 2 and 5 (by method b) exhibits a medium antifungal activity; the other two extracts presents a weak antifungal activity. The obtained extracts present good antifungal effect, even though there are not used essential oils, as the literature suggests.

The presented results allow us to hope in a very effective, less (or non) toxic recipe for the protection of the artifacts against biodeterioration.

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