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Scientific paper
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Natural extracts for solving the issue of biodeterioration of the artefacts

The biodeterioration of the artefacts is a worldwide problem. Each culture's cultural heritage is practically an act of identity; the way it is preserved is an important indicator of the degree of civilization of that community.

All the artefacts (organic, inorganic or mixed) are exposed to various environmental conditions (temperature, pH, humidity, aerobic or anaerobic conditions, etc) that can lead to mould growth. The growth of microorganisms on the artefacts deteriorates theme in various ways (crusts, patina, staining, discoloration, mechanical damage, fouling, soiling, etc.) and leads to tremendous economic and aesthetic loss. The fungal species found in the environment that contaminates objects (Aspergillus, Penicillium, Cladosporium, Aureobasidium, Mucor, etc.) are commonly referred to as environmental mould.

The aim of this investigation is the valorification of some natural selective extracts from plants native in Romania (Allium ursinum and Paeonia peregrina var. romanica) with antifungal properties for biological decontaminations of artefacts.

***Key words**: natural extracts, artefacts, biodeterioration*

INTRODUCTION

Over the years, the term *cultural heritage* changed its meaning (from monuments, archaeological sites and movable heritage collections) to include the tangible heritage (historic areas, vernacular heritage, cultural landscapes) and intangible heritage (living dimensions of heritage, aspects of the physical and spiritual relationship between human societies and their environment) [3].

The milestone of the conservation activities at international level is considered to be the *International Charter for the Conservation and Restoration of Monuments and Sites*, known as the *Venice Charter* 1964, which set the principles governing architectural conservation and restoration [8]. The Charter was since then used as a reference for many conservation documents all over the world [3].

The conservation principles and guidelines, derived from Venice Charter were adopted (as charters, recommendations, resolutions, declarations or statements) by the international organizations responsible for protecting the cultural heritage at international level (*UNESCO–United Nations Educational Scientific and Cultural Organization* and *ICOMOS – International Council on Monuments and Sites*) and ratified by State Parties all over the world. Currently, UNESCO's World Heritage Committee included on the *World Heritage List* 962 properties (745 cultural, 188 natural and 29 mixed) in 157 State Parties [15], properties that fulfil de requirements for their inscription on the World Heritage List, as established by the 1972 World Heritage Convention [15]. The Convention was ratified up to date by 190 State Parties.

The exposure of the artefacts to environmental conditions (temperature, pH, humidity, aerobic or anaerobic conditions, etc.) can lead to mould growth. The moulds can deteriorate objects through the formation of patina with different colours (from dark to green), crusts, staining, discoloration, mechanical damage, fouling, soiling and others.

The most common fungal species found on contaminated objects are *Aspergillus, Penicillium Cladosporium*, *Mucor* and *Aureobasidium* [2, 6, 12, 13].

The aim of this investigation was the valorification of some natural selective extracts from plants native in Romania (*Allium ursinum* – ramsons and *Paeonia peregrina var. romanica*- wild peony) with potential antifungal properties for removing the biodeteriogens affecting the artefacts. The obtained extracts were characterized using analytical techniques (using FTIR and UV-Vis techniques) and in terms of antifungal activity (using the diluted inoculum technique). The fungal species involved in the study were visualized using optical microscopy.

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MATERIAL AND METHODS

Material

Fresh plant material (*Allium ursinum* – ramsons, figure 1 and *Paeonia peregrina var. romanica* – wild peony, figure 2) were harvested from nature, being part of spontaneous flora.

Figure 1 - Allium ursinum – ramsons

Figure 2 - Paeonia peregrina var. romanica – wild peony

To prevent the deterioration of real artefacts, *simulated artefacts* were used, previously demonstrated to replicate the conditions of real infested artefacts [5].

All the reagents used were analytic grade (Merck, Germany). The bistilled water used was obtained in our laboratory.

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. For the visualisation of the fungal growth, a Novex B-series trinocular microscope was used (at a 40x magnification).

The treatments were performed by direct pulverization of the extracts on *simulated artefacts*; the artefacts were then kept for 15 days in conditions propitious for the development of fungal cultures. After this period, samples were collected from the artefacts and the efficiency of the treatment established by the diluted inoculums on the surface of culture media technique.

The technique of the diluted inoculums on the surface of culture media requires the collection of samples and their suspension 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, Romania).

RESULTS AND DISCUSSIONS

The extracts used for the study were obtained from fresh plant material, following three obtaining methods:

a) 20 g of finely cut plant material was mixed in 700 ml of water, under heating and vigorous stirring (*method a*);

b) 20 g of finely cut plant material was mixed in 700 ml 1:1 ethanol: water mixture, under vigorous stirring at room temperature (*method b*);

c) 20 g of finely cut plant material kept in 700 ml 1:1 ethanol: water mixture 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, *Paeonia peregrina var. romanica* - extract 4; extracts obtained by *method b* - *Allium ursinum* - extract 2, *Paeonia peregrina var. romanica* - extract 5; extracts obtained by *method c* - *Allium ursinum* - extract 3, *Paeonia peregrina var. romanica* - extract 6.

When speaking of natural extracts with antifungal activity, the literature data assigns the main role as antifungal agents to the terpene-type compounds, terpene alcohols and derivatives [1]. 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 [10], by the apparition of a specific blue-green ring.

The obtained results are presented in figures 3 (FTIR spectra) and 4 (UV-Vis spectra).

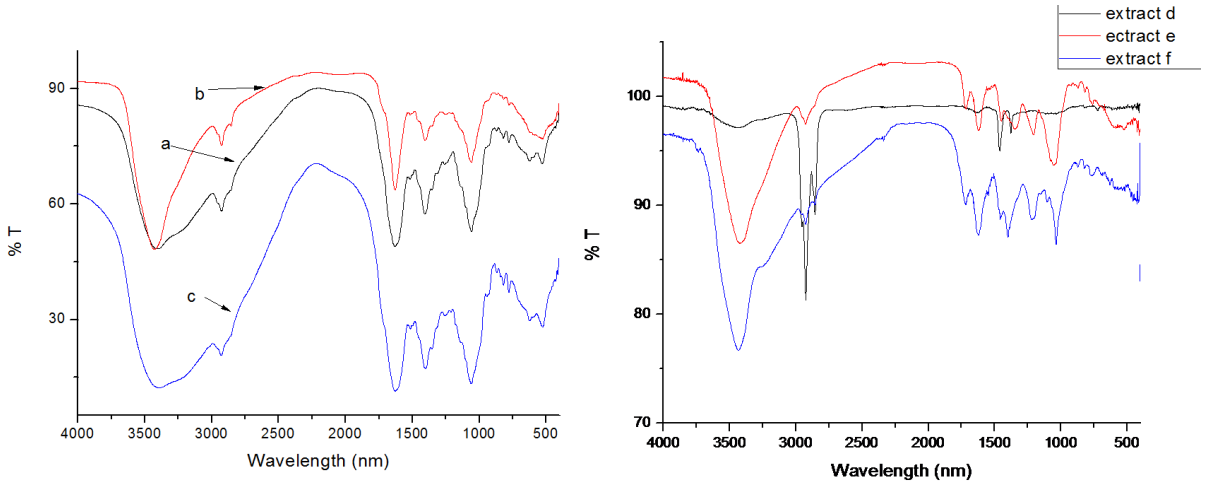

Figure 3 - FTIR spectra of the obtained extracts (left - Allium ursinum and right - Paeonia peregrina var. Romanica)

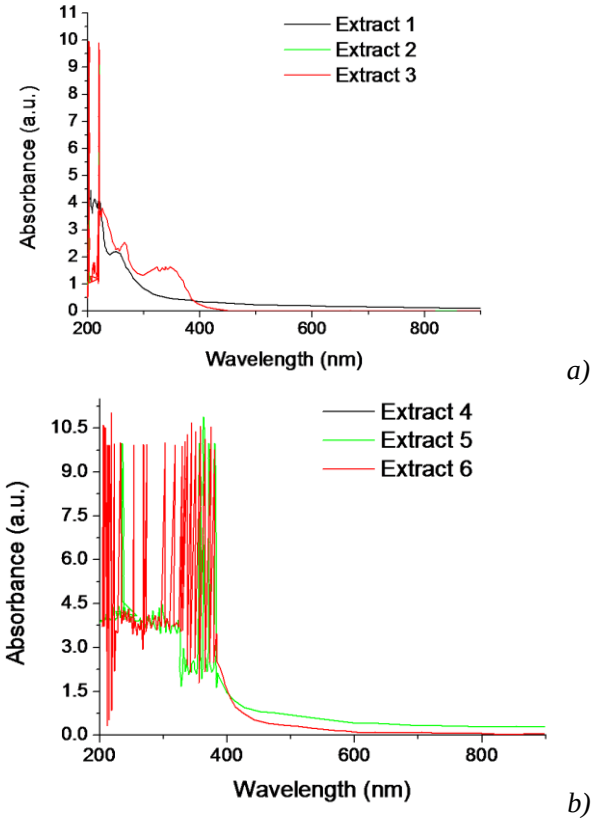

Figure 4 - UV-Vis spectra of the obtained extractc: a) - Allium ursinum and right, b) - Paeonia peregrina var. Romanica)

The FTIR results (figure 3) suggest the presence of specific characteristic compounds for each plant: for ramsons (figure 3 left), 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 $=CH_2$ [7,11,14]; the wild peony extracts (figure 3 right) presents characteristic peaks corresponding to the phenol around 3200 cm^{-1} and 1230cm^{-1}, phenyl CH stretchat 2930 cm^{-1}, ring stretch (benzene ring in aromatic compounds) at 1620 cm^{-1} and the C=O stretching at 1400 and 1030 cm^{-1} were observed, that literature data assign to the presence of paenol/paenoside/paeonolide /paenoniflorin in the wild peony extract [9].

As visible from figure 4, all the extracts exhibit high absorbance in the area 200-300 nm (where most terpenes present absorbance) [4]. The sulphur compounds present in the ramsons extracts presents high absorbance in the range 200-300 nm [7]. The wild peony extracts presents a peak around 530 nm, that literature data assigns to the presence of anthocyanins [17].

After the analytical characterization of the extracts, we focused on the evaluation of the antifungal activity of the selected natural extracts. The *simulated artefacts* were treated with the extracts and after 15 days samples were collected and incubated to evaluate the antifungal potential of the extracts. The incubation time for each sample was seven days, except for blank sample, incubated for 96 hours. The results are presented in figure 5.

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 intense antifungal activity (extract 3), some have a medium antifungal activity (extracts 2 and 4), while the rest (extracts 1, 5 and 6) presents a weak antifungal activity.

From these incubated samples, fungal colonies were collected in order to identify the fungal species involved. The funguses affecting the artefacts were

visually identified by their characteristics as *Aspergillus Sp.*, *Penicillium Sp.* and *Mucor Sp.*. In figure 6 are presented the microscopical images of these species.

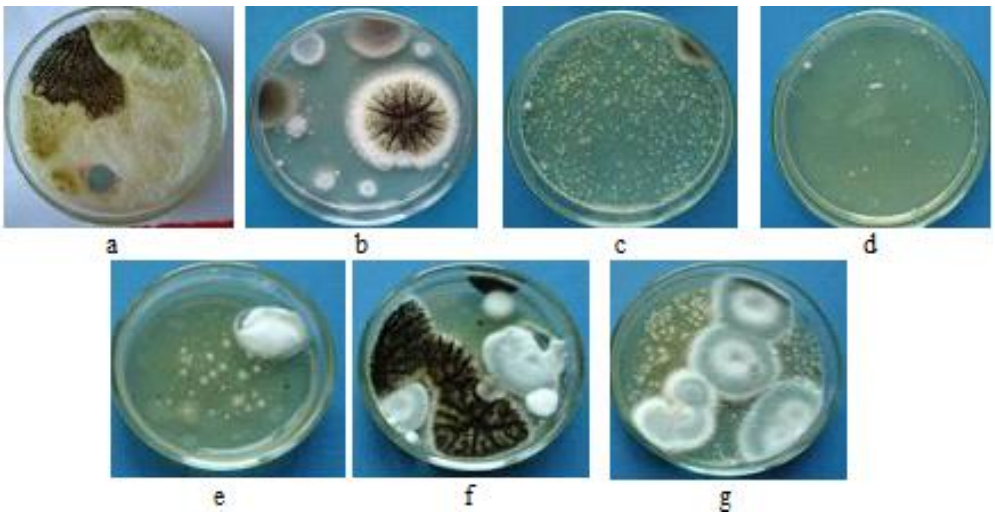

Figure 5 - Results obtained after treatment (solid Sabouraud medium): a) blank sample, b) to g) – samples treated with extracts 1 to 6

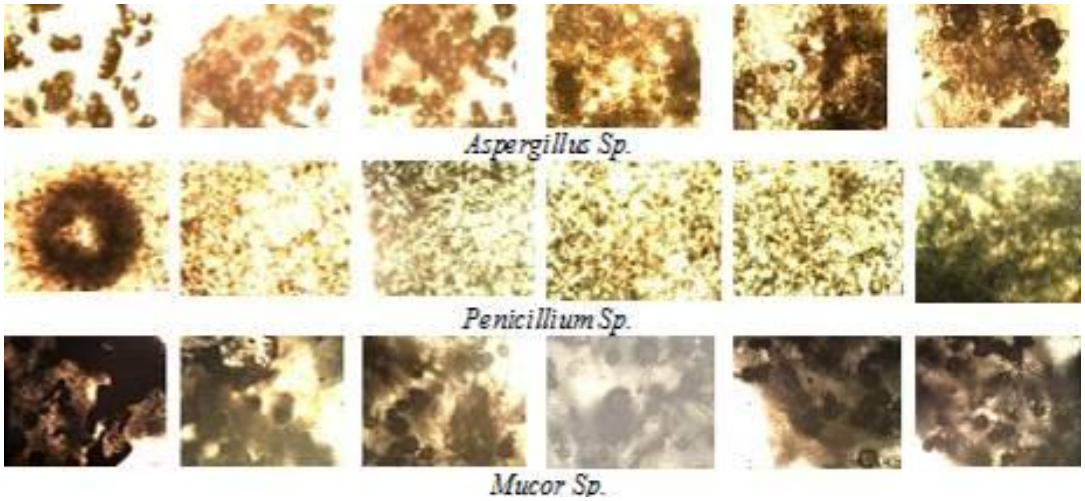

Figure 6 - Microscopical images of fungal species involved in the biodeterioration

CONCLUSIONS

The present paper described the obtaining (by three different methods) and characterization (by FTIR and UV-Vis techniques) of some natural extracts from Romanian native plants (*Allium ursinum* and *Paeonia peregrina var. romanica*). Their efficiency as antifungal materials was evaluated using the technique of the diluted inoculum on the surface of culture media. The best results on *simulated artefacts* were obtained using the following extracts (in the order of their efficiency): 3, 2, 4, 1, 6, 5. Also, the fungal species involved in the process of biodeterioration were identified as *Aspergillus Sp.*, *Penicillium Sp.* and *Mucor Sp.*

From this set of experiments, the extract of *Allium ursinum* obtained by *method c* seems to be the best candidate as antifungal agent. Further studies will be performed using the selected extract.

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IZVOD

PRIRODNI EKSTRAKTI ZA REŠAVANJE PITANJA BIOSTARENJA PREDMETA

Biostarenje predmeta je svetski problem. Kulturno nasleđe svake kulture je praktično čin identiteta; način na koji je sačuvano je važan pokazatelj stepena civilizacije te zajednice.

Svi predmeti (organski, neorganski ili mešoviti) su izloženi različitim uslovima dejstva sredine (temperatura, pH, vlaga, aerobni ili anaerobni uslovi, itd) koji mogu dovesti do rasta buđi. Rast mikroorganizama na predmetima pogoršava ovu pojavu na razne načine (koru, patina, bojenja, diskoloracija, mehanička oštećenja, zaprljanosti, oraštaji, itd), i dovodi do ogromnog ekonomskog i estetskog gubitka. Specijalne vrste gljiva pronađene u sredinama koje kontaminira objekte (Aspergillus, Penicillium, Cladosporium, Aureobasidium, Mucor, etc.) se obično naziva ekološki kalup.

Cilj ovog istraživanja je valorifikacija nekih prirodnih ekstrakata iz biljaka poreklom iz Rumunije (Allium ursinum and Paeonia peregrina var. romanica) sa antigljivičnim svojstvima biološke decontaminacije predmeta.

***Ključne reči**: prirodni ekstrakti, predmeti, biostarenje*

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