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Evaluating the exploitability of several essential oils constituents as a novel biological treatment against cultural heritage biocolonization

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ABSTRACT

This work aimed at evaluating the possible use of several organic compounds as novel conservation products against the biocolonization of cultural heritage materials. In a first step, the antifungal activity of 10 selected essential oil (EOs) constituents was tested against a strain of *Aspergillus niger* collected from a Roman mural painting (Pompeii, Italy). According to antifungal assays, thymol, eugenol and cinnamaldehyde provided a strong and enduring inhibition effect. These properties open the way to the possible exploitability of EOs constituents for middle and long-term protection applications. In this perspective, the three compounds were exposed to different light conditions with the purpose of assessing their stability under photo-oxidation conditions. After ageing, Fourier transform infrared spectroscopy (FTIR) analysis and *in vitro* antifungal assays were performed. The collected results proved that light exposure had a critical negative effect on the antifungal capability of cinnamaldehyde samples. On the contrary, composition and properties of eugenol and thymol were not affected by ageing, confirming their possible use in the future development of long-lasting conservation products.

1. Introduction

Biological colonization represents one of the most dangerous degradation processes jeopardizing cultural heritage. Several kinds of microorganisms, such as algae, bacteria, lichens and molds are capable of colonizing materials of cultural interest, thereby seriously endangering their integrity [1–4]. Among them, fungi have been widely recognized as major biodeteriogens of mural paintings and mortars [5–7]. Despite several fungi species are capable of colonizing walls and frescoes, most of the works published so far on this issue have detected the presence of *Aspergillus niger* strains [2,8,9].

One reason of the widespread of this biodeteriogen lies in its profuse germination of canidiospores (produced from filamentous structures called *Asci*) which, being distributed *via* the air, are able to colonize surfaces located at great distances. In addition, the great adaptability of *Aspergillus niger* makes it capable of tolerating and proliferating over a wide range of temperatures (between 6 and 47 °C) and pH (from 1.4 to 9.8) values [10].

In order to minimize the onset of irremediable damages to the colonized substrate, conservators need to perform specific preservation treatments. Nowadays, the most used method is based on the application of biocides products, followed by the mechanical removal of the treated biological patina. Several chemicals have been used for this purpose, such as acids [11], pyridines [12], quaternary ammonium salts [13] and organometallic compounds [14]. However, some of these products have been banned over time due to their associated environmental and health hazards. To regulate this field, the public administrations from the United States of America [15] and the European Union [16] intervened by adopting specific regulations that indicate all active substances legally employable to protect humans, animals, materials or articles against microorganisms.

Considering that both private companies and public institutions are researching in the development of new biocide products, the list of adoptable chemicals is constantly expanding. Among the products under evaluation to be included in the European Biocidal Products Regulation list (BPR, Regulation (EU) 528/2012) it must be underlined the presence of essential oils (EOs) such as *lavender* (CAS number: 1,245,629–80-4) and *eucalyptus* (CAS: 91,722–69-9) [16]. Their evaluation by competent committees is supported by several scientific works that have proved the effectiveness of those EOs against the growth of several kinds of fungi and bacteria [17–20].

Even though their use has been mainly tested for medical purposes [21–24], some conservation scientists are nowadays assessing the

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possible exploitability of EOs in the field of cultural heritage preservation [25–28]. For example, M. Stupar et al. [29] investigated the biocide capability of *Origanum vulgare, Rosmarinus officinalis* and *Lavandula angustifolia* EOs against fungal strains isolated from several sculptures and monuments, obtaining results comparable to those provided by commercial biocide products.

Despite the promising results obtained so far, it is important to underline that the biocide capability of EOs have been mainly tested *in vitro* rather than on real cultural heritage assets. This is due to the fact that several questions need to be answered before endorsing their use in real case studies. For instance, it is well known that EOs are a mix of tens of different organic compounds, but only a few of them have biocide properties. Whereas the conservation of cultural heritage must respect the principle of minimum intervention [30] (during the implementation of conservation works the use of unnecessary products must be avoided) the biocide activity of single EOs constituents needs to be deepened. Under this requirement, in the first part of this work a comprehensive literature review was carried out to identify the EOs capable of inhibiting the growth of *Aspergillus niger* [31]. Afterwards, the main constituents of each selected EOs were purchased and used to perform *in vitro* antifungal assays.

Furthermore, one of the main issues limiting the use of EOs and their constituents in this field is the lack of knowledge regarding their possible interaction with the environmental context. For this reason the second part of this work had the purpose of characterize the stability of EOs constituents under photo-oxidation conditions [32].

The experiments here summarized represent the first step of a new research line that seek to identify green, effective and stable compounds for the development of long-lasting conservation products capable of preventing biocolonization and biodeterioration of cultural heritage assets.

2. Experimental

2.1. Fungal strain isolation

In this work, the antifungal activity of selected EOs constituents was tested against a wild strain of *Aspergillus niger* collected from a mural painting conserved in the basement of the Ariadne House (Archaeological site of Pompeii, Italy). As shown in analytical work presented by Veneranda et al. (2017) [7], the painting stood out for the presence of critical biocolonization problems. Thus, a biopatina was sampled by means of a sterile swab and cultured in a PDA (potato dextrose agar) Petri dish. Then, metagenomic DNA was isolated using the soil DNA isolation commercial kit. To amplify the region of internal transcribed spacer (ITS), polymerase chain reaction (PCR) and secondary PCR procedures were performed as described by J.L. Leake et al. [33]. For the genomic characterization of the fungi species, the 18S ribosomal RNA gene region was amplified using a Inc. PTC-100 (MJ Research, USA) thermal cycler and DNA sequencing was performed by means of the ABI Prism 3730 DNA analyzer (Thermo Fisher Scientific, USA). Thanks to DNA sequencing and PCR amplification a 99% affinity with the *Aspergillus niger* standard was confirmed by comparison with the National Center for Biotechnology Information (NCBI) database [34]. Once isolated and genomically characterized, the wild strain of *Aspergillus niger* was finally used to prepare spore suspensions by flooding the culture with distilled water and filtering the solution through sterile absorbent cotton wools plugs.

2.2. Selection of antifungal EOs constituents

An exhaustive bibliographic review was carried out with the aim of identifying the main constituent of the EOs that, according to the work of V.C. Pawar and V.S. Thaker [36] provide the most remarkable inhibition effect against *Aspergillus niger*. Accordingly, the following compounds were purchased from Sigma-Aldrich Corp. (St Louis, MO, USA) and used for *in vitro* antifungal assays: thymol (99% purity, main constituent of *thyme* EO [35]), menthol (99%, from *mint sp.* [36]), linalool (97%, from *coriander sp.* [37]), eucalyptol (99%, from *eucalyptus sp.* [38]) cinnamaldehyde (95%, from *cinnamon sp.* [39]), eugenol (99%, from *clove sp.* [40]), cuminaldehyde (98%, from *cumin sp.* [41]), limonene (95%, from *citric plant sp.* [42]), citral (95%, from *lemongrass sp.* [43]), and citronellol (95%, from *rosae sp.* [44]). Their molecular structures is represented in Fig. 1.

2.3. Antifungal activity of EOs constituents

The antifungal activity assessment of fresh and aged compounds was carried out by disc diffusion method [45] and handled under sterile conditions in a Clase II laminar flow cabinet (BioSafety Cabinet Telstar Bio II Advance, Terrassa, Spain).

In order to evaluate the biocide capability of fresh compounds, the *Aspergillus niger* spore suspension was spread over the entire surface of the PDA Petri dishes by swabbing. For each biocide compound, four solutions (with a final concentration of 100%, 10%, 1% and 0.1% *w*/w respectively) were prepared using ethanol as solvent. The solutions were used to impregnate paper discs (about 6 mm diameter), which were finally applied on the Petri dish as represented in Fig. 2. The antifungal activity of the selected compounds was monitored for a month

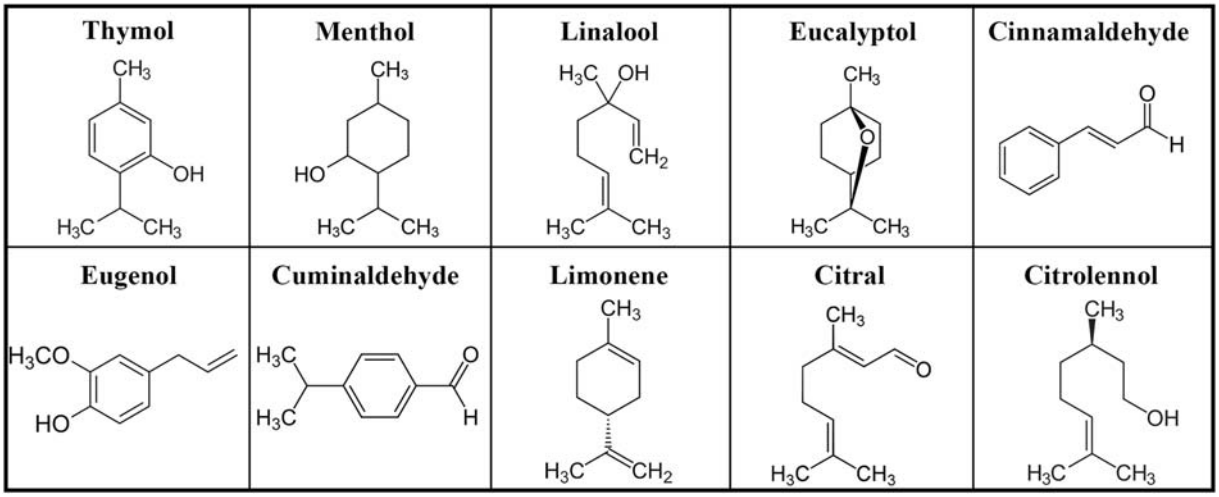

Fig. 1. Molecular structures of the selected EOs constituents.