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Review

Plant Essential Oils as Biocides in Sustainable Strategies for the Conservation of Cultural Heritage

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Abstract: Biodeterioration is a complex network of interactions between macro/micro-biological systems and organic/inorganic substrates involving physical and chemical alterations, strictly related to their metabolic activities. Concerning microbial deterioration, finding a correct approach to counteract this process is often difficult, requiring an understanding of the kind of alterations and the use of methods that respect artwork and human and environmental health. Specific conservative and remedial methods are used for this aim. They comprise physical, mechanical, and chemical methods, as well as, frequently, synthetic chemical biocides, which have obvious limitations because of their toxicity to operators or because they contain polluting substances that persist in the natural environment. New and alternative research has strongly focused on strategies to replace the use of toxic methods with natural products that do not have undesired effects, as well as implementing safe, novel compounds. Several plants contain natural chemical compounds such as oils, phenols, flavonoids, alkaloids, coumarins, tannins, etc., commonly used as drugs, bioactive molecules, and nutrients. Essential oils extracted from plants can be the correct way to prevent the biodeterioration of cultural heritage in a safe manner. This review aims to summarize the latest research on the use of natural essential oils in restoration procedures for cultural heritage, considering them sustainable means with respect to the environment and human health.

Keywords: cultural heritage; aromatic plants; antimicrobial activity; environment sustainability; human health

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1. Introduction

Ancient and modern cultural heritage includes buildings, monuments, artifacts, books, and works of art, as well as traditions, cultural knowledge, and natural heritage such as landscapes and biodiversity. Cultural heritage, such as buildings and residential/historic places or monuments, also comprise what is inside, such as stained-glass windows and frescos. Indeed, movable heritage assets include books, old documents, artwork, machines, clothing, etc. Therefore, cultural assets are made up of some of the most vulnerable materials.

All cultural heritage must be considered worthy of conservation for the future. In fact, it undergoes different deterioration processes induced by biological factors such as micro/macro-organisms (biodeterioration) and physical and chemical agents, both causing changes in the materials with evident aesthetic alterations [1]. Deterioration processes are influenced by several factors that are intrinsic to the materials, such as natural/chemical composition; extrinsic, such as environmental conditions (climate, temperature, lighting); and anthropogenic, such as the cleaning and maintenance methods.

Bacteria, fungi, cyanobacteria, lichens [2–7], mosses, and liverworts [8], as well as insects [2] and higher plants [9,10], can cause problems in cultural heritage maintenance and are found in indoor and outdoor environments [11]. As they contain inorganic compounds such as salts, paper and parchment undergo deterioration processes because of manufacturing procedures and/or inorganic substances in the ink. These impurities have a facility for microorganismal growth [4]. Fungi are the main cause of the evident aesthetic deterioration of paintings, textiles, ceramics, stone, wood, paper, and parchment. In fact, as biodeteriogens, molds and fungi are found everywhere in the historical and contemporary materials of libraries and museums and on stone monuments, frescoes in churches, and hypogeal environments [2]. In this review, we will focus on biodeterioration processes.

Macro- and microorganisms colonize cultural assets because of their metabolic activities and nutritional requirements, inducing different physical and chemical damages. Based on how biodeteriogens settle in artifact materials, different types of damage can be caused, as summarized in Table 1.

Analysis and monitoring measures are the main methods to cope with biodeterioration. The preservation of works of art in indoor environments, i.e., museums, churches, deposits, and libraries, can be implemented through climate control, deep cleaning, and constant microbial monitoring [12–14]. Monitoring the pollution and contamination of the indoor air of museums through aerobiological volumetric analysis was, for example, carried out in order to analyze fungi and other microorganisms at historical, artistic, and cultural sites [3,15,16].

Preservation outdoors is necessary in order to protect different constitutive materials (iron, bronze, marble, stone, etc.) from adverse weather conditions (storms, water, rain, wind, sun), as well as pollution, anthropogenic action, and micro/macro-organism activity (lichens, cyanobacteria, plants, etc.). Furthermore, in outdoor systems, although it may be difficult to monitor and control all the factors that impact the biodeterioration process, it is essential not to underestimate the significance of monitoring the condition of historical assets [17–19].

Table 1. Macro- and microbiological systems colonizing cultural objects and related induced damage to substrata.

Biological Systems	Physical Damages	Chemical Damages	Cultural Heritage Materials	References
Algae and cyanobacteria	Surface alteration	Stone fragmentation, staining	Stone, frescoes, and wood	[6,7,19]
Bacteria	Surface alteration	Release of acids (inorganic and organic), biofilm formation, chromatic alterations	Stone, frescoes, wood, paper, parchment, leather, fibers of vegetal and animal origin.	[2,3,11]
Fungi	Surface alteration	Release of acids (inorganic and organic) and pigments, chelating action	Stone, frescoes, wood, paper, parchment, leather, fibers of vegetal and animal origin.	[4,5]
Mosses and liverworts	Physical intrusion by rhizoids	Production of carbonic acid	Stone (natural, artificial)	[8]
Lichens	Cracks and fissures	Production of organic acids (highly corrosive)	Limestone, sandstone	[5]
Animals and insects	Weathering processes, holes, digging nets, structural damages	Release of metabolic activity products	Parchment and leather paper, stone, wood, fibers of vegetal and animal origin.	[2]
Higher plants	Cracks, detachment, structural changes	Roots excrete organic acids	Natural and artificial stone	[9,10]

Diagnosis reveals and identifies colonizing organisms with the help of biotechnologies through in vitro cultures, microscopic observations, and molecular investigations before decisions are made about treatment methods [20]. For instance, superficial colonies can be removed from the artwork with simple cleaning methods, while spreading colonization necessitates a severe biocide treatment [1].

Many techniques for removing harmful microorganisms have been considered by microbiologists and restorers. Recently, Cappitelli et al. [21] created an overview of the most applied and green methods used to control cultural assets' biodeterioration, including chemical (synthetic biocides), physical (UV exposure, gamma radiation, laser removal, heat, microwave [22], and biological (natural products) methods.

The use of proper biocides is regulated by the European Biocide Directive (98/8/EC: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1998L0008:20080926:EN:PDF, accessed on 14 April 2023). Unfortunately, traditional biocides are toxic to operators and the environment, have low long-term effectiveness, and are not specifically selective against microorganisms, sometimes also promoting biocide-resistant communities [21]. Despite their toxicity, traditional biocides such as formaldehyde releasers, quaternary ammonium compounds, and isothiazolinone, are still largely employed to prevent biodeterioration. Furthermore, gamma rays, which are very effective against fungi and their spores, affect many materials; thus, their application is restricted [4].

Plants contain many compounds and essential oils (EOs) mostly used as well-known biocides for medical/pharmaceutical and food uses [23,24]. In a recent publication, the authors summarized a large number of plants native to Ecuador but that are also diffused all over the world and that are considered important as traditional medical drugs, even if they contain volatile EOs and for which pharmacological/toxicological studies are still lacking [25]. Concerning the Lamiaceae plant family, several studies highlight the antimicrobial activity of its derivatives (EOs and hydro-alcoholic extracts) in counteracting microbial colonization and insect infestations in artifacts of an organic and inorganic nature that are exposed to indoor or outdoor environments [26–29]. The antimicrobial action of natural compounds is due to active phytochemical compounds, comprising EOs, phenols, coumarins, tannins, terpenes, and flavonoids, which are responsible for various pharmacological activities with different models of action [30–32].

In aromatic plants, the volatile secondary metabolites give off a distinctive smell/taste, which also characterizes their related natural products. Various extraction methods can be performed using water, aqueous and non-aqueous solvents, supercritical fluids, and organic and green solvents [33]. About 3000 EOs have been obtained from angiosperms such as the Asteraceae, Lamiaceae Myrtaceae, Rutaceae, and Zingiberaceae families [34].

These natural compounds do not alter the artwork's nature and are mainly non-toxic to humans and the environment, as they are an eco-sustainable approach [35], hence the idea of using these plant derivatives in the conservation and restoration of cultural heritage.

In this review, we report sustainable methods used for the control of the microbial biodeterioration of cultural heritage, evaluating plant extracts and their uses, particularly referring to the most recent research on EOs. Articles were collected from journals indexed in major databases, including Scopus (https://www.scopus.com/), Web of Science (https://www.webofscience.com/wos/), and PubMed at the NCBI (https://pubmed.ncbi.nlm.nih.gov/), using single and combined keywords and considering the most recent research.

2. Brief Historical Notes on Plant EOs

The history of EOs dates back many centuries, as they were used by Egyptian, Chinese, and Indian botanists and physicians for religious and medical purposes [36–38].

In the mural paintings found in the prehistoric caves of Lascaux, France, the use of medicinal plants was first witnessed. Egyptians used aromatic oils daily as early as 4000 B.C. Extracts from plants were used as medicinal products and ointments. The use of plant essences has been documented in China for 2800–3000 B.C. and in India with the medicine called "Ayur Veda". In Ayurvedic texts, over 700 natural products, such as cinnamon, ginger, myrrh, thyme, and sandalwood have been found [39].

The Greeks recorded knowledge of EOs in 400–500 B.C. For example, the physician Hippocrates (460–377 B.C.) noted the effects of many plant extracts. Galen was another Greek scholar who wrote several books, dividing medicinal plants into different medicinal categories, which are known as "Galenic preparations". Ibn Sina, an Arabic physician, used aromatics such as camphor and cloves for medical treatments. More recently, the French Chemist René-Maurice Gattefossé coined, in 1928, the term "Aromatherapie", indicating therapy based on the use of EOs as antiseptic treatments, which are nowadays well known [39].

In the present day, the medicinal properties of some families of plants are known (Apiaceae, Asteraceae, Cannaceae, Combretaceae, Euphorbiaceae, Piperaceae, Solanaceae, Rutaceae, Myrtaceae, Zingiberaceae, etc.) [34], but recently, research has focused on the newly discovered antimicrobial activity of EOs extracted from certain species [40]. One of the most well-known families of plants with antimicrobial activities in its EOs is Lamiaceae, or Labiateae, represented all over the world by about 3000 species and spread particularly throughout the Mediterranean area. This family includes herbaceous, annual, and perennial plants that are traditionally used as aromatic plants. Among them, the best known are basil (*Ocimum basilicum*), rosemary (*Rosmarinus officinalis*), oregano (*Origanum vulgare*), marjoram (*Origanum majorana*), sage (*Salvia officinalis*), thyme (*Thymus vulgaris*), mint (*Mentha piperita*), lemon balm (*Melissa officinalis*), nepitella (*Calamintha nepeta*), hyssop (*Hyssopus officinalis*), and lavender (*Lavandula angustifolia*) [41].

Medicinal plants have a prominent role in the development of newer drugs because of their effectiveness, low side effects, and low costs compared with synthetic drugs, exhibiting a protective function for humans. It is well known, based on traditional literature, that EOs obtained from plants have been used as spices, cosmetics, and therapeutic molecules, and they have antioxidant, anti-inflammatory, and anticancer effects [42–44]. EO extracts may be taken from different parts of plants, such as leaves, bark, aerial parts, flowers, fruits, and branches, with diverse properties and components [45].

3. Antimicrobial Effects of EOs: In Vitro Assays

Studies have been conducted in order to test the activity of many plant derivatives against microorganisms (such as fungi, bacteria, and insect pests) associated with the biodeterioration processes of cultural heritage [46]. Particularly, in vitro methods such as Agar Disc Diffusion and Well Plate Diffusion have been extremely useful in studying the effects of plant products on microorganisms grown in laboratories, and we will report in the following part on some of these studies.

The antifungal effects of *T. vulgaris* L., *Thymus tosevii* L., *Mentha spicata* L., and *M. piperita* L. (Lamiaceae family) EOs against micromycetes in food, plant, animal, and human pathogens have been tested, and the results demonstrated that *Thymus* and *Mentha* possess antifungal activity useful for natural fungicides [47]. In this study, EOs extracted from thyme, mentha, and a commercial fungicide (control) were used to test their antimicrobial activities. The results showed that the commercial fungicide had much lower antifungal activity than the EOs.

The antimicrobial effects of EOs extracted from cassia, eucalyptus, nutmeg, thyme, and sage were tested in vitro against *Alternaria alternata* at different concentrations. Antifungal activity was revealed for both cassia and thyme EOs, even if at different degrees of inhibition, with cassia oil being more efficacious than thyme oil. Long exposure for 3–6 days at the maximum doses induced the irreversible inhibition of fungal growth [48].

Some EOs containing terpenes and terpenoids show inhibitory activity against *Staphylococcus aureus*. In fact, carvacrol has specific effects on *S. aureus* and *Staphylococcus epidermidis*. Perilla oil suppresses the expression of the *Staphylococcus* enterotoxin A and B. Geraniol has shown good activity in modulating drug resistance in several Gram-negative bacteria species [49].

The antibacterial activity of EOs extracted from the leaves of the *Gliricidia sepium* tree (from Kerala, India) was assayed in vitro against *Escherichia coli* and *Pseudomonas aeruginosa*. Among the diverse concentrations of extracts, the highest concentration exhibited maximum activity against the bacteria [32].

Interesting results were obtained in a study involving three plant products, *Melaleuca alternifolia* (tea tree oil), *Allium sativum* L., and *Calamintha nepeta* L. extracts, which were assayed in vitro to determine their antimicrobial effects against four bacteria and fungi (*Bacillus subtilis*, *Micrococcus luteus*, *Penicillium chrysogenum*, *Aspergillus* sp.). In each case, there were differences in the effects on the microorganisms, as, for example, tea tree oil had biocide activity against *P. chrysogenum* but not against *Aspergillus* sp.; *C. nepeta* was active against bacteria, showing very low activity against fungi; a strong antimicrobial activity was performed by *A. sativum*, with biocide activity against *M. luteus* and *B. subtilis* [50].

The EOs extracted from *Cinnamomum verum* have an antimicrobial effect against the human pathogens *Acinetobacter baumannii*, *E. coli*, *S. aureus*, and *P. aeruginosa*. Most of the other oils (basil, clove, galangal, ginger, lemongrass, lime) used in this study were less effective against *P. aeruginosa*, while those obtained from basil, cinnamon, and clove had strong antimicrobial activity against *A. baumannii* at different concentrations [51].

Moreover, the antifungal activity of EOs from many Lamiaceae plants was assessed against fungal colonies, and more than half of these had good activity against fungi. The best activity has been attributed to EOs from *Clinopodium*, *Lavandula*, *Mentha*, *Thymbra*, and *Thymus* [52].

4. Antimicrobial Activity of EOs on Organic Cultural Asset

Generally, cultural heritage is made up of two main matrices: organic and inorganic. The organic ones include paper, parchment, textiles, wood, wool, and silk, while the inorganic ones comprise natural and artificial stones. Microbial colonization affects all these cultural assets. Therefore, in the following, for simplicity, we will address discoveries on the antimicrobial activity of EOs obtained by research on organic and inorganic cultural assets.

Extracts from plant matrices (*A. sativum* L., *Arctium lappa* L., *Centaurea cyanus* L., *Cichorium intybus* L., *Eucalyptus citriodora* Hook, *Medicago sativa* L., *Pinus caribaea* Morelet, *Piper auritum* Kunth, *Plantago major* L.) were tested for their antimicrobial activity against Bacillus sp. colonies, isolated from the surface of photographic paper stored in the Historical Archive of the Museum of La Plata, Argentina [53]. The different plant extracts showed diverse inhibition halos, with Eucalyptus, Arctium, and Piper being the most effective against bacteria. Lavin and colleagues [54] evaluated the antifungal activity of *O. vulgare* L. and *T. vulgaris* L. extracts against *Scopulariopsis* sp. and *Fusarium* sp. discovered on paper documents in the Historical Archives of Buenos Aires (Argentina). The authors found that the two essential oils tested completely inhibited fungal growth.

The antimicrobial activities of different EOs were analyzed against fungi and bacteria sampled from repository air and documents from the National Archive of Cuba and the Historical Archive of the Museum of La Plata, Argentina. The best fungicide activity was found after treatment with anise and garlic oils at all concentrations assessed, whereas oregano oil was also active in preventing fungus sporulation. Instead, laurel and orange sweet oils were ineffective in counteracting fungal growth. High antibacterial activity was shown by clove, garlic, and oregano oils against *Enterobacter agglomerans* and *Streptomyces* sp., while *Bacillus* sp. growth was effectively countered only by clove and oregano oils [55]. In addition, the antimicrobial activity of *Ocimum basilicum* plant derivatives (EOs and hydro-alcoholic extracts) was investigated against *Aspergillus* sp., *Penicillium* sp., and *Mucor* sp. colonizing paper artifacts. The authors tested EOs extracted with three methods, and the results highlighted that the most efficacious method against microorganisms was the one using a high temperature of extraction [56].

Casiglia and co-workers defined the chemical composition of the EO extracted from aerial parts of *Moluccella spinosa* (Lamiaceae) collected in Sicily, testing its antimicrobial activity against several Gram-positive and -negative bacteria (*Bacillus* sp., *Staphylococcus* sp., *Streptococcus* sp., *Klebsiella* sp., *Escherichia coli*, *Pseudomonas* sp.) on the yeast *Candida albicans* and on molds [57]. They found that *Bacillus cereus*, *B. subtilis*, and *S. epidermis*, infesting historical textile materials were affected by the treatment with this oil, showing reduced bacterial cell vitality.

Moreover, the chemical composition of the EO from *Anthemis secundiramea* Biv. collected in Sicily was outlined, and its activity against the bacterial and fungal colonization of historical art crafts was efficacious. The high antimicrobial activity of *Thymus capitata* L. on historical artifacts was also found because of its prevalent carvacrol composition [58,59].

O. vulgare EO antimicrobial activity was assayed against seven *Aspergillus* species colonizing different substrata (paper, silk, and stone) in Serbia, even comparing its biocide activity with commercial products. The anti-Aspergillus potential activity of the *O. vulgare* EO was higher than that of the commercial one [60].

Recently, Palla and co-workers assayed *O. vulgare* and *T. vulgaris* EOs against fungal colonization and insect infestation in wooden artifacts. A thyme EO was applied to counteract *Aspergillus flavus* colonies spreading on the base of a wooden sculpture, while thyme and oregano EOs were applied against *Anobium punctatum* insects infesting the heads of wooden puppets (18th century) [61] kept in the International Puppet Museum of Palermo, Italy. The results of these studies highlight that exposure to the EOs' volatile compounds strongly acts on the vitality of fungal colonies and as a repellent against insects.

T.vulgaris and *Pelargonium graveolens* EOs were assessed to restrict the growth of *Aspergillus niger*, *Penicillium chrysogenum*, and *Trichoderma viride* colonizing pinewood trees, and the results confirmed the potential use of EOs in the protection of wood to prevent mold colonization [62].

Regarding wood materials, Antonelli et al. [63] reported the use of three EOs (*Cinnamomum zeylanicum*, *Thymus serpyllum*, and *T. vulgaris*) as possible novel biocides on waterlogged, biodeteriorated archaeological wood. The efficacy of the oils was assessed via cultural analyses of microorganisms such as fungi (*Chaetomium* sp., *Fusarium* sp., *Aspergillus japonicus*, *Stachybotrys chartarum)* and bacteria and via molecular investigation through next-generation sequencing. The study showed that the EOs caused a significant decrease in fungi grown in vitro and decreased microbial colonization in archaeological wood.

Ferreira et al. [64] used *Lavandula luisieri* extract as an antimicrobial agent in preventing the deterioration of textiles, using it as a potential alternative to packaging materials usually utilized to preserve museum collections. In addition, *L. luisieri* EO, consisting of a camphor and balsam aroma, was demonstrated [65] to have antimicrobial properties against *Aspergillus* spp., *Candida albicans*, and bacteria *(Staphylococcus* sp. and *Streptococcus pyogenes*). The results emphasize the antifungal properties of these natural products.

A recent study focused on the disinfection of the Tholu Bommalu leather puppets (International Puppets Museum of Palermo, Palermo, Italy), colonized by fungi and bacteria. The artifacts were exposed to the volatile compounds of a *T. vulgaris* EO in an ad hoc Plexiglas chamber under controlled environmental and vacuum conditions. A less invasive but, at the same time, fast and replicable disinfection protocol was defined. The results showed that exposure to volatile compounds under vacuum was an efficient method, acting in reduced times compared with environmental conditions [29]. *T. vulgaris* extracts were successfully used to counteract microbial colonization in a wood sculpture from Mali (Africa) exposed at the International Puppet Museum of Palermo, Italy [28]. Specifically, by combining exposure to the EO with the preliminary application of a related *T. vulgaris* hydro-alcoholic extract, the antimicrobial activity of the EO was enhanced.

In a pioneer study, Gatti et al. [66] proposed the use of oregano and clove EOs in removing microbial stains (*Aspergillus*, *Penicillium*, and *Cephaloteca* fungal genera and the *Bacillus* bacterial genera) from the surface of a biodeteriorated canvas painting. Specifically, the volatile organic compounds of these EOs were used to remove the undesired layer, avoiding direct contact between the paint surface and the EO solutions.

5. Antimicrobial Activity of EOs in Inorganic Cultural Assets

EOs extracted from different plants (fennel, lemon, marjoram, rosemary, and spearmint) have been tested against yeast colonies found in limestone and granite blocks (Royal Tombs, Tanis, Egypt) at different concentrations; spearmint oil showed good antimicrobial activity against isolated yeast strains (*Candida albicans*, *C. lipolytica*, *Lodderomyces elongsporus*, and *Saccharomyces cerivisie*) [67]. The EOs of *Syzygium aromaticum*, *Citrus limon*, and *Menta* *piperita* exhibited high antifungal activity against *Aspergillus niger* and *Geotrichum candidum* growth, which are commonly found in buildings and their indoor environments [68]. Natural products from *Anethum graveolens*, *Cymbopogon citrates*, and *Juniperus oxycedrus* had good activity in controlling fungal colonization (*Alternaria alternate, Aspergillus niger*, and *Fusarium oxysporum*) in Egyptian stucco decorations [69].

The antimicrobial activities of *Lavandula angustifolia*, *O. vulgare*, and Rosmarinus officinalis EOs have been assayed against several fungal colonies (*Aspergillus niger*, *Aspergillus ochraceus*, *Bipolaris spicifera*, *Epicoccum nigrum*, *Penicillium* sp., and *Trichoderma viride*) isolated from stone and wooden substrata. The strongest antifungal activity was related to the *O. vulgare* EO, while moderate activity was shown by *R. officinalis* and *L. angustifolia* EOs [70].

In a case study by Rotolo et al. ("Casa di Leda", Greco–Roman site, Solunto, Italy) [26], a thick biofilm was revealed during the restoration project under the mosaic tesserae, causing tessera detachment. This biofilm was analyzed, and a complex microbial *consortium* was found containing bacteria (*Bacillus*), fungi (*Alternaria*, *Aspergillus*), as well as cyanobacteria (*Chroococcus*) and green algae (*Chlorella*). *T. vulgaris* and *O. vulgare* EOs were successfully utilized, preliminarily with in vitro and ex situ assays, and subsequently in situ tests, proving to be efficient against this complex biofilm.

Thymus capitata, an aromatic plant growing in the Mediterranean area with pharmacological properties, was collected on the island of Malta. The extracted EO (mainly composed of phenolic monoterpene carvacrol) was applied to counteract the microbial colonization of stone surfaces (cement grit, ceramic, marble), revealing good activity against green algae and cyanobacteria [71].

The pesticide efficiency of EOs extracted from different plants, including Agastache Gronovius, Hyptis Jacquin, *Lavandula* L., Lepechinia Willdenow, *Mentha* L., *Melissa* L., *Ocimum* L., *Origanum* L., *Perilla* L., *Perovskia Kar*., *Phlomis L.*, *Rosmarinus L.*, *Salvia L.*, *Satureja* L., *Teucrium* L., *Thymus* L., Zataria Boissier, and Zhumeria Rech (Lamiaceae family), was reviewed by Ebadollahi et al. [27], referring to the antimicrobial effects of their main chemical components, including terpenes and aliphatic phenylpropanoid.

Fidanza and Caneva [46] summarized the assessment of several EOs or other substances of plant origin, the different methods of application, and the tested organisms (algae, cyanobacteria, fungi), describing the efficiency of these products. They also highlighted the variability of results and analyzed both different methods of application and used doses, both with sometimes incoherent results.

Another interesting review describes some advancements regarding cultural heritage biodeterioration in stone monuments, first analyzing the microorganisms causing the deterioration of the cultural heritage, then the methods used to counteract the biodeterioration, and finally new antimicrobial applications such as green nanoparticles, plant EOs, and organic/inorganic biocides that are the most convenient for the safe conservation of cultural heritage [72].

The most recent studies concerning the most active known EOs with antimicrobial properties were summarized in detail by Winska et al. [40]. In total, 250 commercial EOs were analyzed, and more than 10 of these possessed high antimicrobial activity, correlated with aromatic, phenolin, and alcohol compounds. The time of action of these molecules is limited because of their high volatility. However, the low toxicity level of these natural products makes them a real alternative in the cosmetics and food industries [40].

An interesting study addressed the problem of the biodeterioration of natural cultural heritage sites such as caves because of the development of a great variety of microorganisms, including bacteria and fungi. The antimicrobial activities of eighteen EOs isolated from Greek plants were tested in vitro against microorganisms (bacteria and fungi) found in a Greek cave; the EOs of two *O. vulgare* plants showed the most inhibitory action on microbial growth [73].

Using an alginate hydrogel, encapsulating different concentrations of EOs, the authors developed a suitable protocol in order to reduce the volatility of the components. In fact, compared with *Lavandula angustifolia*, a *T. vulgaris* EO had the greatest inhibitory effect, especially if encapsulated within the hydrogel applied at different times to cyanobacterial biofilms in stone cultural heritage sites. This innovative protocol for in situ applications allowed for the definition of the concentration of EOs with antimicrobial efficacy and could also be applied to the vertical surfaces of cultural heritage sites for the removal of the biofilm [74].

6. Use of Mixed EOs Compared with Single Ones on Cultural Heritage and Food

Another important point to underline is the efficacy of plant pure essences compared with a blend of EOs. Several studies have reported different efficacies in the biological activity of EOs because of their diverse compositions in terms of natural chemical compounds [75]. EOs from thyme and oregano plants, containing terpens, have been reported as the most effective biocides when compared with other compounds contained in plants [70]. Eugenol isolated from volatile clove extracts mixed with emulsifiers was tested in situ on biofilms detected at two heritage sites in South Korea (Royal Tombs of the Joseon Dynasty) and Laos (Vat Phou temple). It showed great antifungal activity, reducing up to 80% of the microbial activity [76].

Recently, the antifungal properties of a new emulsion (Zege = Zeylantium green emulsion) based on *Citrus aurantium* L. *var. amara* and *Cinnamomum zeylanicum* (respectively, from flowers and bark) EOs were assayed via in vitro and in situ methods. It was effective against fungal colonies in modern paintings (*Rhizopus stolonifera*, *Penicillium* sp., *Alternaria alternate*, and *Aspergillus* sp.). The Zege emulsion showed encouraging results, confirming the in situ antifungal effectiveness [77].

EOs from basil, cloves, eucalyptus, thyme, pine tree, and tea tree were assayed in comparison with the traditional biocide Preventol®RI50 (ammonium quaternary salts) against a complex biofilm layer constituting pioneer microorganisms such as cyanobacteria, chlorophyte, and green algae (*Chlorella*) that colonize stone surfaces. They were applied to mosaics of the XIX room of the "Insula delle Muse" at the Archaeological Park of Ostia Antica (Rome, Italy). Interestingly, biocidal actions were revealed for all the tested products; however, EO mixtures had a synergistic and stronger biocidal effect compared with the single oils. The best results were found with thyme, pine tree, and tea tree products [78].

In a recent work, the authors evaluated the antimicrobial activity of EOs extracted from eucalyptus, lemongrass, oregano, peppermint, and rosemary plants against fungal colonies (*Cladosporium cladosporoides, A. fumigatus,* and *P. chrysogenum),* which are commonly found on papers in the Vojvodina Archives, Novi Sad (Serbia). Particularly, a mix in a 1:1:1 ratio of oregano, lemongrass, and peppermint was used in situ, demonstrating the inhibition of fungal growth on the archive papers [79].

Two mixtures containing EOs extracted from *T. capitatus* L., *S. aromaticum* (L.) Merr. and L.M.Perry, *C. zeylanicum* Blume, and O. *vulgare* subsp. *Hirtum* were applied with promising results to an outdoor marble statue in Silvanus (National Archaeological Museum of Florence) by using three different carriers (agar-agar, Politect®, and Carbogel) to enhance their action. This work represents an important precedent for in situ applications of EOs to stone monuments [80].

Synergistic or antagonistic effects can be the result of interactions between natural plant compounds and can be revealed by in vitro studies, especially those carried out on microorganisms colonizing food. Presently, studies regarding the synergistic antifungal effects of natural plant compounds have focused on the interactions of phenols, terpenes, and terpenoids. Specifically, the synergistic effect of linalool and caryophyllene has been evaluated, and a 10:1 ratio was the best dose for enhancing the antifungal activity of *Michelia alba* EO against *A. flavus* in brown rice [81]. Moreover, a synergistic effect showing enhanced efficiency against *A. flavus*, *A. parasiticus*, and *P. chrysogenum* was observed after combining oregano and thyme EOs. Furthermore, peppermint mixed with tea tree oils had a synergistic effect against *A. niger* colonizing food [82].

However, it is still difficult to predict the antimicrobial effectiveness of mixtures, as each EO is a complex combination of different chemical substances, and the interactions between single components can produce synergistic and/or antagonistic antimicrobial results, as reviewed by Tian et al. [83].

The huge number of scientific papers concerning the use of EOs and other plant derivatives highlight their potent effects as biopesticides for several industrial applications (agriculture, cosmetics, food, medicine, and pharmaceuticals). Plant derivatives are a source of biopesticides; valid alternatives to chemical synthetic products; and safe for humans, the environment, and cultural assets. Their use in the field of conservation and the restoration of cultural heritage, that is, the control of microbes spreading through cultural-heritage-dedicated environments (such as archives, deposits, libraries, museums, and exposition halls) is summarized in Table 2.

Table 2. Plants sources and common/family names and the constitutive materials of cultural artifacts on which EOs tested with related references. Scientific nomenclature can be referred to at http://www.worldfloraonline.org/tpl/kew-2757870 (accessed on 10 April 2023) and https://www.actaplantarum.org/flora/flora_info.php?id=8737 (accessed on 10 April 2023).

Plant Essential Oil	Common Name	Family Name	Artifact Material (References)
Allium sativum L. *Arctium lappa* L. *Cichorium intybus* L. *Centaurea cyanus* L. *Eucalyptus citriodora* (Hook) *Medicago sativa* L. *Plantago major* L. *Pinus caribaea* Morelet *Piper auritum* Kunth	Garlic Burdock Chicory Centaury Lemon-scent. gum Alfalfa Plantain Pine Mexican pepper	Amaryllidaceae Asteraceae Asteraceae Compositae Myrtaceae Fabaceae Plantaginaceae Pinaceae Piperaceae	Paper [53]
Thymus vulgaris L.	Thyme	Lamiaceae	Leather [29]
Origanum vulgare L. *Thymus vulgaris* L.	Oregano Thyme	Lamiaceae Lamiaceae	Paper [54] Wood [61]
Allium sativum L. *Citrus sinensis* (L.) Osbeck *Laurus nobilis* L. *Pimpinella anisum* *Origanum vulgare* L. *Syzygium aromaticum* (L.)	Garlic Sweet orange Laurel Anise Oregano Clove	Amaryllidaceae Rutaceae Lauraceae Apiaceae Lamiaceae Myrtaceae	Repository air and documents [55] Stone [80]
Ocimum basilicum L.	Basil	Lamiaceae	Paper [56]
Anthemis secundiramea Biv.	Coastal chamomile	Asteraceae	Historical art crafts [59]
Moluccella spinosa L.	Spiny lemon balm	Lamiaceae	Textile [57]
Thymus capitata L.	Headed thyme	Lamiaceae	Historical art crafts [58,80]
Origanum vulgare L.	Oregano	Lamiaceae	Paper, silk, stone [60]
Pelargonium graveolens (Thunb.) *Thymus vulgarus* L.	Geranium Thyme	Lamiaceae Geraniaceae	Wood [62]

Table 2. *Cont.*

Plant Essential Oil	Common Name	Family Name	Artifact Material (References)
Cinnamomum zeylanicum Blume *Thymus serpyllum* L.s.s, *Thymus vulgaris* L.	Cinnamon Wild thyme Thyme	Lauraceae Lamiaceae Lamiaceae	Stone [80], wood [63]
Lavandula luisieri L.	Lavender	Lamiaceae	Textiles [64]
Origanum vulgare L. *Syzygium aromaticum* L.	Oregano Clove	Lamiaceae Myrtaceae	Canvas paintings [66]
Citrus limon (L.) Osbeck *Menta piperita* L. *Foeniculum vulgare* Mill. *Origanum majorana* L. *Salvia rosmarinus* Spenn.	Lemon Spearmint Wild fennel Marjoram Rosemary	Rutaceae Lamiaceae Apiaceae Lamiaceae Lamiaceae	Egypt Royal Tombs [67]
Citrus limon (L.) Osbeck *Menta piperita* L. *Syzygium aromaticum* L.	Lemon Spearmint Clove	Myrtaceae Rutaceae Lamiaceae	Buildings [68]
Melaleuca alternifolia Sm.	Tea tree	Myrtaceae	Stucco [69]
Lavandula angustifolia Mill. *Origanum vulgare* L. *Rosmarinus officinalis* L.	Lavender Oregano Rosemary	Lamiaceae Lamiaceae Lamiaceae	Stone, wood [70]
Thymus vulgaris L. *Origanum vulgare* L.	Thyme Oregano	Lamiaceae Lamiaceae	Mosaic tesserae [26]
Thymus capitata L.	Headed thyme	Lamiaceae	Stone surface [71]
Lavandula angustifolia Mill. *Thymus vulgaris* L.	Lavender Thyme	Lamiaceae Lamiaceae	Stone [74]

7. Discussion, Conclusions, and Future Directions

Cultural heritage has a central role from historical, traditional, political, and social points of view. The real value of cultural and historical assets, present all around the world, is inestimable and must be preserved for future generations. The deterioration of cultural objects is strictly related to each geographical location and environment, and they also suffer from natural and anthropogenic processes. Processes induced by biodeteriogens are not only harmful to artwork but can be dangerous to operators, visitors, and restorers because of their allergenic potential and ability to cause infections in humans [84]. Human health risk assessment in cultural heritage is a topic that has been addressed by Pyzic et al. [85], who considered it an important point in the protection of people visiting or working in museums and other cultural heritage assets. Moreover, the use of commercial chemical biocides in order to counteract biodeteriogen growth is now highly discouraged because of their toxicity to the environment and human health. In fact, from this point of view, specific operators (conservators and restorers), in addition to visitors, are the most exposed to toxic biocide effects [4,86].

The application of control systems at cultural heritage assets first requires the continuous monitoring and then diagnosis of eventual biodeteriogens, i.e., knowledge of colonizing microorganisms as well as their interactions with heritage materials. However, monitoring and diagnosis are not always enough to control cultural heritage health, and treatments are sometimes needed or encouraged. The study of green restoration has focused attention on the effectiveness of various natural substances that have low environmental impacts and are safe for humans, in addition to being economical. According to the available literature, EOs are a potential alternative to synthetic biocides because of their natural and unharmful compositions [40,60,78,79].

This review intended to highlight some applications of EOs on cultural heritage assets, evaluating their antimicrobial activity. The antimicrobial activity of plant extracts is being explored, and more studies should be performed in order to evaluate their successful application regarding the biodeterioration of cultural heritage assets. Thus, further investigations should focus on EOs and microorganisms and their relationships with environmental factors such as light, temperature, oxygen, pH, humidity, etc.

Furthermore, there is no uniform literature on EO applications and no shared protocols for the research community. Moreover, it is very important to take into account the critical points of using EOs, such as their potential toxicity; their volatility and, consequently, their inefficacy; the concentrations and the timing of treatments; the opportunity to use mixed EOs (possibly more efficacious than single ones); and the methods of their use, as well as considering the different natures of the constitutive materials of cultural heritage.

Regarding the toxicity of EOs, Petraretti et al. [87] carried out experiments on EOs investigating their efficiency and ecotoxicological features. They evaluated the ecotoxicological profile of four fungal metabolites with potential antimicrobial properties regarding monumental artworks. They tested them on bacteria, algae, crustaceans, and nematodes, revealing the relatively lower toxicity of these compounds, especially when compared with commercial biocides used for the conservation of cultural heritage.

The use of green biocides such as EOs is a valid alternative to synthetic biocides, allowing us to control the spread of microbial communities in cultural heritage assets using low-toxicity products that are easy to handle and environmentally sustainable [88,89]. Gu and Katayama [90], in their book, illustrate a collection of articles focused on green and sustainable strategies for heritage conservation.

The use of green substances is also very important in preserving human health. Focusing on the colonizers of organic and inorganic cultural assets, almost all microbial species reported in this review may act directly as human pathogens or cause indirect opportunistic infections. In Tables 3 and 4, we reported microorganisms that contribute to the formation of consortia or complex biofilms in organic or inorganic substrata, representing potentially harmful agents to operator and visitor health.

Finally, we will report a brief analysis regarding the countries of origin of the studies on EOs included in this review. The results, shown in Figure 1, demonstrate that studies on EOs are most frequently from Europe (Italy, Portugal, Serbia, Malta, Polonia, Greece, and Romania) than other continents (America, Africa, and Asia). These findings show that research on alternative products to synthetic chemical biocides is needed around the world. Certainly, both the geographical area in which the artifacts are exposed and the correlated thermo-climatic environmental conditions must be evaluated, both for open-air and indoor cultural heritage assets.

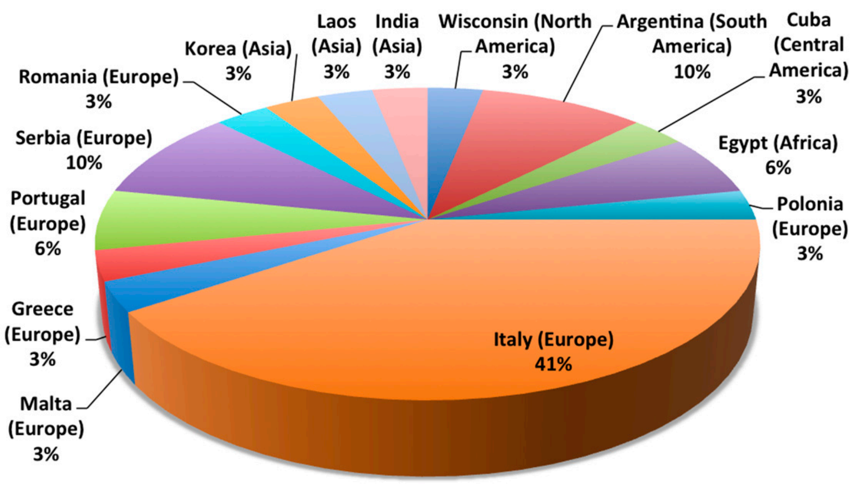

Figure 1. Pie chart of the countries included in this study.

Table 3. Microorganisms that colonize organic cultural assets that are potentially pathogenic to humans or cause opportunistic infection. The constitutive artifact materials and related references are also shown.

Pathogenic Organisms Colonizing Organic Cultural Assets		
Artifacts	**Species**	**Kingdom**
Wooden sculpture [28]	*Micrococcus* sp. *Aspergillus* sp., *Streptomyces* sp.	Monera Fungi
Leather puppets [29]	*Bacillus* sp., *Georgenia* sp., *Ornithinibacillus* sp. *Streptomyces* sp.	Monera Monera
Photographic [53] Paper documents [54]	*Bacillus* sp., *Scopulariopsis* sp. *Fusarium* sp.	Monera Fungi Monera
Repository air and paper documents [55]	*Enterobacter agglomerans*, *Bacillus* sp. *Streptomyces* sp., *Aspergillus* sp.	Monera Fungi
Paper artifacts [56]	*Aspergillus* sp., *Penicillium* sp. *Mucor* sp.	Fungi Fungi
Historical textiles [57]	*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus epidermis*	Monera
Silk, paper [60]	*Aspergillus* sp.	Fungi
Wooden sculptures [61]	*Aspergillus flavus*	Fungi
Pinewood [62]	*Aspergillus niger*, *Penicillium chrysogenum*, *Trichoderma viride*	Fungi
Paper artifacts [63]	*Chaetomium* sp., *Fusarium* sp., *Aspergillus japonicus* *Stachybotrys chartarum*	Fungi Fungi
Oil paintings [66]	*Bacillus* sp. *Aspergillus* sp., *Cephaloteca* sp., *Penicillium* sp.	Monera Fungi
Modern paintings [77]	*Rhizopus stolonifer* *Penicillium* spp., *Alternaria alternate*, *Aspergillus* sp.	Fungi Fungi
Historical papers [79]	*Cladosporium cladosporides*, *Aspergillus fumigatus* *Penicillium chrysogenum*	Fungi Fungi

Table 4. Microorganisms that colonize inorganic cultural assets that are potentially pathogenic to humans or cause opportunistic infections. The constitutive artifact materials and related references are also shown.

Inorganic Cultural Assets		
Artifacts	**Species**	**Kingdom**
Mosaic tesserae (Sicily) [26]	*Bacillus* sp. *Alternaria* sp., *Aspergillus* sp. *Chroococcus* sp. *Chlorella* sp.	Monera Fungi Cyanobacteria Green algae
Limestone, granite block [67]	*Candida albicans*, *C. lipolytica*, *Lodderomyces elongsporus*, *Saccharomyces cerivisie*	Protists
Building and indoor environment [68]	*Aspergillus niger*, *Geotrichum candidum*	Fungi
Stucco ornaments [69]	*Alternaria alternata*, *Aspergillus niger*, *Fusarium oxysporium*	Fungi
Cement grit, ceramic, marble [71]	*Apatococcus lobatus*, *Chlorella ellipsoidea*, *Nostoc* sp., *Chroococcus lithophilus*, *Gleocapsa compacta*	Green algae Cyanobacteria
Caves and hypogea [73]	*Firmicutes* sp., *Actinobacteria* sp., *Bacillus* sp. *Rhodococcus* sp. *Fusarium* sp., *Clonostachys* sp., *Acremonium* sp., *Doratomyces* sp., *Cephalotrichum* sp., *Cladosporium* sp., *Penicillium* sp., *Talaromyces* sp., *Xenoacremonium* sp.	Monera Monera Fungi Fungi Fungi Fungi
Mosaic tesserae (Rome) [78]	*Chlorella* sp.	Green alga

In conclusion, improvements in microbiological and biotechnological studies in the field of cultural heritage are encouraged focusing on obtaining standard procedures of EOs applications for cultural objects and concerning diagnostic methods and the monitoring of their long-term effective activities.

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