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Review

Stealing from Phytotherapy—Heritage Conservation with Essential Oils: A Review, from Remedy to Sustainable Restoration Product

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Abstract: Essential oils represent a good alternative to chemical biocides as they have antiseptic, antibacterial and antifungal properties, inhibiting the formation and proliferation of biofilms which can occur on outdoor and indoor surfaces of ancient and modern artworks. In this review, we illustrate how their antimicrobial properties, known since antiquity in phytotherapy, have been studied and tested for conservation purposes since the 1970s. In vitro tests on a wide range of plant extracts and in situ applications of specific volatile compounds have shown selective antibacterial and antifungal properties after the individual action of pure components or as the synergic effects of pre-determined mixtures. The review emphasizes the broad spectrum of materials—organic and inorganic—that essential oils can be applied to as biocides and finally emphasizes how the demand for commercial solutions has rapidly grown in bioconservation. The review demonstrates how research on the subject has been powerfully boosted by the ecofriendly and harmless character of essential oil applications, which makes them one of the most sustainable options in heritage conservation nowadays. The review elucidates how research is developing novel solutions for the application of EO blends—like encapsulation and microemulsions—and their optimization in commercial products for heritage conservation.

Keywords: essential oil; biocide; plant; cultural heritage; preservation; bioconservation; biodeterioration

Citation: Reale, R.; Medeghini, L.; Botticelli, M. Stealing from Phytotherapy—Heritage Conservation with Essential Oils: A Review, from Remedy to Sustainable Restoration Product. *Sustainability* **2024**, *16*, 5110. https://doi.org/10.3390/su16125110

Academic Editor: Asterios Bakolas

Received: 11 May 2024
Revised: 9 June 2024
Accepted: 12 June 2024
Published: 15 June 2024

1. Introduction

As Benelli and co-workers [1] state in their review on neem-derived products, the interest in the use of natural biocides started in the 1930s and developed until the 1950s, when synthetic insecticides were introduced. However, as sustainability and green practices have been playing a greater role in environmental care in the last two decades, more and more solutions have come from natural compounds. A recent review [2] reports that ~75% of the documents exploring the biopesticide potential of essential oils (henceforth, EOs) have been published in the last five years, with an annual growth rate of 20.51%. These choices also reflect the tendency to minimize the use and disposal of synthetic substances, whether toxic or polluting. As an example, natural biocides have been used to protect food supplies [3], and recent studies have suggested that they have good potential to sanitize virus- and/or bacteria-contaminated environments [4–6]. Contemporarily, the increase in insect resistance and the arrival of endogenous species in Europe, which has resulted in the ineffectiveness of traditional biocides and the use of massive dosages, has somewhat favored research into new-generation pesticides. In cultural heritage, it all began with the protection of cellulose-based materials colonized by microorganisms: since the 1970s, thymol has been applied in biocidal fumigation treatments for archival documentation [7,8]. However, the use of EOs on materials other than paper only started about two decades ago. Research at the MA and doctoral levels has promoted experimentation with pure oils or mixtures on several materials: stone [9–62]; paintings on different supports—paper [63], canvases [64–67] and walls [65,68–73]; and, more rarely, glass and metals [57,74,75].

And yet testing EOs in conservation activities is still rare, although this step is fundamental to validating protocols and comparing their efficiency with respect to traditional systems, usually chemical and often toxic products.

2. The Review Methodology

This review aims to report how EOs have evolved from remedies to sustainable restoration products and become a good alternative to chemical biocides because of their antiseptic, antibacterial and antifungal properties, which inhibit the formation and proliferation of biofilms. The review also highlights how EOs can bring other advantages, such as high volatility, negligible residues and environmental sustainability, and hence guarantee the safety of both professionals and artworks.

The review is meant to provide an overview of past and current methodologies that involve the use of EOs as biocides, with a special emphasis on those that are commercially available, and their potential. The research was conducted using several online database systems, such as PubMed, Google Scholar, ResearchGate, AATA-GC, Publons and the ICCROM Library, using the following keywords: essential oils, plants, cultural heritage and preservation. We narrowed the focus of the investigation to open-access and digitalized publications with the aim of fostering open science.

The review essentially builds on two questions to advance research on the use of EOs in heritage conservation: What have we—present-generation conservators and conservation scientists—learnt from phytotherapy? Can we provide efficient sustainable options to next-generation conservators and conservation scientists? Conceived as a chronological journey to report on past, current and developing trends, the review (1) begins with a brief history of phytotherapy, (2) describes our current knowledge of EOs and their biological activity and (3) focuses on the most recent research progress in heritage conservation to (4) describe current practices and predict future directions.

3. EOs Serving Cultural Heritage: A Brief History, from East to West

Natural substances like oils, herbs and plants are commonly used worldwide for the conservation of organic products. Because of its ancient history and wide range of applications, this practice has constantly evolved in potential and specificity. Aromatic plants have been used widely, for example, in the food industry to improve the shelf life and safety of perishable food [76,77], in cosmetics [3,78,79] and spiritual practices [80], and for healing purposes in traditional medicine [81].

In tropical areas, where biodeterioration has a high impact, heritage conservation has always promoted the development and use of methods based on herbs and natural repellents stolen from phytotherapy, these being considered safer for human health than chemicals. A recurrent method for book preservation in India was to envelop them in red or yellow fabrics, or store them in cedarwood boxes, which offered not only insecticidal and antifungal properties [82,83] but also protection against dust and environmental fluctuations. Among these colored fabrics, those made with the red dye, derived from turmeric [84], were good insect repellent, while those containing the yellow dye were good germicide because of curcumin, i.e., Curcuma longa, whose antibacterial properties are well known [85]. For the same reason, it was also quite common to find bags containing herbs, seeds, leaves or roots nearby manuscripts on library shelves. Source plants varied depending on the geographical area in India, Sri Lanka and other places in South Asia, and usually demanded revision when their efficiency lowered. Neem wood was chosen for building shelves and protecting books from parasites, while its dried leaves were placed between pages/covers of precious manuscripts or in the wooden chests/cupboards they were stored in, with cloves and peppercorn [84].

In the southernmost state of India, Tamil Nadu, medical practices were in vogue for palm-leaf manuscript conservation, and oils were extracted from cedar wood, citronella and lemongrass, olive, eucalyptus, sandalwood, turpentine, and palm leaves [86,87].

In Sri Lanka, best practices have been written for the conservation of the same type of manuscript. These draw inspiration from ancient recipes, in which *Madhuka longifolia* seed oil (*Ericales: Sapotacea*) or Mee oil and *Vateria copallifera* (*Malvales: Dipterocarpaceae*) resin oil or dummala (alternatively, dummela) oil are frequently reported. Of these, dummala oil, distilled from the fossilized resin of the Hal tree (*Gymnopetalum integrifolium*), is recommended by paper conservators to improve the flexibility of palm leaves and keep bacteria, mold and insects away [88,89]. This modern and well-established practice has its origin in a hardly divulged formula that produces an aromatic oil for the preservation of palm leaves from distilled dummala mixed with a small amount of *Goraka Maliyam*, a resin from the *Garcyna Cambogia* tree, and *Divul* (*Limonica Accidissima*) and *Devadaru* (*Erytroxylum monogynum*) roots [90]. Other natural products also available in the West have been suggested in ancient conservation, sometimes stealing from cookery: a combination of sweet flag, cumin, cloves, pepper, cinnamon and camphor. Other agents, rare in the West but common in the Eastern tradition, include karanja (*Pongamia glaba*) seeds, nirgundi (*Vitex negundo*), ashwagandha, Indian ginseng (*Withania somnifera*), Acorus calamus, ajwain (*Trachyspermum ammi*), henna (*Lawsonia inermis*) and sugar apple (*Annona squamosa*) seeds [91]. Overall, natural products have always been preferred in Asian cultures, and they have been imported into the Western world, especially in the colonial period, although with incorrect names and later abandonment in favor of chemical products [84].

4. Definition, Chemical Composition and Biological Activity of Pure Compounds

4.1. Definition

The International Organization for Standardization, whose standard ISO 9235 [92] was conceived in 2013 to define natural raw materials used in EO production and their derivative substances [80], provides a good definition of EO. The ISO in fact assimilated the definition given by the AFNOR (Association Française de Normalisation) in 1998, according to which an essential oil is a product 'obtained from vegetable raw material either by distillation with water or steam, or from the epicarp of Citrus fruits by a mechanical process, or by dry distillation', followed by separation from the aqueous phase using physical methods. An 'essence' is any natural secretion from vegetal tissues with a typical fragrance due to specific volatile aromatic compounds in vegetal cells. The term 'oil' refers to the lipophilic nature, i.e., hydrophobicity and viscosity, of these substances. About 250,000–500,000 is the number of estimated plant species on Earth, 10% of which have aromatic properties [93]. EOs are stored in the cytoplasm of some secretion cells, like hair, gland, oleaginous and resinous cells, and channels in different tissues. Most of them are liquids with no color, although their chromatic features vary in a wide spectral range from yellow to dark brown. Chamomile EOs are an exception, showing a typical blue-purplish color because they contain chamazulene, an aromatic compound originating from wet distillation.

Unlike plant oils, they are volatile compounds with high refractive indices and optimal rotations, as they are rich in asymmetric compounds. With some exceptions, their densities are lower than the density of water. Despite their hydrophobic nature, they are highly soluble in fat, alcohol and organic solvents [80]. EOs vary in composition, type, provenance and production.

4.2. Composition

EOs are an example of how the synergic activity of several biomolecules can foster plant survival. It is known that EOs have great antimicrobial and antioxidant potential. Some scholars explain this feature as resulting from plant extracts or secondary metabolites secreted by plants against different stress factors. The antimicrobial effect of these substances comes from their composition, which mainly includes phenolic compounds (ca. 85%). The latter have been identified in around 8000 plant products and are essential for cell physiology and metabolism, ensuring plant survival through protection against pathogenic agents and predators. The antimicrobial and antioxidation properties of phenolic compounds were extensively described in the early 2000s [94–96]. However, important roles are also played by other subclasses, like flavonoids, phenolic acids and lignans [97,98].

Chemically, an EO contains different and complex structures produced by photosynthesis following two possible routes:

- The metabolic route of mevalonic acid with isoprene multiplication (isopentenyl pyrophosphate);
- The biosynthesis of shikimic acid, when some deviations from the biomolecule can give phenylpropane-derived aromatic compounds like eugenol, anethol, cinnamic aldehyde, etc.

These two routes give rise to two main categories for the classification of all the compounds in EOs: hydrocarbons (mainly mono-, sesqui- and diterpenes) and oxygenated compounds (alcohols, oxides, aldehydes, ketones, phenols, acids and esters/lactones). High variability in composition and output within these classes can be explained by 'internal' factors, i.e., the species, tissues and level of growth of the plant, but also the cultivation method, harvest time and environment (weather, soil, etc.). Other 'external' factors can also play a role, for example, the extraction process and the storage and packaging. Because of their complexity, EOs have a very specific response depending on the mixture of chemical compounds they contain. The biological effect depends on different compounds, whose contribution can be defined based on the main chemotype that produces the toxic effect on microorganisms.

As recently reviewed by Russo and Palla [99], some plant families are particularly well known for their oleaginous species. The main ones are *Apiaceae*, *Lamiaceae*, *Lauraceae*, *Myrtaceae*, *Poaceae* and *Rutaceae*. However, there are EOs deriving from other plants that are worth mentioning for their antimicrobial properties, such as the geranium oil from *Pelargonium graveolens* (*Geraniaceae*) or the sandalwood oil from *Santalum* spp. (*Santalaceae*) [3]. Knowing the exact composition is not only important for the ISO to regulate the world market, it is also essential to distinguish similar or adulterated EOs [80].

4.3. Activity

We have already mentioned how the antimicrobial activity of an EO depends both on the plant it derives from and its chemical composition. Despite this specificity, it is still possible to find common features and make some general observations. For example, the majority of EOs act as inhibitors when their concentration is well below 5% (*v/v*), their efficiency growing with concentration. When they act, they tend to be bactericidal, meaning that microorganisms are inhibited and killed approximately at the same concentration [100], in contrast to bacteriostatic agents that inhibit growth but do not kill. Most EOs additionally have a relatively fast antimicrobial activity. Cellular death occurs at the same or a higher concentration than that required for bactericidal or fungicidal activity. Finally, lots of them have broad-spectrum activity, as they can be effective against a wide range of bacteria and fungi.

The last two decades have brought a greater understanding of the effects EOs and their components have on microorganisms. Overall, EOs act by compromising the integrity and function of the membrane, with the loss of cellular homeostasis and the leakage of intracellular components, eventually causing cell death. The extent of these effects depends on time and dose. It has been observed that high concentrations can rapidly cause serious effects, while low concentrations cause non-lethal effects which may become lethal when the time of exposure is longer [101].

The first interaction between an EO component and a microbial cell is likely to be the passive diffusion of its molecules through the cellular wall of a Gram-positive bacterium or a fungal cell and the outer membrane of a Gram-negative bacterium. Depending on the lipophilic or hydrophilic nature of each EO component, this can cause the alteration of some membrane properties. More specifically, lipophilic properties favor the diffusion of EOs through the cellular wall and membrane in microbial cells. Cells become permeable because of the interruption of the several layers of phospholipids, polysaccharides and fatty acids. Not only can lipids, proteins, cell membranes and walls suffer damage by EOs, but cytoplasm can also be clotted [102,103]. The following are some examples:

- Carvacrol, a chemical compound present in the essential oil of *Origanum vulgare* (oregano), thyme, pepperwort and wild bergamot, can cause cell death through the depletion of the intracellular ATP pool [104];
- Terpinen-4-ol, the major compound in tea tree oil (*M. alternifolia*), is responsible for the inhibition of cell respiration and the leakage of potassium ions, which determines the instability of membrane structures [105];
- The phenylpropanoid cinnamaldehyde can inhibit bacterial cell division [106,107].

Even in fungi, specific effects that compromise their vitality, virulence and cell integrity have been observed. Terpenes desegregate and decrease the level of order in lipid chains when they insert into acyclic fatty chains of the lipid bilayer. This event disrupts van der Waals interactions and consequently alters the physical properties of the membrane and swelling of the membrane bilayer occurs [108]. In fact, changes in the fluidity of the membrane can be intended as the first effect of a treatment with EOs. It has been shown that carvacrol, thymol, γ-terpinene and p-cymene can result in higher fluidity in model membranes because they reduce the lipid melting temperature. This new fluidity and expansion of a membrane can affect its integrity because intracellular constituents such as hydrogen, potassium and sodium can be released. Their loss, and a decreased membrane potential, are the earliest signs of exposure to antimicrobial compounds [101]. Aldehydes differ in action from the other chemical classes because they can have lethal activity even when the damage to the membrane is not extensive [109].

Despite causing great damage to the membrane, most EOs are not thought to cause destruction of the cell wall in Gram-positive organisms and yeasts or to the outer membrane of Gram-negative bacteria. This type of damage is called cell lysis and refers to the rupture or destruction of the cell wall such that the cell's shape is no longer retained. Extensive damage or eventual lysis of the cell wall have been documented using scanning electron microscopy for several bacterial species after their interaction with EOs [110,111]. This is also the reason why EOs do not bring chromatic alterations if compared to benzalkonium chloride, for example.

4.4. Extraction

In the past, extensive use has been made of hydro-distillation to extract EOs. This is the most common method for direct extraction of traditional oils [81], which implies boiling the plant material in water. Contrarily, in modern distillation, steam passes through it. This process, which is often preferred to derive EOs from several parts of a plant, implies that both the temperature and the risk of decomposition are lower. During steam distillation, the plant volatile compounds are vaporized and then condensed by cooling, producing two immiscible phases. The aqueous phase is known as hydrosol, aromatic water or hydrolat and contains odoriferous compounds in low concentrations; the oleaginous phase has a higher concentration of odoriferous compounds, which are sometimes colored and often biologically active. The latter is the essential oil.

Other processes have been proposed for the extraction of EOs—like the solid–liquid extraction commonly known as the Naviglio principle [112]—or solely for the enhancement of traditional methods. The latter can be achieved using ultrasound or microwaves by accelerating the extraction with a solvent or using supercritical fluid extraction. Overall, it can be stated that the composition of an EO as a final product also depends on industrial parameters. An EO is a distilled product which does not correspond to a pure compound and hence a specific chemical formula. It is in fact a mixture of individual constituents, whose proportions vary considerably, but which share a similar physical behavior in given conditions. For steam distillation, this means that the compounds are all volatiles in normal conditions—or at least at the vapor pressure that occurs at temperatures lower than 150 °C [113]—while they are insoluble or poorly soluble in water [114].

4.5. Analysis and Identification

Chromatography plays a major role in the characterization of EOs. This might involve the use of gaseous (GC) or liquid (LC) methods, eventually combined with a flame ionization detector (GC-FID) [30,80,115,116] or with spectrometric techniques (GC-MS) [21,38,57,117–122]. GC-MS can improve the separation and hence the detection and identification of volatile compounds [123]. In fact, gradual improvement in the separation of specific compounds has been fostered by the progressive introduction of packed, capillary and multidimensional gas chromatography, so that GC-MS has become the most common method for the characterization of plant extracts tested for conservation purposes. In cultural heritage, there are several examples of characterization using a combination of chromatographic methods [17,78]. For example, Palla et al. [78] used GC and GC-MS with apolar and polar columns to determine the main compounds in *Origanum vulgare* and *Thymus vulgaris* for an in vitro study on the inhibition of fungal colonization (*Aspergillus flavus*) and insect infestation (*Anobium punctatum*).

Conventional detectors such as FID may fail to identify a component when the retention-time property of a compound is not enough to reliably confirm its identity, especially with EOs and their complex composition. However, they are known to offer a well-characterized response relationship, which can be combined with the mass spectrometry patterns of components with the same retention time [123]. In fact, in medicine and the food industry, GC-MS has been coupled with GC-FID to characterize the antimicrobial effects of *Kielmeyera coriacea* [124] and to compare the antiseptic and antioxidant properties of Greek oregano and common oregano [104].

More recently, GC-MS has also been proposed for the assessment of encapsulation efficiency in different kind of gels—alginate, psyllium–alginate and purified psyllium–alginate beads [125]—after their treatment with acid solutions and separation of the supernatant via ultrasonic bathing and centrifugation. Not only can the analysis confirm whether all the compounds identified in a pure EO are retained after encapsulation, it can also estimate emission signals (%) over time, which describe the stability and homogeneity of the encapsulation system.

The identification of volatile compounds is usually made through the comparison of mass spectral data with reference libraries, such as the NIST or NBS/Wiley [126,127], FFNSC 2 [78], and in-house databases. Confirmation of an identification comes from the estimation of linear retention indices (LRIs), usually calculated with n-paraffin standards or using the standard method involving retention times (tRs) of n-alkanes injected after the EO [30]. The comparison of RIs with values reported in the literature using the SciFinder database has also been suggested for correct identification [78]. Conversely, the quantification of volatile compounds is based on the calculation of the relative areas of individual components after peak deconvolution and normalization [61]. Percentage values should be calculated as the means of at least three injections per sample [116].

4.6. Assessing Biocidal Activity

For EOs, testing and evaluation of biocidal activity is particularly challenging because they are highly volatile, largely insoluble in water and overall characterized by compositional complexity. The most common systems used to assess the biological activity of EOs are diffusion and dilution methods [128].

A frequently used and standardized diffusion method is the Kirby–Bauer test. This is probably the easiest method for a quick screening. This culture-based microbiological assay uses Petri dishes with agar, on which bacteria are swabbed. A known quantity of the EO to be tested is placed in a reservoir, i.e., a filter paper disk or a hole punched in the agar medium. The diffusion of the EO in the agar around the reservoir creates a zone of inhibition if the organism is killed or inhibited by the concentration of the EO, and this area can be measured and compared to standard diameter values for the tested species. Because of the relatively little amount of EO required, the Kirby–Bauer test has been used to test an extensive number of EOs and EO compounds [9,10,21,32,129]. However, this method is not ideal for compounds that are insoluble in water. Moreover, it must be remembered that methodological variations in the diagnostic procedure can imply the impossibility of comparing results from different laboratories [130].

Agar dilution and broth dilution methods are nowadays more frequently used to assess the activity of new antimicrobial agents because of their higher precision, standardization and reproducibility. For EOs, the experimental sample is suspended in a medium like sterile distilled water [57] and then inoculated on the surface of a solid growth medium in Petri dishes. The procedure allows the determination of the exact minimal dose that inhibits microbial growth and the evaluation of synergic, antagonistic or null action when two or more agents are combined. The interpretation of results is the assessment of microbial activity on the growth medium, to which scalar concentrations of EOs are added. Using dilution methods, the minimum lethal concentration (MLC or MIC), that is, the lowest EO concentration that completely inhibits growth, can be estimated and expressed in µg/mL or U.I./mL. The effect is assessed in micro-dilution wells or tubes and detected by the unaided eye [131]. However, it is important to note that when water is used as medium, the insolubility of some EOs in aqueous media might be a factor prejudicing the effectiveness of such tests [132].

When a broth medium (macro- or micro-dilution) is used, one can estimate the minimum bactericidal or fungicidal concentration, the MBC or MFC, respectively. Both parameters refer to the lowest concentration of EO needed to kill 99.9% or more of the initial inoculum.

Although dilutions methods are also affected by methodological variability, their results have higher comparability than those of the Kirby–Bauer test because the efficacy of dilution methods is given as EO concentration and not as the dimensions of the inhibition zone. However, the results of both diffusion and dilution methods are affected by the presence of a solubilizing agent and the microorganism to be tested in vitro [133].

5. EOs in Heritage Conservation: State of the Art

Cultural heritage represents the expression of human evolution and is evidence of human life, culture and history through tangible and intangible artworks. The first class includes archaeological sites and monuments, as well as movable art objects and daily utensils, clothes and weapons [134]. They can be made of organic (paper, leather, parchment, textiles and wood) and/or inorganic (glass, metal, ceramics and stone) compounds, which are prone to suffering different types of biodeterioration or biodegradation because of the 'versatile metabolic abilities' [135] of living organisms like bacteria, archaea, fungi and lichen [136].

5.1. Fighting Biodeterioration

Allsopp et al. [137] have pointed out the different meanings of biodeterioration and biodegradation. The first can be understood as 'any undesirable change in the properties of a material caused by the vital activities of organisms', while biodegradation is 'the harnessing, by man, of the decay abilities of organisms to render a waste material more useful or acceptable'. The latter has taken a different meaning in conservation and has become predominantly a synonym for biodeterioration [138].

These processes can be prevented by direct or indirect methods. Direct methods include mechanical, physical, biological and biochemical strategies, even though chemical processes, i.e., active ingredients in solution, are most frequently applied [139,140]. Indirect methods aim at impeding or delaying the colonization of an object by acting on several factors: environmental parameters, through control and periodic monitoring, and conservation treatments, used to reduce material porosity, roughness and water content. An example is the passive approach to biostabilization, which prevents the adhesion of microorganisms to a substrate, while the active approach implies biocides.

'Biocide' is a general term applied to those substances that can be defined as toxic because they cause the death of a living organism in a more or less specific way. Chemical treatment with liquid biocides (or gaseous fumigation) can be the only viable option when it is not possible to control environmental parameters or use mechanical or physical methods. However, as Sterflinger and Piñar pointed out in 2013 [141], a thorough assessment is necessary prior to their application. It is mandatory to determine whether the removal of a microbial community could cause secondary undesirable effects and more harm to the object. Some undesired effects are color variations and redox reactions of the chemical compounds in the substrate. Furthermore, after the biocidal treatment, the microbial community might become more dangerous and resistant than its precursor because microorganisms have shown the ability to adapt to growing biocide concentrations and to display higher resistance to a biocide treatment when they are incorporated in a matrix of extracellular polymeric substances [142]. If the concentration of the biocide is high, a few species in the biofilm could survive after the treatment and show less sensitivity and resistance to the biocide, but there is a good probability that lethal mutations will develop. When the biocide concentration is low, the number of surviving organisms can be higher and there might be a wider range of species with appropriate responses to the treatment and which go on to develop mutations. Continuous and even sporadic exposure to sub-lethal concentrations of biocides increases the probability of mutation [143].

Problems other than biocide resistance might occur. For example, a study on benzalkonium chloride, a common biocide in conservation, has shown that the presence of a biofilm is evidence of resistance, as it has been observed that this biocide has longer carbon chains (C_{18} or C_{14}, compared to C_{12}). Long-chain compounds show higher hydrophobicity, limiting the penetration of the molecule in the hydrophilic matrix, with a loss of bactericidal efficiency against *P. aeruginosa* biofilms [144]. Other components in the extracellular matrix, like enzymes, can have active roles in the neutralization of toxic compounds. According to Bridier and co-authors [142], the three-dimensional structure of a biofilm also determines its resistance to disinfectants. In this study, the biofilm in fact showed a more heterogeneous response because of its chemical and physiological heterogeneity and a multifactorial response, as several mechanisms contributed to its survival.

A great number of synthetic biocides, such as pentachlorophenol (PCP), tributyltin oxide (TBTO) and zinc carboxylate, are no longer available on the market because of their environmental and human hazard, as they create waste and are carcinogenic. Consequently, research is now focused on non-harmful and non-toxic compounds [78]. Studies since the 1970s have provided evidence that plant-derived biocides can represent a safer method to control the microbial deterioration in cultural heritage [145].

5.2. Repellent, Pesticide and Biocide Activity in Plant-Derived Compounds

We have already mentioned how EOs have been widely used as repellents, insecticides and biocides in the past and in different cultural contexts. In addition to these well-known properties, in the last two decades, several studies have pointed out their effective action against pathogens in the research for cultural heritage. In fact, a technical note by Collis published in 1970 [7] had already suggested the use of thymol for document fumigation. Paper and archival document conservation can be seen as a pioneer research strain that first developed fumigation methods. Because these materials are based on organic compounds, they represent a good ground for the development and feeding of insects, molds, fungi and bacteria. No matter what the infestation problem is, the impact and growth of the biological threat on documents and manuscripts is highly dependent on the monitoring of their conservation environment but also on a direct control method, which must act rapidly and safely for both artworks and conservators [8]. And thymol seemed to meet all these requirements as early as the 1970s.

Thymol is the common name given to 2-isopropyl-5-meta-cresol, a natural crystalline monoterpenoid phenol. Its vapor can kill active or dormant fungi and their spores when released in a fumigation chamber for an appropriate time. This treatment has no negative effects on paper or parchment, except when they are coated with oil paint or varnish. As happens with new technologies, the efficacy of thymol in conservation has been tested with relatively low control, with random applications and uncontrolled treatment cycles. The result is a great controversy surrounding the extent and level of efficacy of thymol in conservation. In 1975, the biologist M.L. Florian and the conservator B. Byers systematically researched the subject. They concluded that thymol is definitively a fungicide and introduced a simple and practical method for the fumigation of paper and parchment with cheap and common materials like a steel storage cabinet [146]. The thymol chamber further developed in the following years, and the different methods of thymol application to control fungus infections in cultural property were revised by Baer and Ellis in 1988 [147].

In other areas, predominantly in the Eastern world, a secular tradition exists and has used plant products as biocides or repellents with continuity, for example, in India. Since 1966, the Indian Association for the Study of Conservation of Cultural Property (IASC) has greatly fostered research in conservation through seminars, yearly meetings, conferences and workshops. This effort culminated in the publication of the journal *Conservation of Cultural Property in India* (New Delhi), which has collected papers on the application of EOs since the 1980s. Examples of experimental protocols for the use of neem oil [148,149] and Artemisia oil [150] as insecticides and repellents in museums can be found in the journal. The research also converged in the International Conference on Biodeterioration of Cultural Property (20–25 February 1989) held at the National Research Laboratory for Conservation of Cultural Property in Lucknow in collaboration with ICCROM and INTACH. Follow-up meetings were held in 1993 and 1995 (Biodeterioration of Cultural Property 2 and 3), the last seeing the participation of Thailand, whose representatives reported on the influence of plant products on insect activity. At the same conference, a study on *Pongamia glabra* oil, also known as Karanja gum, highlighted its efficacy against some of the most important biothreats in museum environments, the furniture carpet beetle (*Anthrenus vorax*) and the silverfish (*Lepisma saccharina*) [148]. The Conference Proceedings also document how de-oiled neem seeds and custard apple seeds can protect artefacts in storage and display cases against *Anthrenus vorax*. The insecticidal activity can be effective in 18–24 h against silverfish (0.5% concentration) and in 40–48 h against the carpet beetle (1% c.). When used against *Anthrenus vorax*, the oil can inhibit egg hatching [151]. In the 1995 Conference Proceedings [152], other remarkable studies include Dhawan's research into the antifungal activity of some EOs like betel (*Piper betel*), camphor, citronella, cloves and eucalyptus on palm-leaf manuscripts stored in archives, libraries and museums; Pandey and Srivastava's use of 15 vapor EOs, which determined that the highest efficacy was achieved with a high content of cinnamic aldehyde and thymol (*Cinnamomum cassia* and *Trachyspermum ammi*); Nilvilia and Wangchareontrakul's experiments with some traditional Thai plants, like 'plai' (*Zingiber cassumunar*) and 'long pepper' (*Piper longum* L.), which showed that the first has insect-repellent activity and is an insecticide against *Lepisma saccharina* and that the second is highly deadly to the American beetle (100% mortality in 24 h). The renewed interest in natural substances for conservation because of their antibacterial and antifungal properties was finally expressed by the first review on the subject by Chingduang et al. included in the Conference Proceedings [152].

One year later, Goldberg included tobacco and camphor in the list of pesticides and fumigants in use at the National Museum of Natural History, Smithsonian Institution, proving that a tendency to include plant-based products had also diffused to the USA [153].

In the 1990s, while researchers were discussing the structure, use and risks of thymol because it seemed to negatively affect paper, gum arabic and iron gall ink after treatment [154], in the UK, researchers were keeping an eye on Indian traditional conservation practices, which were said to include the use of citronella and turmeric as insect repellents on palm-leaf manuscripts at the Saraswati Mahal Library in Thanjavur, South India [155].

In 1997, the French association for research on graphic art, ARSAG, organized a Conference on 'Conservation, science and evolution, results and perspectives', where Rakotonirainy et al. [156] presented their work on thermos-sprayed fungicides (terpineol and carvone) for the disinfection of contaminated books in storage rooms. An important contribution by the same leading researcher came a year later [157], when several EOs (laurel, wormseed, citronella, eucalyptus, lavender and sage) were studied for their fungicidal and fungistatic activity against the most common fungal strains in libraries, archives and museums, testing different application methods. In this systematic work, the Danish research team demonstrated that direct contact with the oil is more effective than contact with the vapor phase, that synthetic products are not necessarily more successful than natural ones, and that fungal sensitivity varies according to different factors like species and stage of fungal development but also depends on the supports on which fungi grow.

Later, in the Eastern area, several authors dealt with the quality of EOs to be used in preventive conservation, as well as the standardization and protection of chemotypes, for which analysis and identification is so important, as remarked in Section 4.5. For example, Singh et al. [158] reported on the insecticidal practice of using camphor and dried neem leaves placed next to or in stored textiles in several Southern Indian Museums.

This section aims at summarizing the most important contributions to the topic, providing graphs to show the growing interest in the application of EOs in conservation, especially after 2000 (Figure 1).

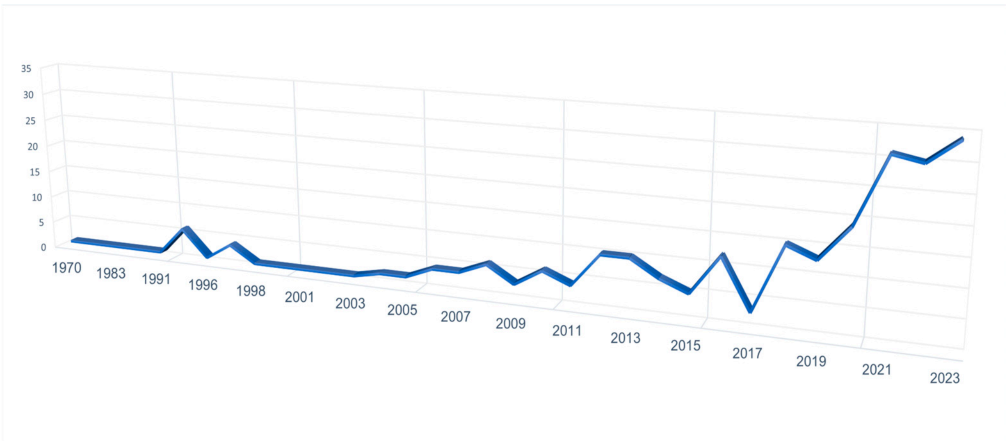

Figure 1. Pivot chart built from Table S1 representing the increasing interest (1970 to 2023) in EOs and their application in the conservation of cultural heritage.

Since the 1970s, several countries have faced the challenge of developing new methods for conservation based on plant products (Figure 2). Not only Italy, with its long tradition of conservation, has extensively researched the subject (contributing 29% of the analyzed works); important contributions have also come from Egypt (15%), India (8%), Romania (7%), Argentina (5%), Portugal, Korea, Spain and France (3%).

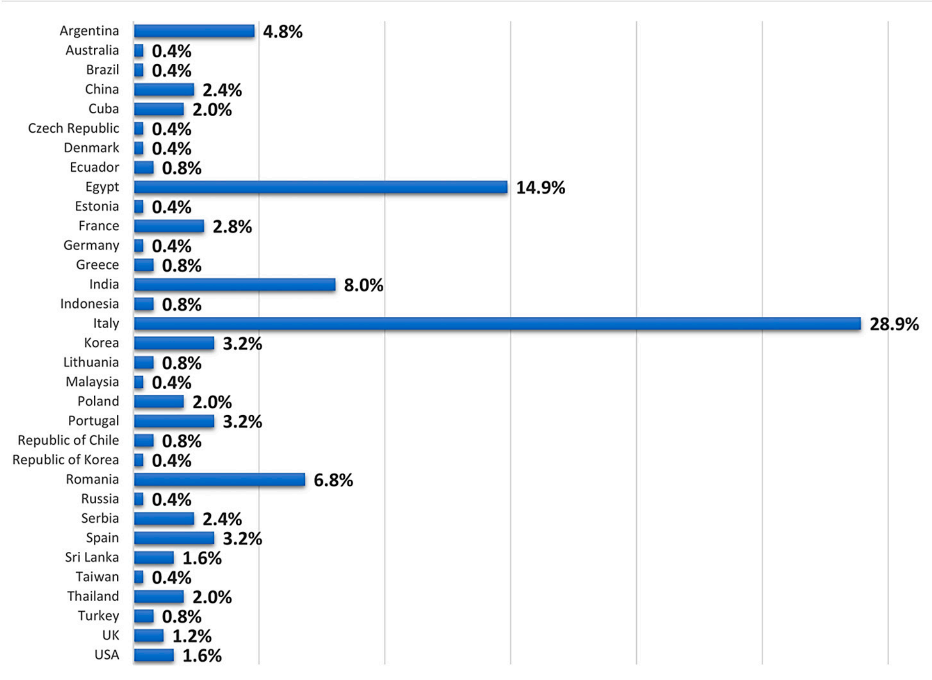

Figure 2. Bar chart illustrating the leading countries involved in research into EOs for cultural heritage. The plot is based on Supplementary Table S1.

The greatest focus has been on applications for natural and artificial stone artworks (see Figure 3), currently representing the most popular research strand (29%). However, it is not by coincidence that archival materials like paper, photographs and graphics [57,91,96,117,121,122,125–127,154,157,159–183] represent the second major area of application (23%), partly because research in conservation started with them (Section 3). Other research teams have dealt with a wide range of artworks, particularly those made with organic materials [119,184–186] (5%), with a special focus on wood [61,70,78,120,187–191], leather or parchment [115,192–195], and textiles [196–201].

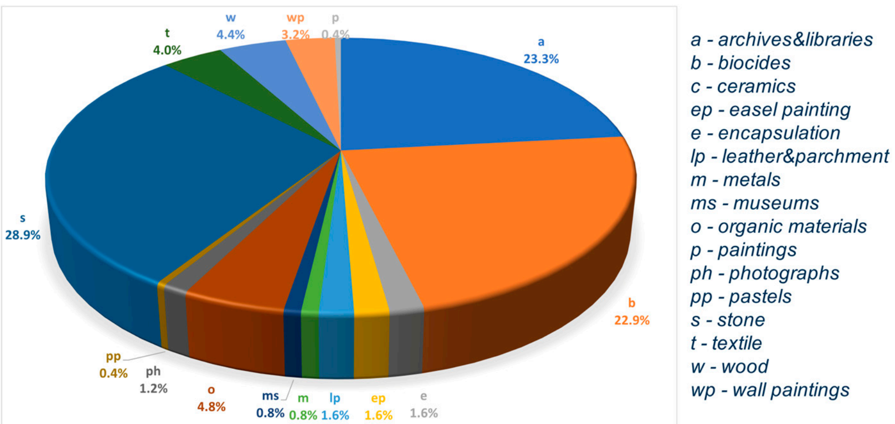

Figure 3. Pie chart built from Table S1 showing how EOs have been tested and applied in conservation since 1970.

Of all the tested EOs, the most common is certainly thyme, for which applications on all types of materials except photographs have been reported (Table S2). However, basil, clove, garlic and lavender have gained a certain popularity over the years. Experiments have been conducted with these EOs on the most-explored materials, like archive documents and stone. Interestingly, for less-studied materials like metals, less common EOs have been proposed [57,74,75], such as onion (*Allium cepa*), ramson (*Allium ursinum*), black mustard (*Brassica nigra*), carob (*Ceratonia siliqua*), longan (*Dimocarpus longan Lour*), olive (*Olea europaea*), black locus (*Robinia pseudo-acacia* L.) and sugarcane (*Saccharum officinarum*). Despite results showing moderate efficacy against bacteria for Allium species, leaf extracts of *C. siliqua* and *S. officinarum* have shown potential to act as corrosion inhibitors [75].

It is also interesting to note how the research community has not limited its attention to objects in themselves and their consistency but has extended its evaluations to the environments in which objects are stored or preserved with in vitro and in situ experimentation.

5.3. Novel Application Mechanisms (Encapsulation, Gels and Emulsions)

Pioneers in the preventive fight against infestation in libraries and archives using EOs are the French research team that has studied the optimization of experimental conditions for the encapsulation of volatile compounds [160]. Applications started with food preservation [131], and as early as 1997 it was suggested that microencapsulation using colloids like gelatine or gum arabic could partially solve EOs' sensitivity to oxygen, light, moisture and heat [160]. Most likely, the French study was taken as an example to investigate the encapsulation of the essential oil of citronella in gum arabic and gelatine for antifungal purposes a few years later [202]. Even in recent years, encapsulation continues to be studied for its great potential to lower the action of environmental agents while favoring more efficient conservation treatments with controlled release [116] and for the possibility of application even on vertical surfaces, for example, on monumental stone. The method has been tested using other environmentally friendly biocides (usnic acid and zosteric acid sodium salt) in silica nanosystems to control the development of biological patina outdoors [203], although research has also focused on inclusion complexes with EOs and β-cyclodextrin (β-CD) [116]. Results are promising and show that *T. vulgaris* can have similar antifungal activity, whether pure or encapsulated in β-CD, and an encapsulation efficiency higher than 50%, laying the ground for future experimentation. The evaluation of a system's efficacy should also consider how it impacts the inner structure of the treated material. On textile fabrics, for example, tensile tests can help establish whether an application of microemulsions can cause variations in their mechanical properties [204].

Thyme and cinnamon EOs have also been added to microemulsion systems as anticorrosive and antifungal agents for copper artworks [205]. This application method seems to reduce the corrosion rate of treated metallic plates, a small amount of EO (approximately 1%) being enough to inhibit the growth of fungi. Recently, cinnamon oil has also been commercialized with *Citrus aurantium* L. *var. amara* in the Zege emulsion, which has shown good potential as an antifungal product for modern paintings [206]. Pickering emulsions from the industry have also been proposed for stabilization. When particles assemble at the water–oil (W/O) interface, emulsion droplets are protected by a mechanical barrier, and they are less prone to coalescence. The method requires that 'the dimensions of the adsorbed particles are at least an order of magnitude smaller than the emulsion droplet size', as clarified by Gagliano Candela and co-workers in 2019 when they reported the results of Pickering emulsion tests for the application of *T. capitata* essential oil [38]. Emulsions can be prepared with water and different commercial clays and applied on outdoor materials (ceramics, marble and cement grit) exposed to biological agents such as green algae and cyanobacteria. Positive results have been observed even four months after treatment. A recent application was first tested in vitro to determine the most effective mixture of EO and hydrolate and then applied on a Renaissance painting to determine the spray formulate action against the microbial community colonizing the canvas. The treatment finally consisted of a uniform emulsion of *C. aurantium* var. *amara* hydrolate and *Cinnamomum zeylanicum* essential oil [207].

An alternative strategy to prevent EO volatilization is offered by hydrogels, which additionally offer the advantage of vertical application. It is not by coincidence that hydrogels were first tested for stone treatment [48], and it was shown that in an alginate hydrogel, *T. vulgaris* maintained antimicrobial efficacy against cyanobacteria even at a concentration of 0.1% (*v/v*). Thyme is in fact very commonly used to test immobilization mechanisms, one of the most recent involving loaded chitosan particles. In vitro and in situ tests by Wang and co-authors [51] demonstrated that with chitosan nanoparticle encapsulation, thymol improved thermal stability and antimicrobial properties, as well as controlled release. PVA gellan-based hydrogels have been alternatively proposed for the cleaning of biocolonized stone surfaces. The experimental system can be crosslinked with $CaCl_2$ and enriched with a surfactant for the encapsulation of EOs or the active principle (or a combination of both). Tests have shown that encapsulated *O. vulgare* can be most effective against different biofilms and eventually has a synergic action with *T. vulgaris* [208]. However, assessment of the composition of a biofilm before any test is performed is of paramount importance, as the cleaning efficacy of EO hydrogels is strongly linked to the colonizing agent.

The latest biopolymer tested for the encapsulation of EOs in conservation is alginate, which has shown good release control, although its emulsifying capacity is low. An alginate–thyme EO hydrogel system has recently been tested both in vitro [209] and in situ [210]. The latter consisted of small-scale tests on the mosaic 'Le Professioni e le Arti' by Fortunato Depero (Roma, Italy), colonized by phototrophic microorganisms and fungi. The performance was said to be excellent and comparable to the alternative hydrogel system containing sodium dichloroisocyanurate, leading to the total disappearance of microorganisms without any aesthetic alteration of the substrates. Attempts to improve the emulsification have been made by mixing alginate with a plant of the *Plantaginaceae* family (*Plantago psyllium*), which resulted in high swelling and higher encapsulation efficiency. Encapsulation of cinnamon oil in these gel beads has preliminarily shown that all the EO components are retained after encapsulation and an efficient release against *Saccharomyces cerevisiae* yeast cell. A negative side effect can be the color change of the treated surface, an effect that occurred when the system was preliminarily tested with one-shot release and a disintegration treatment at pH = 1 [125]. Future studies may focus on verifying whether this non-desirable effect can be avoided in more systematic conservation treatments.

An atypical treatment for wood conservation deserves special mention: it has been shown that EOs (*Thymus capitatus*) can be incorporated into microcrystalline cellulose formulations based on almond-shell cellulose pulp to prepare stuccos with biocidal properties against fungal colonization and insect infestation [211].

6. Commercially Available Products, Their Potential and Application

As professionals working in heritage conservation have begun to comprehend the potential of green solutions and their progressive evolution into green nanotechnology against biodeterioration [212], the demand for commercial solutions has rapidly grown. This research strand has been powerfully boosted by the ecofriendly and harmless features of EO applications, which make them the most sustainable option.

As a result, several EO-based products are now commercially available for conservators:

A. BACTIGAS® (BOC Ltd., North Ryde, Australia) was originally developed to sanitize air-conditioning ductwork. The product contains 0.3% tea tree oil (*Melaleuca alternifolia*) and 2.7% ethanol in carbon dioxide propellant (97%). It can spread throughout inaccessible areas and foster reductions in general microorganisms. Research by the Australian Western University and the University of NSW has proved the efficacy of the EO from *Melaleuca alternifolia*, an Australian indigenous plant, against a wide range of bacteria and fungi [132]. A low dosage (1 $g/m^3/24$ h) is enough to reduce mold, bacterial and starch growth on walls and other surfaces, thus lowering maintenance costs. A study published in the AICCM bulletin in 2003 gave preliminary indications regarding the application of BACTIGAS® to control recurrent mold in large-object storage areas [213].

B. Essenzio© (IBIX Biocare, IBIX S.r.l., Lugo, Italy) is a blend of essential oils, mainly oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) extracts. It is said to be biodegradable and biocompatible and that it can suppress the growth of moss by splitting weed cells up to their structure, similar to a root (hyphae), and then eliminating them. The company reports that the product is safe to use on all types of structures and artefacts for the removal of moss, algae, lichens and fungi. The product is applied with a back-pack sprayer pump or garden sprayer, without dilution. In 2022, it was compared to other innovative biocides in a test of their effectiveness against biodegradation of a Roman mosaic in the Archaeological Park of Ostia Antica [214]. Analytical results showed a weak response of all natural biocides compared to the chemical products. More recently, Essenzio© has been tested on mock-up samples designed to represent the wall paintings of a semi-hypogeum room in the archaeological park of Baia, Italy, with the aim of comparing the efficacy of modern treatments, including essential oils, to UV-C irradiation for the removal of an artificial biofilm [215]. Experimental results showed that a 50% dilution and application for 1.30 h did not cause remarkable color change to the painted surface, making the product preferable to UV-C irradiation. In a recent intervention on the fountain mosaic 'Le chaos et la source de vie' by Claude Rahir (1984), Essenzio© has been designated as the biocide to be used, particularly on mosses, algae and lichens [216].

C. BIOTERSUS® (Exentiae s.r.l., Catania, Italy) is the result of a collaboration among some of the most important laboratories of conservation science and conservation of stone. Originally designed and tested on the stone monuments in the Vatican Gardens [19], BIOTERSUS® is a 100% organic, solvent-free and non-toxic product. It contains the active ingredients of several standardized EOs—carvacrol, cinnamic aldehyde, eugenol and thymol from *Cinnamonum zeylanicum* (0.25% *v/v*), *Eugenia caryophyllata* (0.5% *v/v*), and *Corydo thymus capitatus* (0.4% *v/v*). It is commercialized in solution and must be diluted for the removal of biological patinas (algae, fungi and lichens) from more or less sensitive stone surfaces. On a Roman mosaic, it has shown moderate biocide activity [214].

D. YOCOCOIL (YOCOCU APS, Rome, Italy) is a mix based on a combination of the EOs described as the most promising in the conservation literature: *Eucalyptus globulus* (0.25% *v*/*v*), *Thymus vulgaris* (0.4% *v*/*v*), *Eugenia caryophyllus* (0.5% *v*/*v*) and *Ocimum basilicum* (0.25% *v*/*v*). Macchia and co-workers, who were the first to test this product in 2022 [47], suggest the addition of 8.5 g of NEVEK® and Tween®20 at 0.3% *v*/*v* and dilution with distilled water for its application on ancient mosaics. The authors have shown that YOCOCOIL, with Preventol® RI50, has the best biocidal activity compared to other commercial products [47,214].

7. Final Remarks and Future Directions

In Otero's perspective on the future of heritage conservation [217], the UN 2030 Agenda for Sustainable Development was recalled in addressing the importance of two actions: (1) the promotion of 'democratization, open science, open-access learning opportunities, productive work, equitable quality interdisciplinary'; (2) the need to safeguard our heritage 'as represented by one of the 169 specific targets of the Sustainable Development Goals (SDG 11.4)'. In line with Otero's perspective and the UN 2030 Agenda for Sustainable Development, in this review, we aimed to prove how heritage conservation has recently tended—and is still moving—toward more sustainable strategies, using open-access resources. Research into EOs shows how applications can be effective on a wide range of materials. The list of countries researching the subject proves that it realistically represents a bridge between developed and developing countries, as both have been long familiar with the antimicrobial properties of EOs and have shared their knowledge on the subject. Less recent as well as current and developing research trends also highlight a constant exchange of knowledge between science and practice: EOs from phytotherapy are now commonly used in heritage conservation, as much as green nanotechnology is now serving heritage conservation to boost innovative and more sustainable solutions. In fact, green conservation practices are already preferred by Italian authorities, which support the application of green products in heritage conservation, as evidenced by (1) the often-explicit requirements to include EOs and green solvents as binding conditions in public tenders and (2) the promotion of companies that use sustainable materials in their activities. Greater collaborative efforts, at the private and public levels, shall be made in the future to develop and improve application strategies. Because EOs are highly volatile, maintaining the surface contact between the biofilm and the biocide solution, i.e., ensuring that the application time is as long as possible, is a key factor. Furthermore, research into sustainable biocide treatments shall move towards the design of high-retention systems to maximize the yield of active molecules and the efficacy of treatments. Finally, sharing research outcomes and the impact of novel application treatments in real case studies will promote the advancement of mutual and uniform practices and their constant improvement.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su16125110/s1, Table S1: List of relevant contributions cited in this review and included in statistics with a pivot table and charts; Table S2: List of the EOs applied in cultural heritage studies with their common names and the materials they have been tested on.

Author Contributions: Each author has made substantial contributions to the conception and design of this work and has drafted the work and substantively revised it. All three authors have approved the submitted version (and the version substantially edited by the journal's staff that involved the authors' contributions to the study). They all agree to be personally accountable for their own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved and documented in the literature. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the Supplementary Materials. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors would like to express their gratitude to the anonymous reviewers for their time and useful comments.

Conflicts of Interest: The authors declare no conflicts of interest.

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