Review

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# Essential Oils and Cultural Heritage Conservation: Are They Safe, Environmentally Friendly, Sustainable, and Negligibly Toxic?

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Posted Date: 7 October 2025

doi: 10.20944/preprints202510.0496.v1

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*Review*

# Essential Oils and Cultural Heritage Conservation: Are They Safe, Environmentally Friendly, Sustainable, and Negligibly Toxic?

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**Abstract**

Microbial colonization of heritage materials is a well-known conservation issue. When necessary, it is removed using mechanical, physical, or chemical methods, with biocide formulations being a common choice. The need to reduce dependence on conventional biocides has led to the exploration of innovative alternative methods and new formulations with biocidal properties for the conservation of heritage objects. Alternative approaches include natural compounds such as plants' essential oils.While these natural options show promise, they present challenges—such as inconsistent effectiveness, possible toxicity, and the need for thorough compatibility testing with historic materials. Therefore, although some concerns are legitimate, the "run" to alternative substances is a growing concern as well. A comprehensive selection and examination of international papers from the past two decades on this subject has been conducted. The detailed and critical analysis of existing data on essential oils, hydrolates, and other plant-derived extracts experimented to prevent and/or eradicate the colonization of microbial communities on heritage objects focused on the effect on microorganisms in controlled environments; in situ applications on microorganisms; encapsulation in hydrogels and emulsions; toxicity and ecological impact; alterations of heritage materials. The review also discusses the advantages, limitations, and practical implications of these strategies.

**Keywords:** control of microbial colonization; cultural heritage objects; essential oils; hydrolates; efficiency; toxicity; environmental impact

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## 1. Introduction

The weathering of cultural heritage objects is influenced by a complex combination of physical, chemical, and biological factors. The colonization of heritage materials—such as textiles, paper, wood, and stones—by microorganisms is a well-documented aspect of conservation. When necessary, biological growth is removed using mechanical, physical, or chemical methods, with biocide formulations being a common choice. However, concerns regarding toxicity and environmental impact are significant factors when selecting biocides. Moreover, resistance of biofilms to environmental stressors, including antimicrobials, is emerging as a worldwide concern, affecting various fields.

In recent years, there has been a growing interest in exploring alternatives to conventional biocides used in conserving artworks. The term "biocide" has garnered a negative perception among many conservators, primarily due to health and safety concerns. It is worth underling that biocides are among the most rigorously regulated and controlled chemicals due to their potential effects on human health and the environment. Despite extensive testing and strict regulations before biocides are approved for sale and labeling, concerns about their health effects persist. However, it is important to note that "biocide" refers to a wide and diverse range of substances, and the term itself does not inherently imply negativity. As the control of biological growth is often necessary, the need to reduce dependence on conventional biocides has led to the exploration of innovative alternative methods and new formulations with biocidal properties for the conservation of heritage objects.

 doi:10.20944/preprints202510.0496.v1



Alternative approaches include natural compounds such as plants' essential oils. While promising, these approaches face challenges such as variability in efficacy, potential toxicity, and the need for extensive testing to ensure they are compatible with historic materials. Therefore, although some concerns are legitimate, the "run" to alternative substances is a growing concern as well, as it will be further discussed.

Essential oils (hereinafter referred to as EOs) are highly concentrated distillation products of aromatic plants known for their strong antimicrobial and pesticide properties. EOs are volatile and lipophilic substances, which means they can effectively penetrate cell membranes. Despite a centuries-long history of use, their antimicrobial mechanisms have not been fully elucidated.

Other plant derivatives have been experimented with in the field of cultural heritage conservation. Hydrolate extracts (also known as hydrosol, floral water, aromatic water, or herbal water) are by-products of the steam distillation process of EOs. They are colloidal suspensions composed of a continuous phase, the distilled water, and a dispersed phase, the emulsion of essential oil droplets and water-soluble components. Their composition can significantly differ from that of the respective EOs [1]. They are much more diluted and have lower antimicrobial activity, but they are safer and can be used in situations where EOs are not suitable. Phytocomplexes, which are blends of molecules with a wide range of actions, remain quite unexplored in this field.

EOs are compounds produced in distinct parts of plants (flowers, leaves, stems, seeds, fruits, roots, bark) as secondary metabolites. They are complex mixtures of hydrocarbons, alcohols, aldehydes, esters, ethers, ketones, oxides, phenols, and terpenes. Their applications span the food industry, agriculture, pharmacology, aromatherapy, and cosmetics. More than 90% of global production is utilized by three major industries worldwide: food flavoring, perfumery, and pharmaceutics [2].

The effectiveness of essential oils relies heavily on their quality. As Holmes [2] notes, for an essential oil to be considered bioactive, it must meet four essential standards: it should have a verified biological identity, be pure, come from intact source plant material, and be obtained through a well-understood extraction process.

An additional key point is the concept of chemotype (ct.), which describes a group of plants within the same species that differ from others in that species based on the chemical makeup of their secondary metabolites. For instance, *Thymus vulgaris* has many chemotypes, among them ct. thymol, ct. linalool, ct. geraniol, ct. thujanol, ct. paracymene, ct. cineole. They differ in the dominant constituents and, consequently, in their characteristics.

Although plant-based products are often viewed as more environmentally friendly and safer than chemical alternatives, there is insufficient scientific evidence to fully support this belief [3]. This highlights the need for broader safety evaluations to better understand their ecological impact. With increasingly strict international regulations concerning the environmental effects of chemicals, chemical mixtures, and new products—such as the REACH regulation in the European Union and the globally adopted GHS by the United Nations—additional research will be hopefully conducted to produce scientific data on the effects of EOs, hydrolates, and other plant-derived extracts as emerging sources of bioactive compounds [3].

This review aims to provide a detailed and critical analysis of existing data on plant EOs, hydrolates, and other plant-derived extracts experimented to prevent and/or eradicate the colonization of microbial communities on heritage objects, and it focuses on various related aspects: i) effect on microorganisms in controlled environments; ii) in situ applications on biofilms and lichens found on different heritage materials; iii) encapsulation in hydrogels and emulsions; iv) toxicity and ecological impact; v) alteration of heritage materials; vi) how all this information impacts their use on cultural heritage objects. The review also discusses the advantages, limitations, and practical implications of these strategies, fostering a constructive dialogue among conservation professionals and supporting the development of sustainable approaches to managing biological colonization on stone heritage.

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To achieve this, a comprehensive selection and examination of international papers from the past two decades on this subject was conducted. The literature search included the following scientific research databases:

Jstor, using the advanced search with terms “cultural heritage” AND “biodeterioration” AND “control methods,” AND “essential oils,” FIELDS all fields;

Scopus, TITLE-ABS-KEY with the search terms “biodeterioration,” “cultural heritage,” “control methods”, and “essential oils”.

Google Scholars and ProQuest, using keywords “biodeterioration,” “cultural heritage,” “control methods”, and “essential oils.”

In a later phase, combinations of keywords were used. Additional papers were identified by consulting the reference lists of the selected articles. I would like to highlight the work of Peter Holmes, a medical herbalist and essential oil therapist. He has authored several renowned textbooks on herbal and essential oil medicine, including the two-volume series titled ‘Aromatica: A Clinical Guide to Essential Oil Therapeutics’ (2019) [2]. These books have been an invaluable source of information on EOs for me. Another very important, comprehensive and evidence-based resource on the safety of EOs has been the book ‘Essential Oil Safety: A Guide for Health Care Professionals’ by Robert Tisserand and Rodney Young (2013) [4], which is a landmark reference in the field of aromatherapy and essential oil research.

## 2. Effect of Essential Oils, Hydro-Alcoholic Extracts and Hydrolates on Microorganisms in Controlled Environments

The antimicrobial activity of EOs has primarily been assessed through laboratory experiments using various methods. Common approaches include the disk diffusion test and the tube dilution test. Regarding the first, Petri dishes containing growth media, microorganisms, and EO-impregnated filter paper disks are incubated under optimal environmental conditions. Regarding the tube dilution test, serial dilutions of the antimicrobial agent are made in a liquid growth medium inoculated with microorganisms and then incubated. A further method adopts the evaporation of EOs instead of direct contact with microorganisms. Filter paper discs soaked with EOs are placed on the lids of Petri dishes where microbial colonies grow on specific media. The minimum inhibitory concentration (MIC) is determined as the lowest concentration of an EO at which microbial growth is no longer visible compared to the control.

In certain cases, microorganisms are inoculated onto samples designed to simulate cultural heritage materials (such as wood, paper, stone, wall paintings, canvas paintings, etc.). Treatments are applied not only to bio-colonized surfaces but also to uncolonized surfaces to investigate possible interactions between EOs and the materials themselves.

All these simulated laboratory tests offer significant advantages over in situ studies, as they allow for reproducibility and comparison of results before and after treatment. In other words, they simplify the complexity of nature to make it more understandable.

The target microorganisms of these tests were sampled from artworks made of organic and inorganic materials (paper, wood, canvas, wall paintings, stone, etc.). Most were fungi, a few bacteria (heterotrophic and phototrophic) and algae.

For clarity and ease of reading, I refer to EOs by their common names throughout the text. The taxonomic names of the plants along with the EOs common names are listed in Table 1.

**Table 1.** Botanical name of plant source and common names of the essential oils reported in the reviewed papers. Essential oils extracted from plants marked with an asterisk are classified by the U.S. FDA as “Generally Recognized as Safe” (GRAS), in accordance with 21 CFR Part 182.

| Scientific Name | Synonym | Common Name |
|---|---|---|
| *Allium sativum* L. | | Garlic |
| *Boswellia* spp. | | Frankincense |
| *Calamintha nepeta* (L.) Savi | | Calamint |

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| | | |
|---|---|---|
| **Cinnamomum cassia* (L.) J.Presl | | Cassia |
| **Cinnamomum verum* Presl | *C. zeylanicum* Blume | Cinnamon |
| *Citrus aurantium* L. ssp. *amara* Engl. | *Citrus aurantium* L. ssp. *aurantium* L. | Bitter orange |
| **Citrus limon* (L.) Burm. F. | | Lemon |
| *Crithmum maritimum* L. | | Sea fennel |
| *Cyanus segetum* Hill. | | Cornflower |
| **Cymbopogon citratus* (DC.) Stapf | | Lemongrass |
| *Eucalyptus globulus* Labill. | | Eucalyptus |
| **Foeniculum vulgare* Mill. | | Fennel |
| *Glycyrrhiza glabra* L. | | Liquorice |
| *Grindelia robusta* Nutt. | | Gumplant |
| *Hamamelis virginiana* L. | | Witch hazel |
| *Lavandula angustifolia* Mill. | | English lavender |
| **Lavandula latifolia* Medik. | | Spike lavender |
| *Lavandula stoechas* L. | | French lavender |
| *Lavandula viridis* L'Hér. | | Green lavender |
| *Melaleuca alternifolia* Maiden & Betche | | Tea tree |
| **Melissa officinalis* L. | | Lemon balm |
| **Mentha piperita* L. | | Peppermint |
| *Mentha pulegium* L. | | Pennyroyal |
| *Mentha suaveolens* Ehrh. | *Mentha rotundifolia* var. *suaveolens* (Ehrh.) Briq. | Apple mint |
| *Monarda citriodora* Cerv. ex Lag. | | Lemon bergamot |
| *Monarda dydima* L. | | Scarlet beebalm |
| *Monarda fistulosa* L. | | Wild bergamot |
| **Nigella sativa* L. | | Black cumin |
| **Ocimum basilicum* L. | | Basil |
| *Origanum vulgare* L. | | Oregano |
| *Origanum vulgare* L. subsp. *hirtum* | | Greek oregano |
| ***Origanum vulgare* L. subsp. *viridulum* (Martrin-Donos) Nyman** | *O. heracloticum* L. | Green oregano |
| **Pelargonium graveolens* L'Hér. | | Geranium |
| *Pinus cembra* L. | | Pine tree |
| **Rosmarinus officinalis* L. | | Rosemary |
| **Salvia officinalis* L. | | Sage |
| *Satureja montana* L. | | Winter savory |
| *Satureja thymbra* L. | | Pink savory |
| *Syzygium aromaticum* (L.) Merr. et L.M. Perry | *Eugenia caryophyllata* Thunb. | Clove |
| *Thymbra capitata* (L.) Cav. | *Thymus capitatus* (L.) Hoffmanns. & Link, *Coridothymus capitatus* (L.) Cav. | Conehead thyme |
| *Thymus mastichina* (L.) L. | | Mastic thyme |
| **Thymus serpyllum* L. | | Wild thyme |
| **Thymus vulgaris* L. | | Common thyme |
| **Thymus zygis* Loefl. Ex L. | | White thyme |

Several EOs are referenced across the reviewed literature, but some—such as EOs of cinnamon, clove, common thyme and oregano—are mentioned more frequently and are recognized for their superior antimicrobial effectiveness also at low concentrations [5–23]. Also, fennel EO (20% v/v in water) demonstrated good antifungal efficacy [24]. Additionally, some studies have evaluated the individual components of various EOs. Table 2 presents the essential oils, the hydrolates, their

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concentrations, the microbial species they targeted in the in vitro experiments, and the associated references.

It is worth mentioning the contrasting results obtained with Essenzio©, a commercial product composed of oregano EO. Some papers wrongly report that it is a blend of oregano and common thyme [23,25–27]. Used to treat a biofilm of Chlorophyceae and cyanobacteria on wall painting model samples, it showed effective at a dilution of 50% in demineralized water [26], while undiluted it was instead ineffective on microalgal biofilms [23]. Quite strange opposite results.

There are EOs with extremely low antifungal activity such as spike lavender and rosemary EOs (applied either undiluted or dissolved in dimethyl sulfoxide at different concentrations) [18,21]. Other examples are those of clove EO (5-10 %) that was just partially efficient on brown-rot and white-rot fungi [28] and basil EO [18] that was found to be ineffective over extended periods (more than 168 hours) [29]. The authors suggested that treatments based on this EO should be applied more frequently.

**Table 2.** This table presents the essential oils, the hydrolates, their concentrations and the microbial species they targeted in the in vitro experiments. The associated references are listed in chronological order. Further details can be found throughout the text.

| Essential oils, hydrolates and extracts (plant source) | Concentration | Microorganisms | References |
|---|---|---|---|
| EO and hydro-alcoholic extracts of basil | 15 μL | *Aspergillus* sp., *Mucor* sp., *Penicillium* sp. | [29] |
| English lavender, oregano, rosemary | 0.1-2.0 μLmL$^{-1}$ for oregano, 10.0-100.0 μLmL$^{-1}$ for the other two EOs | *Aspergillus niger*, *A. ochraceus*, *Bipolaris spicifera*, *Epicoccum nigrum*, *Penicillium sp.*, *Trichoderma viride* | [5] |
| Tea tree EO, calamint and garlic extracts | 100%, 50%, 25%, 12.5% | *Bacillus subtilis*, *Micrococcus luteus*. *Aspergillus* spp., *Penicillium chrysogenum* | [38] |
| Hydrolates of apple mint, bitter orange, conehead thyme, cornflower, Greek oregano, green oregano, gumplant, lemon balm, lemon bergamot, rosemary, sage, scarlet beebalm, wild bergamot, winter savory, witch hazel | diluted at a 1:2 ratio in gels | *Aspergillus sydowii*, *Cladosporium sphaerospermum*, *Penicillium chrysogenum* | [48] |
| Cinnamon | 5.625 μLmL$^{-1}$ for fungi, and 22.5 μLmL$^{-1}$ for bacteria | *Aspergillus niger*, *Penicillium funiculosum*, *Trichodema viride*. *Bacillus megaterium*, *Pseudomonas fluorescens*, *Streptomyces rutgersensis* | [6] |
| Common thyme, clove, geranium | 0.25, 0.5, 0.75, 1 μLmL$^{-1}$ | *Aspergillus awamori*, *A. flavus*, *A. niger*, *A.oryzae*, *A. tamari*, *A. terreus*, *A. ustus*, *A.wentii*, *Curvularia clavate*, *Fusarium oxysporum*, *Mucor fuscus*, *Penicillium citrinum*, *P. glabrum*, *P. oxalicum*, *Rhizopus oryzae*, *Stemphylum vesicarium* | [7] |
| Cinnamon, common thyme, wild thyme | 3%, 1%, 0.7%, 0.5% | *Aspergillus japonicus*, *Chaetomium* sp., *Fusarium* sp., *Stachybotrys chartarum* | [8] |
| Basil, fennel, lemon, rosemary, sage | 1.56-100 μLmL$^{-1}$ | *Alternaria alternata*, *A.tenuissima* | [36] |
| Common thyme, oregano | 50%, 25%, and 12.5% (v/v) | *Aspergillus flavus* | [60] |

doi:10.20944/preprints202510.0496.v1



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|---|---|---|---|
| English lavender EO, Liquorice alcoholic leaf extract | ELAV (5% v/v), LIQ (10 and 30% v/v). | *Leptolyngbya* sp., *Scytonema julianum*, *Symphyonemopsis* sp. *Actinobacteria*, *Bacteroidetes*, *Proteobacteria* | [34] |
| Lemon, common thyme | 5%, 10%, and 15% in ethanol 70% (v/v) | *Aspergills niger*, *Fusarium solani*, *Penicillium cyclopium* | [10] |
| Oregano, pink savory | 0.1, 0.2 and 0.5% (v/v) | *Bacillus* sp., *Paenibacillus* sp., *Stenotrophomonas* sp. *Cladosporium* sp., *Clonostachys* sp., *Fusarium* sp., *Penicillium* sp. | [11] |
| Common thyme, sea fennel | 50% and undiluted | *Bacillus* sp., *Georgenia* sp., *Ornithinibacillus* sp., *Streptomyces* sp. | [12] |
| Clove, oregano | Diluted with ethanol 70% in the ratio 2:1 | *Bacillus* sp., *Penicillium* sp. | [13] |
| Calamint, common thyme, oregano in hydrogels | 1-2% | *Bracteacoccus minor*, *Chlorella* sp., *Stichococcus bacillaris*. *Aphanocapsa* sp., *Isocystis* sp., *Leptolyngbya cebennensis* | [32] |
| Calamint, common thyme, French lavender, green lavender, mastic thyme, rosemary, sage | 5µL of undiluted EOs | *Aspergillus niger*, *Cladosporium* spp., *Exophiala* sp., *Fusarium oxysporum*, *Penicillium* spp. *Rhodotorula* sp. *Bacillus* sp., *Arthrobacter* sp. | [44] |
| Common thyme | 12.5%, 25%, 50%, and 100% (v/v) | *Bacillus* sp., *Streptococcus* sp. *Aspergillus* sp., *Penicillum* sp. | [14] |
| Common thyme, oregano | 5.63 and 7.5 $\mu LmL^{-1}$ | *Alternaria alternata*. *Staphylococcus epidermidis*, *Rhodotorula mucilaginosa* | [15] |
| Rosemary hydro-alcoholic extract | 0.78, 1.2, 1.56 mg/mL | *Aspergillus clavatus*, *Penicillium chrysogenum*. *Arthrobacter globiformis*, *Bacillus cereus*, *B. thuringiensis* | [37] |
| Bitter orange hydrolate and cinnamon EO | 28 $\mu L/cm^2$ | *Alternaria alternata*, *Aspergillus niger*, *Aureobasidium pullulans*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Penicillium citrinum* | [40] |
| Black cumin, clove, common thyme, geranium, lavender, lemongrass | 0.1, 0.25, 0.5, 1, 2 $\mu LmL^{-1}$ | *Pseudomonas protegens*, *P. putida*, *Serratia odorifera*. *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Cladosporium halotolerans*, *Penicillium crustosum*, *Trichoderma viride* | [16] |
| Cinnamon, scarlet beebalm, wild bergamot EOs in hydrogels. Hydrolates of bitter orange, lemon bergamot, scarlet beebalm | 2-0.06 % v/v and 50-1.6 % v/v respectively for EOs and Hys | *Alternaria alternata*, *Aspergillus niger*, *Aureobasidium pullulans*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Penicillium citrinum* | [17] |
| Common thyme and English lavender encapsulated within an alginate hydrogel | 0.1% (v/v) | *Brasilonema* sp., *Leptolyngbya* sp., *Oculatella subterranea*, *Scytonema julianum*, *Symphyonemopsis* sp. | [43] |
| Common thyme (a) or thymol (b) in hydrogels | (a) 0.25 % or 0.1 %, (b) 0.18 % or 0.07 % | *Leptolyngbya* sp., *Oculatella subterranea*, *Scytonema julianum* | [45] |
| Clove, oregano | 7.5% | *Acremonium*-like fungus, *Cladosporium* sp., *Fusarium oxysporum*, *Mortierella* sp. *Rhodococcus* sp., *Streptomyces avidinii* | [39] |
| EO and hydro-alcoholic extract (HAE) of oregano | 50% EO and 100% HAE | *Bacillus* sp., *Streptomyces* sp. *Terribacillus* sp. *Alternaria* sp., *Aspergillus* sp., *Chaetomium* sp. | [19] |
| Mixture of oregano, lemongrass and | 0.78% | *Aspergillus fumigatus*, *Cladosporium cladosporoides*, *Penicillium chrysogenum* | [30] |

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| | | | |
|---|---|---|---|
| peppermint in ratio 1:1:1 | | | |
| Eucalyptus, lemongrass, oregano, peppermint, rosemary | 0.78 and 6.75% | *Aspergillus fumigatus*, *Cladosporium cladosporoides*, *Penicillium chrysogenum* | [30] |
| Fennel, green lavender, mastic thyme, pennyroyal | Undiluted | *Aspergillus versicolor*, *Cladosporium cladosporioides*, *Roussoella* sp., *Stagonosporopsis* sp., *Paraconiothyrium variabile*. *Cystobasidium minutum*, *Vishniacozyma globospora*. *Pseudomonas* sp., *Micobacterium* sp. | [35] |
| Cinnamon, eucalyptus, frankincense, geranium, lavender, lemongrass, mint, rosemary, tea tree, thyme | 0.125, 0.25, 0.5, 0.75, 1 μL/mL | *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus* | [21] |
| Fennel, green lavender, mastic thyme, pennyroyal | 20% (v/v) | *Bacillus mobilis*, *B. wiedmannii*. *Cladosporium cladosporioides*, *Penicillium brevicompactum* | [24] |
| Basil, cinnamon, common thyme, English lavender, oregano, tea tree. Essenzio© | 0.5% and 5% (v/v). Essenzio© undiluted | *Acinetobacter junii*, *Aeromonas rivipollensis*, *Chryseobacterium contaminans*, *Ensifer adhaerens*, *Enterobacter quasihomaechei*, *E. sichuanensis*, *E.* sp., *Exiguobacterium mexicanum*, *Pantoea agglomerans*, *Pantoea ananatis*, *Pseudomonas alkilphenolica*, *P. chengduensis*, *P. lalkuanensis*, *P. mosselii*, *P. oryzihabitans*, *P. resinovorans*, *P. sediminis*, *P. soli*, *Serratia liquefaciens*, *S. rubidaea*, *Shigella flexneri*, *Stenotrophomonas lactitubi*. *Fusarium chlamydosporium*, *Paecilomyces lilacinus*, *Penicillium chrysogenum*, *P. citreonigrum*, *P. miczynskii* | [23] |

Basil, tea tree, and English lavender EOs at 5% (v/v) resulted almost ineffective against a microalgal biofilm [23]. EO of sea fennel (undiluted) showed poor activity against some bacteria [12].

It has also been found that the chemical composition of the EO from a plant species can vary. Gas chromatography-mass spectrometry (GC-MS) analysis highlighted compositional differences between basil EO obtained commercially and that produced in the laboratory. The lab-produced oil had higher levels of eucalyptol and $\alpha$-bergamotene, while the commercial oil contained more linalool [29]. Similarly, in a recent study [30], the chemical composition of oregano EO was found to differ from that reported in previous research. Notably, terpinene, identified by this study as the most abundant compound, was not listed as the dominant component in other studies. According to the authors [30], this variety in chemical composition can be due to the different geographic origins of EOs since the studies have been conducted in more than twenty different countries. Another illustrative example comes from two separate studies: while Arantes and coauthors [31] identified 1,8-cineole as the primary component in calamint EO, Genova and coauthors [32] found this compound present at just 0.5%.

These findings underscore a crucial point: the antimicrobial properties of EOs can vary significantly depending on the production method, which influences their composition, as well as on the geographic origins and age of the plants from which the oils are derived [10]. Unfortunately, numerous studies fail to specify or offer only limited information about the chemical composition of the EOs utilized in their experiments. Each EO has a unique chemical profile that significantly impacts its antimicrobial effectiveness. Altering the proportions of these chemical constituents can result in mixtures with entirely different levels of efficacy. Thus, it is crucial to disclose the full

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composition of an EO to ensure accurate interpretation of results [33], yet this information is overlooked or insufficiently detailed in some scientific studies.

Studying the effects of English lavender EO (5% v/v) on cyanobacteria, a recovery rate of 20% was noticed [34]. According to the authors, this was not due to new growth but rather to the repair of the microorganisms' photosynthetic structures. A second treatment was applied, and notable results were observed after 76 days. This study highlights the initial efficacy of essential oils followed by the microorganisms' ability to restore their structures. Such findings have implications for the required quantities of essential oils, the frequency of applications, and the resistance of microorganisms to the damaging effects of these oils—contrary to claims made by many other studies.

Some studies have evaluated the effectiveness of EOs in comparison to conventional biocides, which are listed in Table 3. When compared with the commercial biocide Biotin® T some EOs showed lower antimicrobial effect. It is the case of mastic thyme, pennyroyal, fennel and green lavender EOs that inhibited fungal and bacterial growth 64%, 32%, 30% and 25% less than Biotin® T (1% v/v) despite their higher concentration (20%) [35]. The comparison with conventional biocides such as benzalkonium chloride and Preventol® RI80, on the other hand, favored basil EO, as larger quantities of the two biocides were required to inhibit the growth of the tested fungal strains [36].

**Table 3.** Overview of biocidal products reported in the reviewed literature.

| Product Name | Main Components | Concentration / Notes |
|---|---|---|
| Bioban© TM 104 | Didecyldimethylammonium chloride and octyl-isothiazolinone | Antimicrobial blend |
| Biotin© R1+R2 | R1 iodopropynyl butyl carbamate dissolved in diethylene glycol monobutyl ether; R2 n-octyl isothiazolinone and terbutryn dissolved in 2-butoxyethoxy ethanol | Antimicrobial blend |
| Biotin© T | Didecyldimethylammonium chloride (1), octyl-isothiazolinone (2), isopropanol (3), formic acid (4) | (1) 40-60%, (2) 7-10%, (3) 15-20%, (4) 1-2.5% |
| NewDes© 50 | Didecyldimethylammonium chloride | 50% aqueous solution |
| Preventol© RI 50 | Alkyl benzyl dimethyl ammonium chloride (benzalkonium chloride) | ~50% aqueous solution |
| Preventol© RI 80 | Alkyl benzyl dimethyl ammonium chloride (benzalkonium chloride) | ~80% aqueous solution + 2% isopropyl alcohol |

Cinnamon, oregano, and common thyme EOs at a 5% concentration caused a significant reduction in the photosynthetic activity of algal biofilms. This effect lasted for up to two months after treatment and was comparable in effectiveness to biocidal treatments using Bioban® TM 104 Antimicrobial and benzalkonium chloride, both applied at 3% (v/v) [23].

Calamint EO and its active component pulegone were better than Preventol® RI80 applied on a biofilm composed of green algae and cyanobacteria [32].

A few experiments were conducted on hydro-alcoholic extracts such as that of rosemary [37] or hydrolates such as that of bitter orange [17]. They inhibit the growth of fungi and bacteria, but the quantity needed is far higher than that of EOs - mg/mL vs µg/mL or quite high concentrations. Some did not even show any antimicrobial activity, as was the case of extracts of mastic thyme, pennyroyal, fennel, and green lavender [35]. Better results were obtained using alcoholic leaf extract of liquorice (10 and 30% v/v) and a mixture of it (10%) with English lavender EO (5%) when applied twice on photosynthetic and heterotrophic microorganisms [34]. Stronger antimicrobial activity was performed by garlic extract at different concentrations (100%, 50%, 25%, 12.5%) compared to tea tree EO and calamint extract [38].

The production of hydro-alcoholic and water extracts of basil through three methods resulted in different compositions that had varying levels of antifungal effectiveness [29].

 doi:10.20944/preprints202510.0496.v1



Both painted and unpainted samples of granite and gneiss, as well as replicas of wall paintings of Etruscan tombs, were inoculated with fungi and heterotrophic bacteria. These samples were then treated (three applications) using emulsified oregano and clove bud EOs at a concentration of 7.5% (v/v) in water, with Tween 20 and Span 20 as emulsifiers [39]. Following the initial application, there was a sharp reduction in viable cells on stone samples, while no notable change was seen in wall painting samples compared to the controls. This difference may be related to how long the product remained in contact with the substrates. According to the authors, the high porosity of stone samples likely allowed the product to penetrate deep into the substrate, away from the colonized surface, making repeated applications necessary to achieve meaningful biocidal effects. After two months, wall painting samples treated with clove bud EO developed a widespread and dense fungal growth. The researchers suggested that the emulsifiers may have served as nutrients for the surviving cells. Even so, after the first application, fungi *Cladosporium* sp. and *Penicillium* sp. were detected in all samples, and three treatments were still insufficient to completely eliminate the fungi [39].

### *2.1. Emulsions and Mixtures of Essential Oils*

To determine whether combining multiple essential oils could enhance their effectiveness, some studies have assessed mixtures of EOs. “Zeylantium green emulsion” (Zege) made of bitter orange hydrolate and cinnamon EO (from bark) was effective on fungi growing on canvas samples at concentration of 28 μL/cm$^2$ [40]. Using a mixture of oregano, lemongrass and peppermint EOs in ratio 1:1:1, the lower minimal inhibition concentration (0.78%) and better efficiency during a vapor test compared with individual EOs was proven on fungi commonly found on archive papers [30]. Nevertheless, accurately predicting the antimicrobial effectiveness of essential oil mixtures is highly challenging. Each essential oil contains many chemical components, and the interactions among them can lead to either synergistic or antagonistic effects against microbes [41]. This effect also appears with mixtures of EOs components, as shown by the application of blends of thymol, carvacrol, and pulegone on a biofilm covering a travertine wall [42]. In this case, both positive and negative interactions were observed: while some individual active compounds were more effective than the EOs they naturally occur in, combining these compounds actually reduced their overall effectiveness.

### *2.2. Essential Oils Functionalized on Carrier Materials - Hydrogels*

The use of carrier materials for delivery of antimicrobial EOs has been studied. According to many authors, the method allows even low amounts of EOs to be efficiently antimicrobial likely because it reduces the volatility of EOs’ components.

An alginate hydrogel loaded with common thyme EO at 0.1% (v/v) had the greatest inhibitory effect of photosynthetic efficiency compared to English lavender EO [43]. In contrast, complexation of common thyme EO with β-cyclodextrin did not show important levels of fungal inhibition [44]. The same EO (0.1% and 0.25%) or thymol (0.07% and 0.18%) were incorporated into a hydrogel composed of 5% alginate (w/w) and 0.2% calcium chloride (w/w) and demonstrated significant effectiveness against cyanobacterial biofilms for up to six months post-treatment [45]. Thymol-loaded in chitosan nanoparticles had a better performance than free thymol against fungus *Aspergillus niger* [46]. Thymol, carvacrol, and eugenol have been functionalized on crystalline structures of β-cyclodextrins and phenazine-based cocrystals, offering a novel approach to managing microorganisms that degrade paper. The phenazine-carvacrol cocrystal was the most effective treatment against some fungal species [47].

Sixteen EOs hydrolates were incorporated into a gellan hydrogel and applied to paper samples that had been inoculated with three different fungal strains. Of these, only two—wild bergamot and bitter orange—demonstrated fungicidal effects [48].

A biofilm developed on granite blocks and composed of green algae and cyanobacteria, the same species found on a historic granitic building in Santiago de Compostela (Spain), was treated with EOs of oregano, common thyme, calamint, and their respective main active components carvacrol, thymol, and pulegone, all of them (2% w/w) embedded in a hydrogel matrix [32]. Calamint and its

 doi:10.20944/preprints202510.0496.v1



active component pulegone proved to be the most effective and yielded similar results, comparable to those of uncolonized granite.

Psyllium and psyllium-alginate beads loaded with cinnamon essential oil (2%) have been suggested as a means to protect antique books, historical documents, and other cellulosic materials such as paper, wood, and textiles from microbial contamination [49].

A general observation about all these results is that biocides, including EOs, frequently show different behavior in laboratory experiments than they do when applied in in situ conditions when targeting the same microorganisms. One reason for this is that the sensitivity of microorganisms to biocides in laboratory settings is typically much higher than in situ conditions. Another reason is that biocides tend to be less effective against sessile microorganisms, such as those forming biofilms, compared to planktonic ones [20]. Biofilms, with their intricate architecture and dynamic nature, create a physical barrier that shields microorganisms from harmful substances like biocides. Moreover, the response of in situ biofilms is influenced by environmental factors [20].

As a result, the dosage needed to eliminate a biofilm in situ might be significantly higher than the amount required to eradicate isolated organisms in vitro. Therefore, due to the substantial differences between laboratory and field conditions, extrapolating results for practical applications often proves challenging. Nevertheless, such studies are valuable in providing a preliminary assessment of the antimicrobial efficacy of EOs.

## 3. In Situ Studies Using Essential Oils, Hydrolates and Plant-Derived Extracts Applications

### *3.1. Inorganic Materials*

Oregano, common thyme, conehead thyme and clove EOs have been among the most widely utilized in in situ experiments, primarily targeting biofilms containing varying proportions of cyanobacteria, green algae, fungi, and black meristematic fungi. Although these EOs generally produced positive results in laboratory studies, their real-world effectiveness varied. For instance, tests with oregano and common thyme EOs individually, in combination, and with the commercial biocide Biotin® T (all at 2% v/v in water) on outdoor marble at Florence Cathedral (Italy) covered with a black biofilm of bacteria and fungi [20] found Biotin T® to be most effective, though the EOs also showed notable antimicrobial effects, sometimes rivaling it. Combining the oils did not increase their efficacy. The authors attributed differences in outcomes to variations in the microbial community composition and local climate.

Three weekly applications by brush of the same previously mentioned EOs and clove EO (all at 5% concentration in a 30/70 ethanol-water solution), along with the commercial biocide Preventol® RI80 (3% in water), were carried out on marble artworks at the Non-Catholic Cemetery for Foreigners in Rome, Italy [50]. These artworks featured a black patina mainly made up of cyanobacteria and some black meristematic fungi, as well as a green patina mostly consisting of green microalgae. Preventol® RI80 completely eliminated microorganisms, while EOs were less effective, leaving 40–70% of cells viable. Similarly, some EOs (eucalyptus, basil, clove, common thyme, pine tree, and tea tree) were applied at a concentration of 0.4% v/v in water on cyanobacteria and green algae growing on a mosaic located at the Archaeological Park of Ostia Antica (Rome, Italy). The best results were obtained by using tea tree, pine tree and common thyme. However, all the EOs had minor biocide activity if compared with Preventol® RI50 (3% v/v in water) [25].

The application of EOs on a marble statue colonized by cyanobacteria, fungi, and lichens proved to be very difficult. The statue was treated using two essential oil blends: one containing oregano, conehead thyme and clove, and the other containing oregano, cinnamon, and clove, each oil at a concentration of 2.25% w/w. These blends were incorporated into three different carriers—agar-agar, Politect®, and Carbogel. The process of removing the biological colonization was highly challenging, requiring four treatment applications for two months to fully remove the microorganisms [51]. Based on the study, I believe that mixtures of EOs do not outperform individual EOs. Additionally, the restoration process was lengthy and involved applying multiple substances to the stone.

Embedding EOs in various carrier materials helps improve their efficacy by reducing evaporation, as previously discussed. Oregano, common thyme, and calamint EOs (and their main phenolic components - thymol, carvacrol, and pulegone) were incorporated at 2% w/w into polyvinyl alcohol-based hydrogels and applied to granite walls with dark and green biofilms [52]. Combinations and individual ingredients were tested. After one month, hydrogels were easily removed, simplifying cleanup. Oregano-containing treatments worked best, especially against algae-dominated biofilms, but were less effective when cyanobacteria were present, likely due, according to the authors, to their higher amount of protective extracellular polymers hindering biocide penetration. An experiment used similar hydrogels (poly(vinyl)alcohol-borax hydrogels) loaded with thyme EO on slabs of Carrara marble and St. Margarethen stone located outdoors and covered by a dark, greenish to blackish biofilm. The oil covered a broad spectrum of activity against bacteria, fungi and algal microorganisms [53].

In one approach, conehead thyme EO was mixed with water at a 1:3 ratio and stabilized with 4% kaolinite by mass [54]. This emulsion was applied to outdoor surfaces of ceramic, marble, and cement grit with green algae and cyanobacteria growth. After treatment, the surfaces were covered with polyethylene film for several hours. The results were very positive: all surfaces remained clean for at least four months. Another approach proposed a release system based on halloysite nanotubes [55]. Areas of outdoors ancient limestone Buddha statues were treated with 5% (w/v) cinnamaldehyde (a component of cinnamon EO)-loaded nanotubes water suspension and after a year the number of microorganisms was greatly reduced.

Liquorice leaf extract (10%) was used on a patina primarily made up of cyanobacteria on stone surfaces from the Domus Aurea in Rome (Italy) [34]. The treatment was administered twice: initially and again after 14 days. While the biofilm nearly recovered by day 14, after the second treatment the yield was 57% lower than the control area by day 76. The authors suggest that this lack of sustained effectiveness indicates that a higher concentration of the extract may be necessary for lasting results, although, as I note, the concentration used was already quite substantial.

Some studies have investigated Biotersus®, a commercial blend of EOs derived from clove (35.0–37.5%), conehead thyme (27.5–30.0%), and cinnamon (15.0–16.5%). In one study, the product was used at concentrations of 1.5% and 1.4% in water to treat crustose lichens growing on stone walls in Persepolis, Iran, and Saluzzo, Italy [56]. It was effective when applied with a cellulose poultice or brushed onto the surface followed by coverage with a paper tissue, a second application of it, and then a final covering with plastic film. Conversely, brushing it on and covering the surfaces only with plastic film did not yield effective results; this was likely due to an insufficient amount of product and incomplete hydration of the lichens. The study emphasized that successful EOs treatments depend on defining an application protocol that ensures thorough hydration of lichens [56]. The same product, diluted to 1.4% in water, was combined with Psyllium seed shells and applied twice on vertical peperino surfaces colonized by cyanobacteria, algae, fungi, and epilithic crustose lichens [57]. The treated areas were sealed with plastic film for a week, and this process completely removed the biofilms, although some lichen remnants persisted. The authors noted that dehydration was a major concern, as it caused some peeling of the Psyllium support. Another study [27] experimented Biotersus® (1.4% in water) along with another commercial product, Essenzio© (undiluted), and two EOs (cinnamon bark and oregano at 0.5% and 1% in a mixture of water and ethanol) comparing them to three chemical biocides used in the conservation of stone artifacts (Biotin© R1+R2 at 3% in ethanol, NewDes© 50 and Preventol© RI50 both at 5% in water). The products were applied by brush on a marble slab colonized by cyanobacteria, microalgae and microcolonial fungi. The results indicate that chemical biocides exhibited biocidal activity greater than 99.5% with a single application, while cinnamon oil (at a 1% concentration) and Essenzio© required two applications to achieve the same level of efficacy. Conversely, oregano oil and Biotersus were not able to effectively reduce microorganism vitality, even with repeated applications.

A study focused on a very important issue in conservation of outdoor artworks: the recolonization of restored surfaces. Although a biocidal treatment initially removed a green biopatina on the plastered surfaces of a historic building in Chiavari, Italy, it proved ineffective over time, as widespread biological recolonization occurred within a few months [58]. So, the study wanted to determine whether further removal treatments should be performed, considering their environmental impacts. To achieve this, the Life Cycle Assessment (LCA) was conducted to examine the challenges of its application in assessing three biocidal/cleaning treatments. While a detailed explanation of the methodology is beyond scope here, it is noted as particularly interesting. The three biocides evaluated were Preventol© RI 50, Essenzio©, and hydrogen peroxide. The first two were applied to the surface three times using a brush, while the third was applied once. None of the treatments completely removed the green patina, which remained especially in binder-rich areas around aggregates. However, Preventol© RI 50 and hydrogen peroxide both reduced the extent of biocolonization by over 90%, whereas Essenzio© reduced it by about 60%. The LCA results indicated that oregano oil, the main component in Essenzio©, had the highest environmental impact across all categories examined, including human health, ecosystem quality, and resource scarcity. The authors emphasize that using natural products does not always guarantee a lower environmental impact than synthetic alternatives. They also suggest that a non-treatment option should be considered, especially since the patina on the recolonized surfaces was primarily epilithic, did not cause cracks or loss of cohesion, and resulted in the surface becoming nearly hydrophobic.

EOs have been also experimented indoors. A recent study [59] evaluated the efficacy of 100% pure tea tree and common thyme EOs at disinfecting indoor air. In an unventilated area of a church in Spain, the EOs were diffused as vapor using doses ranging from 10 drops (about 0.5 mL) to 20 drops (about 1 mL). Tea tree EO delivered the strongest results, reducing fungal and bacterial air contamination by 77.3% and 95.0%, respectively, without leaving any residue on the artistic surfaces.

### 3.2. Organic Materials

Common thyme, clove, oregano, and cinnamon are also key essential oils used in the treatment of organic materials such as paper, wood, and canvas.

Common thyme EO, applied at a concentration of 0.75% v/v, was tested both by spraying and by using impregnated contact sheets on a book cover contaminated with fungi and bacteria [15]. Microbial inhibition proved less effective in the areas treated by spraying compared to those covered with contact sheets. This difference is likely due to the paper slowing the evaporation of the oil's volatile components.

Clove EO was applied at concentrations of 5% and 10% v/v in ethyl alcohol to beechwood sawhorses from a museum collection that were contaminated with fungi. While there was an initial reduction in fungal growth, the fungi began to regrow after 48 days [28].

Three EOs (cinnamon, wild thyme, and common thyme) were tested as possible alternative biocides to use in the preservation of waterlogged archaeological wood [8]. A hydroalcoholic solution containing the oils, 1% in concentration, leads to a significant decrease in the vitality of the microorganisms present in the wood and the storage water and demonstrated antimicrobial effects higher than 73%, reaching 99–100% for cinnamon oil.

A wooden sculpture was exposed for 15-20 days to vapors (from 0.5 mL) of oregano and common thyme EOs to eliminate *Aspergillus flavus* colonies present on the surfaces. The treatment was successful, and no recolonization occurred after eight months [60].

A combined approach was chosen to eradicate the bacterial and fungal colonization spread over the surface of an African sculpture made of kapok wood. The object was preliminarily treated with a hydro-alcoholic solution of common thyme EO followed by the exposure to vapor of the same EO in a dedicated chamber [14]. The treatment successfully removed the microbes.

Two historical books, each exhibiting different levels of microbial contamination, were treated with vapors of common thyme EO at a 10% concentration in dimethyl sulfoxide. It demonstrated stronger bacteriostatic effects (ranging from 12% to 100%) compared to its fungistatic activity (ranging from 0% to 99.3%) and showed biocidal activity against 9 out of 16 microbial strains tested, including 5 fungal strains [61].

An oil painting at the Uffizi Museum in Florence, Italy, was treated on its reverse side by spraying an emulsion consisting of 0.3% (v/v) cinnamon EO and 99.7% (v/v) bitter orange hydrolate [17]. A total of 96 sprays were applied to the artwork. To prevent the compounds from dispersing, Melinex® was folded to enclose the painting, forming a sealed container bag using insulating tape. The treatment was allowed to act for 24 hours and effectively eradicated the fungal contamination.

## 4. Effects of Essential Oils on Material Characteristics

Assessing color alterations has been the primary analytical approach for evaluating the effects of EOs on both inorganic and organic materials. However, interpreting these analytical findings is challenging, as what constitutes an acceptable change in color is often complex and varies depending on the specific object. Visual assessment, although very effective for qualitative evaluation, lacks precision for quantitative evaluation [62]. It is worth noting that the threshold value considered not perceptible by human eye is $\Delta E^*$ 3.5 [63]. Therefore, $\Delta E^*$ values below 4 are generally considered acceptable [64]. The total color difference $\Delta E^*$ between two measurements is the geometrical distance between their positions in the CIE L*a*b* color space where L* indicates lightness, a* is the red/green coordinate, and b* is the yellow/blue coordinate.

Additional analyses were conducted to evaluate the impact of essential oil treatments on various material properties. For stone, researchers examined changes in mineralogical composition and water absorption. For cellulose-based materials, such as wood and paper, they assessed oxidation and depolymerization processes. Paper samples were further analyzed for mechanical and structural parameters, including stretch, tensile strength, tensile energy absorption, burst index, fiber integrity, and the morphology of printed areas. Tables 4, 5 and 6 provide detailed findings from studies examining the impact of essential oils and other plant-derived substances on material color and other properties. For brevity, only the most pertinent results are discussed in the text.

Some studies have demonstrated that EOs are capable of causing noticeable color changes on various materials, even at low concentrations, while others have found no impact on color at all.

**Table 4.** Pigment sensitivity to essential oils.

| .Pigment | Essential oil | Concentration (% v/v) | ΔE*(Color change) | Effect description | References |
|---|---|---|---|---|---|
| Charcoal black, malachite, cinnabar | Clove | 7.5 | >4 | Pronounced color shift | [39] |
| Cinnabar | Oregano, clove, common thyme | 1–10 | <2 | | [18] |
| Hematite | Clove | 1-10 | 2.99 | | [18] |
| Hematite | Clove | 7.5 | >4 | Pronounced color shift | [39] |
| Hematite | Oregano | 1-10 | 2.89 | | [18] |
| Hematite | Cassia | 1-10 | 4.06 | | [18] |
| Hematite | Common thyme | 1-10 | 3.37 | | [18] |
| Oyster shell white | Common thyme, oregano | 1–10 | <1 | | [18] |
| Oyster shell white | Cassia | 3–10 | 5.89 | Yellowing effect | [18] |
| Vinyl blue | Zeylantium green emulsion | Undiluted | 10.3 | Significant color variation | [40] |

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| Alkyd blue | Zeylantium green emulsion | Undiluted | 3-4 | Moderate shifts in unaged samples only | [40] |
| Acrylic blue | Zeylantium green emulsion | Undiluted | <3 | | [40] |
| Alkyd yellow | Zeylantium green emulsion | Undiluted | 12.5 | Noticeable shifts in unaged samples only | [40] |
| Acrylic and vinyl yellow | Zeylantium green emulsion | Undiluted | <2 | | [40] |
| Acrylic, vinyl, alkyd red, and green | Zeylantium green emulsion | Undiluted | <3 | | [40] |

Color Legend $\Delta E^* > 4$ = Significant color change, $\Delta E^* \sim 3–4$ = Moderate change, $\Delta E^*$ 1–3 = Minimal change.

Painted stone samples treated with oregano and clove bud EOs [39] showed significant total color difference ($\Delta E^* > 4$) with notable variability depending on the pigment. Charcoal black, hematite, malachite, and cinnabar exhibited pronounced color shifts when exposed to clove oil (Table 4).

The pigments cinnabar, hematite, and oyster shell white were mixed with 3% Hide Glue©, spread on slide glass and treated by spraying oregano, clove, common thyme, and cassia EOs [18]. Cinnabar treated with oregano, clove, and common thyme EOs showed a slight color change. Thyme and oregano EOs led to minimal color change of the oyster shell white pigment, whereas clove and cassia EOs caused yellowing, and the second caused a strong color change. Hematite got the highest color change by cassia EO, followed by thyme, clove and oregano EOs (Table 4) [18]. With respect to these two studies, it is important to highlight the conflicting outcomes observed for clove EO on cinnabar pigment: while Isola and coauthors [39] reported significant color changes, Lee and Chung [18] found only minor alterations. This discrepancy likely stems from differences in the chemical composition of the clove EOs used in each study.

The application of Zeylantium green emulsion (Zege) (see subsection 2.1) to painted canvas samples [40] induced significant color variation in vinyl blue across both unaged and artificially aged specimens. In contrast, alkyd blue, ocher, and red pigments exhibited noticeable shifts exclusively in unaged samples, suggesting a differential sensitivity to Zege based on pigment composition and aging state. Acrylic, vinyl, and alkyd red and green pigments, and acrylic and vinyl yellow pigments did not show significant color changes (Table 4).

**Table 5.** Effects of essential oils and biocides on stone materials.

| Stone | Essential oils and biocides | Concentration (% v/v) | $\Delta E^*/\Delta L^*/\Delta b^*$ (Color change) | Effect Description | References |
|---|---|---|---|---|---|
| **Limestone** | Green lavender | 20 | $\Delta E^*$ 9.4; $\Delta L^*$ −9.3 | Blurring, staining, marked lightness reduction | [35] |
| **Limestone** | Fennel | 20 | $\Delta E^*$ 2.3; $\Delta L^*$ −2.2 | Staining, lightness reduction | [35] |
| **Limestone** | Pennyroyal | 20 | $\Delta E^*$ 3.4; $\Delta L^*$ −3.3 | Staining, lightness reduction | [35] |
| **Limestone** | Common thyme | 0.075 | $\Delta E^*$ 2.5 | Cumulative risk with repeated treatment | [9] |
| **Limestone** | Clove | 0.1 | $\Delta E^*$ 0.8 | | [9] |

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| **Limestone** | Geranium | 0.1 | ΔE* 4.1 | | [9] |
| **Limestone, tuff** | Biotin© T | 1 | | No noticeable color change; staining only on low-porosity rock | [35] |
| **Granite** | White thyme | 2 | ΔE* ~6 | | [65] |
| **Schist, mortar, granite** | Oregano, common thyme | 2 | | No mineralogical or color changes | [65] |
| **Granite, gneiss** | Oregano, clove, common thyme, cassia | 1–10 | | No color or chemical changes | [18] |
| **Granite, gneiss** | Oregano, clove bud | 7.5 | | Negligible color change; no change in water absorption | [39] |
| **Carrara marble** | Oregano, common thyme, Biotin© T | 2 | ΔE* < 3 | Combination of the two EOs (ΔE*~3). Stone yellowing observed | [20] |
| **Brick, peperino, mortar** | Nanocomposite with oregano EO or eugenol | — | ΔE* < 3 | Surfaces hydrophobic, vapor-permeable; no aesthetic alteration | [66] |
| **Peperino** | BioTersus© | — | — | No color interference | [57] |
| **White sedimentary rock** | Cinnamon Bark & Oregano EOs | 0.5–1 | ΔE* < 2; Δb* < 2 | Possible yellowing from Δb* | [27] |
| **White sedimentary rock** | Biotersus©, Essenzio© | 1.4 and undiluted | ΔE* < 2; Δb* < 2 | Possible yellowing from Δb* | [27] |
| **White sedimentary rock** | Biotin© R1+R2, NewDes© 50, Preventol© RI50 | 3 / 5 | ΔE* < 2; Δb* < 2 | Possible yellowing from Δb* | [27] |

Color Legend ΔE* > 4 = Significant color change, ΔE* ~ 3–4 = Moderate change, ΔE* 1–3 = Minimal change.

Fennel, pennyroyal, and green lavender EOs as well as the biocide Biotin© T (at 1% v/v) were applied four times to limestone and calcareous tuff samples [35]. The limestone samples exhibited color changes. Notably, green lavender EO led to blurring and staining, and a marked reduction in lightness. Fennel and pennyroyal EOs induced staining and a reduction in lightness as well, though the effects were comparatively much less pronounced (Table. 5). The commercial biocide Biotin T© did not cause any noticeable changes in color or tone on the samples. However, blurring or staining was observed only on samples with very low porosity, most likely because nonvolatile components of the EOs remained on the surface of the rock. In rocks with higher porosity, such as tuff, these components are more likely to penetrate the pores, making any color or tonal changes undetectable [35].

EOs of oregano, common thyme and white thyme were applied to schist, mortar, and granite from the Roman wall in Lugo, Spain. The first two did not cause any mineralogical changes or alterations in salt content and original color, while the third resulted in a significant color change in the granite [65]. Similarly, oregano, common thyme, clove bud, and cassia EOs caused no color or chemical changes on granite and gneiss. Water absorption remained unchanged after treatment with oregano and clove bud [18,39] (Table 5).

Specimens of white Carrara marble were treated with oregano and common thyme EOs, a combination of both oils, and the commercial biocide Biotin T. After two years of outdoor exposure, common thyme and the combination of oils gave the highest color difference close to 3 (Table 5) [20]. The values of water absorption were similar for the treated and untreated specimens meaning that the treatments did not cause significant changes in their porosity. However, outdoor marble surfaces at Florence Cathedral treated with the same substances showed some yellowing four months after treatments, which is an undesirable side-effect [20].

Silica nanocapsules separately loaded with oregano EO and eugenol were incorporated into a multifunctional, hybrid nanocomposite coating and applied to samples of brick, peperino, and mortar [66]. Tests indicated that the treated stone surfaces became highly hydrophobic while maintaining their natural permeability to water vapor, confirming the effectiveness of the nanocomposite coatings for protecting stone materials from biodeterioration. Additionally, for all treated stone types, the measured ΔE values were below 3, proving that the coating maintained the stones' original appearance and was aesthetically suitable.

The effect of two EOs (cinnamon bark and oregano) and two EO-based products (Biotersus© 1.4% in deionized water, and Essenzio© no dilution) and of three chemical biocides commonly used in the conservation of stone artifacts (Biotin© R1+R2 at 3% in ethanol, NewDes© 50 and Preventol© RI50 in water) were tested on specimens of a white sedimentary rock. All the products were applied on the specimens' surface twice by brush, three-day interval. ΔE* was always lower than 2 for all the products. However, the Δb* value < 2 of some (Preventol©, Biotersus©, Essenzio©, and cinnamon oil) could suggest the possible progressive yellowing of the surface after repeated applications [27] (Table 5).

Artificially aged limestone samples were immersed for 8 hours in aqueous solutions containing common thyme, clove, and geranium essential oils [9]. The resulting ΔE* values were 2.5 for thyme, 0.8 for clove, and 4.1 for geranium, indicating varying degrees of color alteration (Table 5). However, even a low ΔE* value of 2.5 can be risky, as repeated treatments may cause significant discoloration.

Linalool is a naturally occurring terpene alcohol commonly extracted from lavender, rose, basil, thyme and neroli EOs. In an in vitro experiment, exposure to its vapor caused damage to both silver-gelatin photographic samples and bookbinding leathers [67]. When exposed to air, it becomes unstable and may oxidize into linalool hydroperoxide, a compound known to trigger the degradation of silver-based photographic images. Regarding leather samples, results indicated that linalool modified collagen conditions. Therefore, the authors did not recommend the use of this compound or EOs containing it in storage areas housing these types of materials [67].

**Table 6.** Effects of essential oils and hydrolates on organic materials.

| **Essential oils** | **Material** | **Treatment method** | **Concentration** | **ΔE* (Color change)** | **Observed effect** | **Reference** |
|---|---|---|---|---|---|---|
| Clove, lavender | Gelatin prints | Vapor exposure | Pure EOs | ~5 | Oxidation and depolymerization of cellulose | [68] |
| Clove, lavender | Wood | Vapor exposure | Pure EOs | < 4 | Oxidation and depolymerization of cellulose | [68] |
| Common thyme | Pine wood | Immersion in solution | 0.75 μl/ml | 2.7 | | [9] |
| Clove | Pine wood | Immersion in solution | 1 μl/ml | 4 | | [9] |
| Geranium | Pine wood | Immersion in solution | 1 μl/ml | 9 | | [9] |

| | | | | | | |
|---|---|---|---|---|---|---|
| Common thyme | Historical book | Vapor exposure | 10% in dimethyl sulfoxide | | Improved mechanical properties; increased bulk | [61] |
| Wild bergamot, bitter orange hydrolates | Paper from 18th-century books | Hydrogel treatment | | 1.27 (bergamot), 0.46 (orange) | No fiber damage | [48] |
| Rosemary, lavender | Historical archive paper | Fumigation | 1% v/v (rosemary), 0.4% v/v (lavender) | 0-2.5 | | [69] |
| Common thyme, sage | Cotton and hemp fabrics | Vapor exposure with | Pure EOs encapsulated in ethyl cellulose | | Thyme decreased strength; sage increased cotton strength but decreased hemp strength | [70] |
| Cinnamon | Cotton, linen, silk fabrics | Vapor exposure | Pure EO | 0.84 (cotton), 2.42 (linen), 2.67 (silk) | No change in optical, mechanical, or structural properties | [6] |

Color Legend ΔE* > 4 = Significant color change, ΔE* ~ 3–4 = Moderate change, ΔE* 1–3 = Minimal change.

Vapors of clove and lavender OEs strongly change the color of gelatin prints (Table 6). On wooden objects they induce oxidation and depolymerization of cellulose with changes in its crystallinity [68].

Quite low ΔE* values, 3.82 on a light area and 2.75 on a dark area of a sculpture made of kapok wood, resulted from a preliminary treatment with a hydro-alcoholic solution of common thyme followed by the exposure to vapor of the same EO in a dedicated chamber [14].

Pine wood samples were artificially aged and then immersed for 8 h in water solutions containing common thyme, clove and geranium EOs [9]. The samples showed high ΔE* values with geranium oil indicating that it interfered strongly with pine wood (Table 6).

The stretch, tensile, tensile energy absorption and burst indexes, correlated with book folding endurance, measured on a historical book treated with vapors of common thyme EO were higher than the ones of control paper. Additionally, an increase in overall bulk was observed as the thickness grew. Some parameters did not change after the treatment, e.g., pH and intrinsic viscosity [61].

Paper samples taken from eighteenth-century printed volumes treated with a gellan hydrogel loaded with hydrolates of wild bergamot and bitter orange *showed* minimal color changes [48] (Table.6). Moreover, fiber integrity and morphology of printed areas did not change after treatment.

Treating a historical archive paper by fumigating it with rosemary and lavender EOs proved effective in reducing fungal colonization. Although the initial treatment with rosemary EO did not eliminate *Trichoderma longibrachiatum*, the subsequent use of lavender oil successfully eradicated the fungus [69].

The study by Indrie and coauthors [70] investigated how exposing cotton and hemp fabrics to the saturated vapors of common thyme and sage EOs encapsulated in ethyl cellulose affected their mechanical and morphological properties. For cotton, the application of thyme EO oil decreased tensile strength by 29.9%, whereas sage essential oil increased it by 20% in the warp direction and by 39% in the weft direction. In the case of hemp, tensile strength dropped by up to 36% with sage and 40% with thyme. The authors attributed these effects to the microcapsules, which restrict fiber movement within the fabric, leading to the material breaking more abruptly under higher loads.

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Three artificially aged woven fabrics - cotton, linen and silk (sericin was removed) - were used in a model in vitro study. The treatment with pure cinnamon EO in vapor phase did not change the optical, mechanical and structural parameters of the textiles [6] (Table 6).

When considering the use of cinnamon EO as a preservative in the restoration of waterlogged archaeological wood, its solvent properties must be considered. During experimental trials, the oil caused damage to certain plastic components—specifically, the pumps used for bath recirculation and the plastic accessories of instruments employed to measure suspension density—highlighting its potentially corrosive effect on such materials [8].

## 5. Toxicity, Ecotoxicity and Sustainability of Essential Oils

Most authors of heritage conservation papers highlight the low toxicity and environmental impact of essential oils (EOs), presenting them as sustainable and safe alternatives to chemical biocides. However, they oddly do not reference any supporting studies to substantiate this claim. It appears that one or two initial articles were written this way, and subsequent publications, in their haste to present their own research on this innovative topic, simply echoed the same statements. Toxicity of EOs is desirable when the goal is to eliminate bacteria, algae, or fungi, and these microorganisms and human cells share some characteristics. So, it should not be totally surprising that some of the most useful antimicrobial EOs possess a degree of human toxicity [4].

Some papers [59,71] claim that EOs are classified as "Generally Recognized As Safe" (GRAS) by the US Food and Drug Administration (FDA https://www.fda.gov). This is not correct. Firstly, just some EOs are designated as GRAS – Table 1 lists EOs derived from asterisk-marked plants that are designated as GRAS. Secondly, the GRAS designation indicates that a chemical or substance added to food is considered safe by experts under the conditions of its intended use. In addition, another US regulation, California Proposition 65 (https://oehha.ca.gov/proposition-65/proposition-65-list) restricts the use of certain substances in consumer and lists them on the Proposition 65 List. Essential oils may contain some of those restricted substances. For instance, beta-myrcene and pulegone, found in EOs, are both listed because they might cause cancer. Pulegone was used in reviewed papers that claim it is harmless for the environment and human health [32,42,52].

In an effort to demonstrate the safety of hydrolates derived from *Citrus aurantium* and *Monarda fistulosa*, Di Vito and coauthors [48] report that these plants are included in sources permitting their use in human formulations. Regarding *M. fistulosa* they cite the Plant Guide Database of the U.S. Department of Agriculture that provides standardized information about the vascular plants, mosses, liverworts, hornworts, and lichens of the United States and its territories, but does not list plants permitted for use in human formulations, as the authors claim. It is important to note that *Monarda fistulosa* is not listed as "Generally Recognized As Safe" (GRAS) by the U.S. FDA. In contrast, *Citrus aurantium* is currently classified as GRAS (Table 1).

It is unrealistic to consider EOs safe and harmless merely because they are naturally derived from plants. There is actually scientific evidence supporting their toxicity and ecotoxicity. Recent research has indicated that specific constituents of EOs oils may exhibit genotoxic properties causing damage to DNA and possess carcinogenic potential, contributing to the development of cancer [72], as mentioned above.

As noted earlier, Peter Holmes' books [2] provide extensive and reliable information regarding EOs safety in relation to pharmacology and toxicity. He affirms that "their clinical uses have too short a history, their applications are too varied, and the current dichotomy between clinical experience and scientific research is too great." This indicates that it is incorrect to claim that EOs are safe for humans, as many studies on cultural heritage applications assert. Some EOs pose acute toxicity risks to humans and animals in small doses due to the presence of certain highly toxic components [73]. For example, EOs from *Artemisia absinthium* and *Thuja occidentalis*, both of which contain the neurotoxin thujone, and *Mentha pulegium*, which contains pulegone, another neurotoxin [74].

EOs of clove bud, *Pimenta* berry and leaf, cinnamon leaf, oregano, conehead thyme, chemotypes of *Thymus vulgaris* and winter and summer savory belong in the category of strong skin irritants

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because of their high content in various phenols, including eugenol, thymol and carvacrol [2]. Cinnamon bark and cassia bark oils are strongly irritating to the skin because of their high levels of cinnamic aldehyde. While most EOs do not cause sensitization, the ones mentioned above are known to pose a potential risk for it [2].

The effects of EOs of rosemary, citrus and eucalyptus on acute, developmental, and reproductive toxicity as well as on mucous membrane irritation have been studied by using in vitro (cell cultures) and in vivo (nematode *Caenorhabditis elegans* and hen's egg test) approaches [73]. The results indicated that all three EOs produced similar effects across the evaluated endpoints, with rosemary oil displaying a slightly higher toxicity. Notably, all the tested oils demonstrated pronounced toxic effects even at low concentrations.

Important studies have highlighted results obtained experimenting single components of EOs. Alkenyl phenols exhibit genotoxic and carcinogenic properties [75]. Thymol, 8-cineole (also known as eucalyptol), (αβ)-pinene and limonene induced cytotoxicity and genotoxicity through oxidative damage in diverse types of target cells (see review in [76]).

Non-carcinogenic components may also pose concerns such as potential liver, nerve, reproductive, kidney toxicity, and endocrine disruption [75].

Terpenes are a large group of volatile unsaturated hydrocarbons found, among others, in the essential oils of plants. They are widely used as food additives and in the medical field. Most terpenes, particularly monoterpenes, have high cytotoxic potential shown in various model organisms, while some of them, such as β-caryophyllene, show anti-inflammatory, antioxidant, and cytoprotective effects [77]. Terpinolene, α-terpineol, humulene and linalool are considered among the most toxic terpenes [77]. The cytotoxic effect is primarily caused by plasma membrane disruption, lipid peroxidation, reactive oxygen species (ROS) production, mitochondrial transmembrane potential loss, and mitochondrial impairment [77].

Phenolic compounds like carvacrol, thymol, eugenol, and vanillin are widely used in food and other products and are generally considered safe. Significant biological properties, including antimicrobial, antioxidant, analgesic, anti-inflammatory, anti-mutagenic, or anti-carcinogenic activity, have been described for these substances. However, research has found that carvacrol, thymol, and eugenol can have toxic effects, including oxidative stress, mutagenicity, genotoxicity [78] and hepatic toxicity [79]. In vivo studies show adverse effects after acute and prolonged carvacrol and thymol exposure to mice, rats, and rabbits. Eugenol has caused pulmonary and renal damage to exposed frogs. In humans, these three compounds may cause skin reactions, inflammation, ulcer formation, and slow healing. Vanillin may reduce cell viability at high concentrations [78]. According to the authors, increased exposure to these substances warrants further safety review for both human and environmental health.

Terpenoids and ketones are associated with neurotoxicity and abortive properties; monoterpenoids are associated with renal toxicity; pinene derivatives, trans-anethol, sclareol or alpha-humulene having hormone-like structures raise the risk of endocrine disruption; furocoumarins have phototoxic properties [79].

It is worth examining some characteristics of individual EOs, focusing on those that have been most frequently evaluated in the preservation of cultural heritage. The primary references are the books by Holmes [2] and Tisserand & Young [4], with various other sources cited throughout the text.

Basil is a mild skin irritant.

Cinnamon bark oil is known for being highly irritating and sensitizing to the skin and mucous membranes due to its components cinnamaldehyde and eugenol.

Clove bud is a mild skin irritant. It contains 60–96% of phenols including methyl eugenol that, despite being moderately toxic, has been demonstrated to be genotoxic and carcinogenic according to The National Toxicology Program of the US Department of Health and Human Services. Since 2021, under the Regulation EC No 1272/2008, any product sold in the European Union that contains more than 0.01% methyl eugenol is required to carry a label indicating its presence.

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Oregano is highly dermocaustic (skin irritant). Much commercial oregano oil is extracted from *Thymus capitatus*.

Common thyme (*Thymus vulgaris* L.). Its key constituents include the phenols thymol and carvacrol, glycosides, flavonoids, p-cymene, borneol, linalool, alcohols, rosmarinic acid, saponins, tannins, and terpenoids [80]. Thyme ct. linalool, thyme ct. geraniol and thyme ct. thujanol are terpenol-dominant. They do not irritate the skin, making them more broadly applicable and versatile in practical use compared to other chemotypes. Terpenols, such as geraniol, linalool, and citronellol, are acyclic compounds, which contain an alcohol functional group (-OH) attached to a monoterpene structure. Geraniol, one of the most important molecules used in cosmetic industries for its antimicrobial activities, can induce allergic reactions such as irritant contact dermatitis (see review in [81]). Thyme ct. thymol is phenol-dominant and is highly irritant to the skin and mucosa. Thymol, a terpenoid phenol, is naturally found in the EO of thyme and various species of the genera *Origanum*, *Satureja*, and many others. It induced cytotoxicity and genotoxicity through oxidative damage in several types of target cells (see review in [76]. *Thymus capitatus* EO is particularly rich in carvacrol—typically ranging from 40–50% and occasionally reaching concentrations as high as 74%. It presents a significant risk of cumulative toxicity and is associated with moderate to severe skin irritation and sensitization [2]. Thyme EO has been reported to exhibit notable toxicity, with an $LD_{50}$ of 2.84 g/kg body weight in rats [80]. Although this value may appear high, under the European Union Classification Criteria for Acute Toxicity it corresponds to substances categorized as harmful rather than highly toxic.

Sage contains the toxic substance thujone [82].

The potential toxic effects of EOs and plant extracts on living organisms and ecosystems have been poorly investigated [83]. Recently, some studies have started to focus on non-target organisms, which include all living organisms other than those at which the treatment is directed. The active substances in plant EOs can be harmful to phytoplankton, zooplankton, algae, aquatic vertebrates, soil microorganisms, terrestrial vertebrates, and plants [3]. While EOs and plant extracts are generally considered eco-friendly and safer than synthetic alternatives, some of them have been found to be toxic to non-target organisms [3,74]. Currently, data on the effects of plant-based products is still limited, with most studies concentrating on aquatic systems and, in particular, on *Daphnia magna*. The potential impact on other aquatic organisms, as well as marine and terrestrial systems, remains largely unknown. Ferraz and coauthors [3] anticipate that with the tightening of international regulations, such as REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) in the European Union and the globally implemented GHS (Globally Harmonized System of Classification and Labelling of Chemicals) by the United Nations, there will be an increase in studies producing scientific data on the effects of EOs, hydrolates, and other plant extracts as new sources of bioactive compounds.

The study by Nematollahi and coauthors [84] emphasized the need to consider the potential impact on visitors of volatile organic compounds (VOCs) from EOs. It found that a variety of commercially available EOs with therapeutic claims—including tea tree, lavender, eucalyptus, geranium, peppermint, bergamot, orange, and blended oils—emit VOCs such as acetaldehyde, alpha-phellandrene, alpha-pinene, camphene, limonene, methanol, terpinolene, 3-carene, acetone, beta-phellandrene, ethanol, and gamma-terpinene, with many present in over 90% of the oils. The most common potentially hazardous VOCs included acetaldehyde, limonene, methanol, acetone, ethanol, 3-carene, and toluene. Each essential oil tested released at least nine potentially hazardous VOCs, yet less than 1% of all identified VOCs and hazardous VOCs were disclosed on product labels, safety data sheets, or websites.

## 6. Current Limitations and Future Prospects of Essential Oil Applications in Cultural Heritage Preservation

The use of EOs, plant extracts, and hydrolates in cultural heritage conservation is a fascinating yet highly debated area. Upon reviewing the literature, one cannot help but notice the enthusiasm

 doi:10.20944/preprints202510.0496.v1



with which many studies present the use of these substances. It is as if a long-sought panacea has finally been found for the biodeterioration of cultural heritage objects. Summarizing some of the most cited claims, these natural substances are portrayed as harmless to artifacts, environmentally sustainable, and safe for restorers. Such affirmations appear somewhat misplaced, given the highly contentious nature of the field. The complexity and diversity of factors involved make it exceedingly difficult to draw consistent or generalizable conclusions. Nonetheless, some critical concerns have emerged—including safety, environmental toxicity, and their potential to foster bio-resistance [33,85,86].

Essential oils are chemically complex mixtures, often containing dozens of constituents, and it remains challenging to determine which specific components exhibit antimicrobial activity, or whether such effects arise from synergistic interactions among them. The antimicrobial activity of EOs can differ significantly. The same EO has contrasting effects on microorganisms, as oregano EO on algal and cyanobacterial biofilms. This variability can be attributed to differences in chemical composition resulting from distinct extraction techniques, geographical locations, or the specific plant parts from which they originate. Many active components are volatile organic compounds with limited duration of action, complicating both their management and overall efficacy. This inconsistency raises questions about their reliability. Understanding both the production methods and chemical composition of EOs is an important aspect that is frequently overlooked or insufficiently detailed in some scientific studies. Additionally, research highlights that the antimicrobial activity of EOs is influenced by the particular microbial species being targeted. One example is the commercial product Biotersus, which demonstrated fluctuating levels of effectiveness. Another example is the failure of oregano and clove bud EOs at eliminating fungi of the genera *Cladosporium* and *Penicillium* [39].

A relevant point highlighted in the literature is the ability of microorganisms to recover. Three studies [28,34,39] reported an initial reduction in viable cells followed by the microorganisms' ability to repair and restore their structures. Such findings have implications for the required quantities of EOs, the frequency of applications, and the resistance of microorganisms to the damaging effects of these oils.

Some publications also suggest that these substances are environmentally friendly because they are biodegradable. While plant-derived compounds are generally considered readily biodegradable due to their volatility, there is still a notable lack of research investigating their persistence in the environment or their potential chronic toxicity to non-target organisms, as highlighted by Ferraz and coauthors [3]. As to environmental considerations, results from the application of the Life Cycle Assessment revealed that oregano EO posed a great impact on human health, ecosystem integrity, and resource depletion [58]. The study underscores that opting for natural substances does not necessarily mean they are more environmentally friendly than synthetic products.

Although some of these products show promise, the comparison of results is quite difficult because of the variability of EOs chemical composition, the variety of concentrations used and exposure times, the heterogeneity methods employed to evaluate their antimicrobial effectiveness and the infrequent application of statistical analysis to substantiate the findings [10,87]. Future research should adhere to standardized protocols to enable more comprehensive comparisons across studies and facilitate the ranking of plant-derived extracts based on their safety profiles. Additionally, the effects of these substances on cultural heritage materials warrant more thorough investigation, as highlighted in section 4, since certain EOs have been shown to modify the color, texture, or chemical composition of such materials.

Results described in section 5 demonstrate that further in vitro and in vivo research is necessary to thoroughly examine the safety of essential oils, especially regarding their impact on mammalian and other cell health, their interactions with particular biomolecules, and the underlying mechanisms by which they act [87]. The lack of regulation and standardization in the production and marketing of these products makes it difficult to determine the appropriate doses and concentrations for their safe use [83]. The way EOs are marketed is often unclear. They can be sold individually or as blends,

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and they may be categorized as medicines, food additives, dietary supplements, cosmetics, medical devices, or biocides. Their classification—and the associated safety considerations—raises important public health issues [82]. Currently, EOs are assigned a regulatory status according to their intended use, meaning their marketing category determines how they are distributed. As a result, they appear in a wide range of products, each offering different levels of safety and efficacy assurance, even though the underlying EO is identical. This situation creates a risk that EO-based products not classified as medicines may be sold without adequate guidance or warnings [82]. Evaluation of the toxicity of EOs is currently providing new knowledge of their toxic action. The whole aspect of safety is now being rigorously reviewed, and new European regulations will likely impede the sale and usage of many essential oils [88].

Embedding EOs in various carrier materials represents an innovative and advanced approach to improve their stability and efficacy. Their practical use is often limited by their high volatility, hydrophobicity, and susceptibility to degradation from light, heat, and oxygen exposure [46]. These factors make EOs vulnerable to breakdown, resulting in diminished biological activity [89]. However, many authors indicate that incorporating them into different carrier materials allows even low amounts to be efficiently antimicrobial. Despite these approaches being developed, other challenges remain. Their poor water solubility and strong, persistent odor can pose additional inconveniences for restorers. The duration and costs associated with restoration treatments using EOs may also limit their widespread adoption.

In summary, the application of essential oils in cultural heritage conservation is still in an early stage, facing many difficulties. More systematic long-term follow-up of treatment procedures and consideration of regrowth rates after interventions are essential. Continued research, technological development, and systematic evaluation are required to ensure that these natural products can be used safely and reliably in the protection and restoration of cultural heritage.

Finally, while treatments for biodeterioration are part of standard restoration practices, challenges remain in developing and establishing effective techniques. I think it can be opportune to start a discussion on a topic suggested by Berti and coauthors [58], that is the consideration of a non-treatment option.

## References


1. Tavares, C.S.; Gameiro, J.A.; Roseiro, L.B.; Figueiredo A.C. Hydrolates: a review on their volatiles composition, biological properties and potential uses. *Phytochem Rev* **2022**, 21, 1661–1737.
2. Holmes, P. Aromatica. A clinical guide to essential oil therapeutics Volume 1. Applications and profiles Volume 2. Singing Dragon, 2019.
3. Ferraz, C.A.; Pastorinho, M.R.; Palmeira-de-Oliveira, A.; Sousa, A.C,A. Ecotoxicity of plant extracts and essential oils: A review. *Environ Pollut* **2022**, 292, 118319.
4. Tisserand, R.; Young, R. *Essential Oil Safety: A Guide for Health Care Professionals*. Elsevier Health Sciences, 2013.
5. Stupar, M.; Grbić, M.L.; Džamić, A.; Unković, N.; Ristić, M.; Jelikić, A.; Vukojević, J. Antifungal activity of selected essential oils and biocide benzalkonium chloride against the fungi isolated from cultural heritage objects. *S Afr J Bot* **2014**, 93, 118-124.
6. Matusiak, K.; Machnowski, W.; Wrzosek, H.; Polak, J.; Rajkowska, K.; Smigielski, K.; Kunicka-Styczynska, A.; Gutarowska, B. Application of *Cinnamomum zeylanicum* essential oil in vapour phase for heritage textiles disinfection. *Int Biodeterior Biodegrad* **2018**, 131, 88–96.
7. Geweely, N.S.; Afifi, H.A.; Ibrahim, D.M.; Soliman, M.M. (2019) Efficacy of Essential Oils on Fungi Isolated from Archaeological Objects in Saqqara Excavation, Egypt, *Geomicrobiol J* **2019**, 36(2), 148-168.
8. Antonelli, F.; Bartolini, M.; Plissonnier, M.L.; Esposito, A.; Galotta, G.; Ricci, S.; Davidde Petriaggi, B.; Pedone, C.; Di Giovanni, A.; Piazza, S.; Guerrieri, F.; Romagnoli, M. Essential oils as alternative biocides for the preservation of waterlogged archaeological wood. *Microorganisms* **2020**, 8, 2015.

 doi:10.20944/preprints202510.0496.v1




9. Geweely, N.S.; Afifi, H.A.; Ibrahim, D.M.; & Soliman, M.M. Inhibitory effect of essential oils on growth and physiological activity of deteriorated fungal species isolated from three archeological objects, Saqqara excavation, Egypt. *Geomicrobiol J* **2020**, 37(6), 520-533.
10. Albasil, M.D.; Abeer EIHagrassy, G.M.; Reyad, A.M. Evaluating the antimicrobial activity of essential oils in the conservation of mural paintings. *Conserv Sci Cult Herit* **2021**, 21, 125–148.
11. Argyri, A.A.; Doulgeraki, A.I.; Varla, E.G.; Bikouli, V.C.; Natskoulis, P.I.; Haroutounian, S.A.; Moulas, G.A.; Tassou, C.C.; Chorianopoulos, N.G. Evaluation of plant origin essential oils as herbal biocides for the protection of caves belonging to natural and cultural heritage sites. *Microorganisms* **2021**, 9, 1836.
12. D'Agostino, G.; Giambra, B.; Palla, F.; Bruno, M.; Badalamenti, N. The application of the essential oils of *Thymus vulgaris* L. and *Crithmum maritimum* L. as biocidal on two Tholu Bommalu Indian leather puppets. *Plants* **2021**, 10, 1508.
13. Gatti, L.; Troiano, F.; Vacchini, V.; Cappitelli, F.; Balloi, A. An in vitro evaluation of the biocidal effect of oregano and cloves' volatile compounds against microorganisms colonizing an oil painting—A pioneer study. *Appl Sci* **2021**, 11, 78.
14. Sparacello, S.; Gallo, G.; Faddetta, T.; Megna, B.; Nicotra, G.; Bruno, B.; Giambra, B.; Palla, F. *Thymus vulgaris* essential oil and hydro-alcoholic solutions to counteract wooden artwork microbial colonization. *Appl Sci* **2021**, 11, 8704.
15. Bosco, F.; Mollea, C.; Demichela, M.; Fissore, D. Application of essential oils to control the biodeteriogenic microorganisms in archives and libraries. Heritage **2022**, 5, 2181–2195.
16. Geweely, N.S.; Afifi, H.A.; AbdelRahim, S.A.; Kamh, G.M.I.; Soliman, M.M.; AbdelSattar, M.; Ali, H.M.; Akrami, M.; Salem, M.Z.M. Bioactivities of six plant essential oils against some isolated microbes from an archaeological limestone statue at the Saqqara excavation. *BioResources* **2022**, 17(1), 543-573.
17. Minotti, D.; Vergari, L.; Proto, M.R.; Barbanti, L.; Garzoli, S.; Bugli, F.; Sanguinetti, M.; Sabatini, L.; Peduzzi, A.; Rosato, R.; et al. Il Silenzio: The first Renaissance oil painting on canvas from the Uffizi Museum restored with a safe, green antimicrobial emulsion based on *Citrus aurantium* var. *amara* hydrolate and *Cinnamomum zeylanicum* essential oil. *J Fungi* **2022**, 8, 140.
18. Lee, H.-J.; Chung, Y.-J. Antifungal, antibacterial, and interference effects of plant-extracted essential oils used for mural conservation at Buyeo Royal Tomb No. 1. *Appl Sci* **2023**, 13, 3645.
19. Palla, F.; Bucchini, A.E.A.; Giamperi, L.; Marino, P.; Raimondo, F.M. Plant extracts as antimicrobial agents in sustainable conservation of *Erythrina caffra* (Fabaceae) Historical Trees. *Antibiotics* **2023**, 12, 1098.
20. Santo, A.P.; Agostini, B.; Cuzman, O.A.; Michelozzi, M.; Salvatici, T.; Perito, B. Essential oils to contrast biodeterioration of the external marble of Florence Cathedral. *Sci Total Environ* **2023**, 877, 162913.
21. Geweely, N.S.; Abu Taleb, A.M.; Grenni, P.; Caneva, G.; Atwa, D.M.; Plaisier, J.R.; Ibrahim, S. Eco-friendly preservation of Pharaonic wooden artifacts using natural green products. *Appl Sci* **2024**, 14, 5023.
22. Mohamed, A.A.; Ahmed, M.A.; Korayem, A.S.; Abu-Hussien, S.H.; Bakry Rashidy, W. Antifungal, toxicological, and colorimetric properties of *Origanum vulgare*, *Moringa oleifera*, and *Cinnamomum verum* essential oils mixture against Egyptian Prince Yusuf Palace deteriorative fungi. *BMC Biotechnology* **2025**, 25:4.
23. Ranaldi, R.; Rugnini, L.; Migliore, G.; Tasso, F.; Gabriele, F.; Spreti, N.; Scuderi, F.; Braglia, R.; Di Martino, P.; Pujia, A.; Canin, A. The role of essential oils as eco-friendly strategy to control biofilm collected in the Colosseum (Rome, Italy). *Appl Microbiol Biotechnol* **2025**, 109:48.
24. Mateus, D.; Costa, F.; de Jesus, V.; Malaquias, L. Biocides based on essential oils for sustainable conservation and restoration of mural paintings in built cultural heritage. *Sustainability* **2024**, 16, 11223.
25. Macchia, A.; Aureli, H.; Prestileo, F.; Ortenzi, F.; Sellathurai, S.; Docci, A.; Cerafogli, E.; Colasanti, I.A.; Ricca, M.; La Russa, M.F. In-situ comparative study of eucalyptus, basil, cloves, thyme, pine tree, and tea tree essential oil biocide efficacy. *Methods Protoc* **2022**, 5, 37.
26. Cennamo, P.; Scielzo, R.; Rippa, M.; Trojsi, G.; Carfagna, S.; Chianese, E. UV-C irradiation and essential-oils-based product as tools to reduce biodeteriorates on the wall paints of the archeological site of Baia (Italy). *Coatings* **2023**, 13, 1034.

27. Antonelli, A.; Iovine, S.; Sacco Perasso, C.; Macro, N.; Gioventù, E.; Capasso, F.E.; Bartolini, M. Essential oils and essential oil-based products compared to chemical biocides against microbial patinas on stone cultural heritage. *Coatings* **2024**, 14, 1546.
28. Pop, D.M.; Timar, M.C.; Beldean, E.C.; Varodi, A.M. (2020). Antifungal clove essential oil. *BioResources* **2020**, 15(4), 9474-9489.
29. Fierascu, I.; Ion, R.M.; Radu, M.; Dima, S.O.; Bunghez, I.R.; Avramescu, S.M.; Fierascu, R.C. Comparative study of antifungal effect of natural extracts and essential oils of *Ocimum basilicum* on selected artefacts. *Rev Roum Chim* **2014**, 59(3-4), 207-211.
30. Tomić, A.; Šovljanski, O.; Nikolić, V.; Pezo, L.; Aćimović, M.; Cvetković, M.; Stanojev, J.; Kuzmanović, N.; Markov, S. Screening of antifungal activity of essential oils in controlling biocontamination of historical papers in archives. *Antibiotics* **2023**, 12, 103.
31. Arantes, S.M.; Piçarra, A.; Guerreiro, M.; Salvador, C.; Candeias, F.; Caldeira, A.T.; Martins, M.R. Toxicological and pharmacological properties of essential oils of *Calamintha nepeta*, *Origanum virens* and *Thymus mastichina* of Alentejo (Portugal). *Food Chem Toxicol* **2019**, 133, 110747.
32. Genova, C.; Fuentes, E.; Sanmartín, P.; Favero, G.; Prieto, B. Phytochemical compounds as cleaning agents on granite colonized by phototrophic subaerial biofilms. *Coatings* **2020**, 10, 295.
33. Menicucci, F.; Palagano, E.; Michelozzi, M.; Ienco, A. Essential oils for the conservation of paper items. *Molecules* **2023**, 28, 5003.
34. Rugnini, L.; Migliore, G.; Tasso, F.; Ellwood, N.T.W.; Sprocati, A.R.; Bruno, L. Biocidal activity of phyto-derivative products used on phototrophic biofilms growing on stone surfaces of the Domus Aurea in Rome (Italy). *Appl Sci* **2020**, 10, 6584.
35. Mateus, D.M.R.; Ferraz, E.; Perna, V.; Sales, P.; Hipolito-Correia, V. Essential oils and extracts of plants as biocides against microorganisms isolated from the ruins of the Roman city of Conímbriga (Portugal). *Environ Sci Pollut Res Int* **2023**, 31(28), 40669-40677.
36. Marco, A.; Santos, S.; Caetano, J.; Pintado, M.; Vieira, E.; Moreira, P.R. Basil essential oil as an alternative to commercial biocides against fungi associated with black stains in mural painting. *Build Environ* **2020**,167, 106459.
37. Corbu, V.M.; Gheorghe-Barbu, I.; Marinas, I.C.; Avramescu, S.M.; Pecete, I.; Geană, E.I.; Chifiriuc, M.C. Eco-friendly solution based on *Rosmarinus officinalis* hydro-alcoholic extract to prevent biodeterioration of cultural heritage objects and buildings. *Int J Mol Sci* **2022**, 23, 11463.
38. Rotolo, V.; Barresi, G.; Di Carlo, E.; Giordano, A.; Lombardo, G.; Crimi, E.; Costa, E.; Bruno, M.; Palla, F. Plant extracts as green potential strategies to control the biodeterioration of cultural heritage. *Int J Conserv Sci* **2016**, 7, Special Issue 2, 839-846.
39. Isola, D.; Bartoli, F.; Casanova Municchia, Lee, H.J.; Jeong, S.H.; Chung, Y.J.; Giulia Caneva. Green biocides for the conservation of hypogeal mural paintings raised from Western and Eastern traditions: Evaluation of interference on pigments and substrata and multifactor parameters affecting their activity. *J Cult Herit* **2023**, 61, 116–126.
40. Di Vito, M.; Vergari, L.; Mariotti, M.; Proto, M.R.; Barbanti, L.; Garzoli, S.; Sanguinetti, M.; Sabatini, L.; Peduzzi, A.; Bellardi, M.G.; Mattarelli, P.; Bugli, F.; De Luca, D. Anti-mold effectiveness of a green emulsion based on *Citrus aurantium* hydrolate and *Cinnamomum zeylanicum* essential oil for the modern paintings restoration. *Microorganisms* **2022**, 10, 205.
41. Goni, P.; López, P.; Sánchez, C.; Gómez-Lus, R.; Becerril, R.; Nerín, C. Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. *Food Chem* **2009**, 116, 982–989.
42. Genova, C.; Grottolic, A.; Zoppis, E.; Cencetti, C.; Matricardi, P.; Favero, G. An integrated approach to the recovery of travertine biodegradation by combining phyto-cleaning with genomic characterization. *Microchem J* **2020**, 156, 104918.
43. Ranaldi, R.; Rugnini, L.; Gabriele, F.; Spreti, N.; Casieri, C.; Di Marco, G.; Gismondi, A.; Bruno, L. Plant essential oils suspended into hydrogel: Development of an easy-to-use protocol for the restoration of stone cultural heritage. *Int Biodeterior Biodegrad* **2022**, 172, 105436.
44. Macedo-Arantes, S.; Piçarra, A.; Caldeira, A.T.; Candeias, A.E.; Martins, M.R. Essential oils of Portuguese flavouring plants: potential as green biocides in cultural heritage. *Eur Phys J Plus* **2021**, 136,1106.

45. Gabriele, F.; Ranaldi, R.; Bruno, L.; Casieri, C.; Rugnini, L.; Spreti, N. Biodeterioration of stone monuments: Studies on the influence of bioreceptivity on cyanobacterial biofilm growth and on the biocidal efficacy of essential oils in natural hydrogel. *Sci Total Environ* **2023**, 870, 161901.
46. Wang, X.; Hu, Y.; Zhang, Z.; Zhang, B. The application of thymol-loaded chitosan nanoparticles to control the biodeterioration of cultural heritage sites. *J Cult Herit* **2022**, 53, 206–211.
47. Menicucci, F.; Palagano, E.; Michelozzi, M.; Cencetti, G.; Raio, A.; Bacchi, A.; Mazzeo, P.P.; Cuzman, O.A.; Sidoti, A.; Guarino, S.; Basilei, S.; Riccobono, O.; Peri, E.; Vizza, F.; Ienco, A. Effects of trapped-into-solids volatile organic compounds on paper biodeteriogens. *Int Biodeterior Biodegrad* **2022**, 174, 105469.
48. Di Vito, M.; Bellardi, M.G.; Colaizzi, P.; Ruggiero, D.; Mazzuca, C.; Micheli, L.; Sotgiu, S.; Iannuccelli, S.; Michelozzi, M.; Mondello, F.; Mattarelli, P.; Sclocchi, M.C. Hydrolates and gellan: An eco-innovative synergy for safe cleaning of paper artworks. *Stud Conserv* **2018**, 63, 13-23.
49. Campanella, L.; Angeloni, R.; Cibin, F.; Dell'Aglio, E.; Grimaldi, F.; Reale, R.; Vitali, M. Capsulated essential oil in gel spheres for the protection of cellulosic cultural heritage. *Nat Prod Res* **2021**, 35(1),116-123.
50. Bartolini, M.; Pietrini, A.M. La disinfezione delle patine biologiche sui manufatti lapidei: biocidi chimici e naturali a confronto. *Bollettino ICR* **2016**, 33, 39-48.
51. Spada, M.; Cuzman, O.A.; Tosini, I.; Galeotti, M.; Sorella, F. Essential oils mixtures as an eco-friendly biocidal solution for a marble statue restoration. *Int Biodeterior Biodegrad* **2021**,163, 105280.
52. Genova, C.; Fuentes, E.; Favero, G.; Prieto, B. Evaluation of the cleaning effect of natural-based biocides: Application on different phototropic biofilms colonizing the same granite wall. *Coatings* **2023**, 13, 520.
53. Boccalon, E.; Nocchetti, M.; Pica, M.; Romani, A.; Sterflinger, K. Hydrogels: A 'stepping stone' towards new cleaning strategies for biodeteriorated surfaces. *J Cult Herit* **2021**, 47, 1–11.
54. Gagliano Candela, R.; Maggi, F.; Lazzara, G.; Rosselli, S.; Bruno, M. The Essential oil of *Thymbra capitata* and its application as a biocide on stone and derived surfaces. *Plants* **2019**, 8, 300.
55. Wang, X.; Zhu, C.; Hu, Y.; Zhang, Z.; Zhang, B. Development and application of cinnamaldehyde-loaded halloysite nanotubes for the conservation of stone cultural heritage. *Appl Clay Sci* **2023**, 236, 106886.
56. Favero-Longo, S.E.; Laurenzi Tabasso, M.; Brigadeci, F.; Capua, M.C.; Morelli, A.; Pastorello, P.; Sohrabi, M.; Chaverdi, A.A.; Callieri, P. A first assessment of the biocidal efficacy of plant essential oils against lichens on stone cultural heritage, and the importance of evaluating suitable application protocols. *J Cult Herit* **2022**, 55, 68–77.
57. Isola, D.; Capobianco, G.; Tovazzi, V.; Pelosi, C.; Trotta, O.; Serranti, S.; Lanteri, L.; Zucconi, L.; Spizzichino, V. Biopatinas on peperino stone: Three eco-friendly methods for their control and multi-technique approach to evaluate their efficacy. *Microorganisms* **2025**, 13, 375.
58. Berti, L.; Arfelli, F.; Villa, F.; Cappitelli, F.; Gulotta, D.; Ciacci, L.; Bernardi, E.; Vassura, I.; Passarini, F.; Napoli, S.; Salvatore Napoli, Sara Goidanich. LCA as a complementary tool for the evaluation of biocolonization management: The case of Palazzo Rocca Costaguta. *Heritage* **2024**, 7, 6871–6890.
59. Díaz-Alonso, J.; Bernardos, A.; Regidor-Ros, J.L.; Martínez-Máñez, R.; Bosch-Roig, P. Innovative use of essential oil cold diffusion system for improving air quality on indoor cultural heritage spaces. *Int Biodeterior Biodegrad* **2021**, 162, 105251.
60. Palla, F.; Bruno, M.; Mercurio, F.; Tantillo, A.; Rotolo, V. Essential oils as natural biocides in conservation of cultural heritage. *Molecules* **2020**, 25, 730.
61. Pietrzak, K.; Otlewska, A.; Danielewicz, D.; Dybka, K.; Pangallo, D.; Kraková, L.; Půskárová, A.; Bučková, M.; Scholtz, V.; Ďurovič, M.; Surma-Ślusarska, B.; Demnerová, K.; Gutarowska, B. Disinfection of archival documents using thyme essential oil, silver nanoparticles misting and low temperature plasma. *J Cult Herit* **2017**, 24, 69-77.
62. Pinna, D. Can we do without biocides to cope with biofilms and lichens on stone heritage? *Int Biodeterior Biodegrad* **2022**, 172, 105437.
63. Mokrzycki, W.S.; Tatol, M. Color difference Delta E - a survey. *Mach Graph Vis* **2011**, 20 (4), 383–411.
64. Sasse, H.S., Snethlage, R., 1997. Methods for the evaluation of stone conservation treatments. In *Dahlem Workshop on Saving Our Architectural Heritage*; Baer, N.S., Snethlage, R., Eds.; John Wiley & Sons Ltd., 1997; pp. 223–243.

65. Prieto, B.; Sanmartín, P.; Cancelo-González, J.; Torres, L.; Silva, B. Impact of herbicide treatments on the construction materials in the Roman wall of Lugo, Spain (UNESCO World Heritage Site). *Appl Sci* **2021**, 11, 5276.
66. Privitera, A.; Tuti, S.; Laverdura, U.P.; Duranti, L.; Di Bartolomeo, E.; Taddei, A.R.; Sodo, A. One-step nanoencapsulation of essential oils and their application in hybrid coatings: A sustainable long-lasting treatment of stone materials against biodeterioration. *Prog Org Coat* **2024**, 196, 108759.
67. Rakotonirainy, M.S.; Juchauld, F.; Gillet, M.; Othman-Choulak, M.; Lavedrine, B. The effect of linalool vapour on silver-gelatine photographs and bookbinding leathers. *Restaurator* **2007**, 28, 95–111.
68. Ali, M.; Fawzy, M. The effect of the vapors of clove and lavender oils on the chemical and optical properties of silver gelatin prints in wood frames. *Int J Conserv Sci* **2021**, 12(3), 961-976.
69. Paolino, B.; Sorrentino, M.C.; Pacifico, S.; Garrigos, M.C.; Riccardi, M.G.; Paradiso, R.; Lahoz, E.; Borriello, G. A preliminary study on the efficacy of essential oils against *Trichoderma longibrachiatum* Isolated from an archival document in Italy. *Heritage* **2025**, 8, 187.
70. Indrie, L.; Affandi, N.D.N.; Díaz-García, P.; Haji, A.; Ilies, D.C.; Zlatev, Z.; Taghiyari, H.R.; Grama, V.; Farima, D. Mechanical and morphological properties of cellulosic fabrics treated with microencapsulated essential oils. *Coatings* **2022**, 12, 1958.
71. Romano, I.; Granata, G.; Poli, A.; Finore, I.; Napoli, E.; Geraci, C. Inhibition of bacterial growth on marble stone of 18th century by treatment of nanoencapsulated essential oils. *Int Biodeterior Biodegrad* **2020**, 148, 104909.
72. Narciso, L.; Raggi, C. Oli essenziali e sicurezza: studi di tossicità nei modelli animali. *NATURAL 1* **2023**, 32-38.
73. Lanzerstorfer, P.; Sandner, G.; Pitsch, J.; Mascher, B.; Aumiller, T.; Weghuber, J. Acute, reproductive, and developmental toxicity of essential oils assessed with alternative in vitro and in vivo systems. *Arch Toxicol* **2021**, 95, 673–691.
74. Espadero, M.; Gavilanes, S.; Mosquera, T.; Alvarez, G. Toxicological evaluation of essential oil and ethanolic extracts of *Ruta graveolens* in *Artemia salina*. *Smart Innov Syst Technol* **2022**, 252, 165-174.
75. Sartori Tamburlin, I.; Roux, E.; Feuillée, M.; Labbé, J.; Aussaguès, Y.; El Fadle, F.E.; Fraboul, F.; Bouvier, G. Toxicological safety assessment of essential oils used as food supplements to establish safe oral recommended doses. *FCT* **2021**, 157, 112603.
76. Contini, A.; Di Bello, D.; Azzara, A.; Giovanelli, S.; D'Urso, G.; Piaggi, S.; Pinto, B.; Pistelli, L.; Scarpato, R.; Testi, S. Assessing the cytotoxic/genotoxic activity and estrogenic/antiestrogenic potential of essential oils from seven aromatic plants. *Food Chem Toxicol* **2020**, 138, 111205.
77. Agus, H.H., 2021. Terpene toxicity and oxidative stress. In *Toxicology*; Patel, V.B., Preedy, V.R., Eds., Academic Press, **2021,** 33–42.
78. Fuentes, C.; Fuentes, A.; Barat, J.M.; Ruiz, M.J. Relevant essential oil components: a minireview on increasing applications and potential toxicity. *Toxicol Mech Methods* **2021**, 31(8):559-565.
79. European Food Safety Authority EFSA. Compendium of botanicals reported to contain naturally occurring substances of possible concern for human health when used in food and food supplements. *EFSA J* **2012a**, 10 (5), 2663. Available at: https://efsa.onlinelibrary.wiley. com/doi/epdf/10.2903/j.efsa.2012.2663.
80. Basch, E.; Ulbricht, C.; Hammerness, P.; Bevins, A.; Sollars, D. Thyme (*Thymus vulgaris* L.), Thymol. *J Herb Pharmacother* **2004**, 4(1), 49-67.
81. Romani, M.; Warscheid, T.; Nicole, L.; Marcon, L.; Di Martino, P.; Suzuki, M.T.; Lebaron, P.; Lami, R. Current and future chemical treatments to fight biodeterioration of outdoor building materials and associated biofilms: moving away from ecotoxic and towards efficient, sustainable solutions. *Sci Total Environ* **2022**, 802, 149846.
82. Guerriaud, M. Réglementation des huiles essentielles, un besoin de sécurité. *Actual Pharm* **2018**, 580, 21-25.
83. da Silva, I.I., Jr.; da Silva, N.P.C.; Marrs, J.A.; Cadena, P.G. Essential oils produce developmental toxicity in Zebrafish embryos and cause behavior changes in Zebrafish larvae. *Biomedicines* **2023**, 11, 2821.
84. Nematollahi, N.; Weinberg, J.L.; Flattery, J.; Goodman, N.; Kolev, S.D.; Steinemann, A. Volatile chemical emissions from essential oils with therapeutic claims. *Air Qual Atmos Health* **2020**, 14, 365–369.

85. Paolino, B.; Sorrentino, M.C.; Pacifico, S. Greener solutions for biodeterioration of organic-media cultural heritage: where are we? *Heritage Science* **2024**, 12:334.
86. Celi, D.; Marvasi, M.; Perito, B. Conventional and innovative approaches to black fungi control for stone heritage preservation. *IUBMB Life* **2025**, 77(3), e70010.
87. Radulović, N.; Gencic, M.; Stojanovic, N.; Randjelovic, P.; Stojanović-Radić, Z.; Stojiljkovic, N. Toxic essential oils. Part V: behaviour modulating and toxic properties of thujones and thujone-containing essential oils of *Salvia officinalis* L., *Artemisia absinthium* L., *Thuja occidentalis* L. and *Tanacetum vulgare* L. *Food Chem Toxicol* **2017**, 105, 355–369.
88. Radulović, N. (Ed). Toxic effects of essential oils and their constituents. Special Issue of *Food Chem Toxicol* **2020**.
89. Tanasă, F.; Nechifor, M.; Teacă, C.-A. Essential oils as alternative green broad-spectrum biocides. *Plants* **2024**, 13, 3442.