

## Research article

# Chemical Characterization of Essential Oils With a Biocide Base for Conservation and Restoration

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Essential oils (EOs) are acclaimed for their antimicrobial properties, leading to their multiple applications in various fields. In this work, four aromatic plants were used, namely thyme (*Thymus mastichina* L.), everlasting (*Helychrysum stoechas* Moench), European pennyroyal (*Mentha pulegium* L.) and fennel (*Foeniculum vulgare* Mill.). Hydrodistillation was the extraction method used, and the obtained extracts were composed of a variety of volatile molecules, mainly terpenoids and phenylpropanoids. The EO yields were determined, and the chemical composition of these natural products was obtained by gas chromatography-mass spectrometry (GC-MS) analysis. The yields varied greatly in the range of 0.99 to 4.27% (v/w). The two major chemical constituents of the EOs analysed by GC-MS were as follows: thyme – 1,8-cineole and champhor; everlasting –  $\alpha$ -pinene and limonene; European pennyroyal – pulegone and isomenthone; fennel – trans-anethole and limonene. This preliminary study is an important contribution to the understanding of EO bioactive compounds that are under investigation to establish their ability to control the biocolonization of cultural heritage.

**Keywords:** aromatic plants, chemical characterization, essential oils, green biocides

## 1. Introduction

Humans since prehistorical times left us a cultural heritage as a legacy, which due, mostly to biodeterioration, requires conservation and, in many cases, restoration. The 21<sup>st</sup> Century enlightened politicians and scholars alike onto the importance of preserving such a patrimony, resulting on the investment in research and education from which most chemical and mechanical biodeterioration solutions we use nowadays arose. On the other hand, the 21<sup>st</sup> Century brought, along with a tourism boom, environmental concerns which lead to many studies on the safety and sustainability of chemicals used in

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Conservation and Restoration of cultural heritage elements [1]. Cultural asset safeguard combined with the purpose of cultural heritage use and enhancement are mechanisms to value the large Portuguese historical heritage and to promote the Tourism Industry. Following this protective action, various conservation and restoration interventions aim to minimize the degradation of different materials where the artistic expressions are externalised. Biodeterioration, as the unwanted alterations of various materials, mainly caused by microorganisms, can be observed in art and architectural works holding patrimonial value. This phenomenon is primarily attributed to the proliferation of various microorganisms (bacteria, fungi, algae, lichens) that, alone or in association, through their energy-producing metabolisms, lead to the formation of acidic substances that can attack the physicochemical structures of support [2-4]. This structural degradation encompasses mechanical damages through substrate breakage or cohesion decrease, chemical alterations due to metabolites chemical action and aesthetic damages by the breeding of patinas or crusts. These alterations are directly linked to the climatic and environmental conditions to which the artwork is exposed [5].

In conservation and restoration, different compounds of synthetic origin are traditionally used, however they can exhibit human and environmental toxicity, and cause adverse effects on treated materials. The traditional biocides can be divided into four different groups: pesticides, disinfectants, preservatives, and other biocidal products [6]. For instance, quaternary ammonium compounds, phenols, aldehydes, and alcohols are a clear example of disinfectants, with temporary results and long-term damage to the preserved artifacts [2,7,8]. Environmental awareness, side effects of disinfectants and inhibitors led to the need for new materials with biocidal effects. Biocides with a wide spectrum of activity and low cost, favouring natural materials derived from plants, such as essential oils (EOs) which are generally attributed to easy handling environmental stability and lower toxicities are currently preferred [9-11]. Several plants were already studied with this purpose in different geographies, namely *Origanum vulgare* L., *Thymus vulgaris* L., *Thymus capitatus* L., *Cymbopogon khasans*, *Pimpinella anisum* L., *Syzygium aromaticum* L., *Cuminum cyminum* L., *Allium sativum* L., *Laurus nobilis* L., and *Citrus sinensis* (L.) Osbeck, [12-17]. The EOs contain a wide variety of secondary metabolites able to act against several biological systems. The complex mixture that constitutes essential oils can comprise dozens of compounds, belonging to different families of lipids, including hydrocarbons, alcohols, esters, aldehydes and ketones, phenols, phenolic ethers, and terpenes. The analysis of essential oils shows that terpenes exhibit the highest relative abundance and subgroups such as monoterpenes, sesquiterpenes and diterpenes, among others, can be distinguished. In general, the most abundant

constituent confers the biological activity of the essential oil, although this activity often results from a synergistic action between several constituents [18]. These compounds may be presented as environmentally acceptable pesticides, acting on the microbial cell by different mechanisms such as growth inhibition, cytoplasmic membrane deterioration, metabolism regulation throughout disturbance of enzymatic reactions, affecting the enzyme's synthesis or behaviour. Some isolated chemical components of essential oils have also been studied to prevent biocolonization and improve heritage conservation, such as Thymol, Menthol, Linalool, Eucalyptol, Cinnamaldehyde, Eugenol, Cuminaldehyde, Limonene, Citral, Citronellol, among many others [10, 19, 20].

Environmental concerns along with the need to replace synthetic biocides in Conservation, originated the core idea for a global project (NatBio Project – Natural Biocides for Sustainable Heritage Conservation) which primary goal is the development of new natural materials extracted from endemic or naturalized plants that can be used as biocides in the preservation of cultural and artistic heritage. Altogether the project encompasses several phases and diverse activities starting with the collection of plants, extraction, purification, and characterization of extracts (including EOs). Tests to evaluate biocide potential - *in vitro* laboratory tests and *in situ* tests on monuments are foreseen. Ecotoxicological tests will be carried out aiming the determination of extract solutions' toxicity. Two Portuguese iconic heritage sites will also be intervened during this Project. As main results it is intended to study a group of aromatic plants, to select those whose extract will exhibit higher biocide potential, from which it is possible to formulate an ecological biocide with evident results in the preservation of stone and ceramic materials. The Project combines a team of 14 transdisciplinary researchers from multiple Institutions.

The current paper is part of the early stages of NatBio Project, involving the need to study and analyse the biocide potential of several plants. The focus relies on essential oils obtained by hydrodistillation in a Clevenger type-apparatus from four endemic aromatic plants. The yields in EOs extractions were ascertained and the chemical composition of these natural products was established by GC-MS analysis. Subsequent tests on organic solvent extracts and ulterior assessment of biocide potential of all the extracts are being carried out.

## 2. Material and methods

Raw materials – Four aromatic plants, belonging to different genera, holding different major compounds, and recognised by their antiseptic properties were selected for this

study. From Asteraceae family the *Helichrysum stoechas* (L.) Moench (common name Everlasting; portuguese name Perpétuas-das-areias), from Lamiaceae family the *Thymus mastichina* L. (common name Thyme; portuguese name Bela-luz or Amor-de-Deus) and the *Mentha pulegium* L. (common name European pennyroyal; portuguese name Poejo), and finally from Apiaceae family the *Foeniculum vulgare* Mill. (common name Fennel; portuguese name Funcho-amargo). These plants are autochthonous in the national territory, and their essential oils (EOs) are already industrially produced for the market with other purposes. These products can be applied in pharmaceutical, cosmetic and food industries, besides the usage in wellness aromatherapy or agricultural disinfestation.

The plants were provided by D'Alenguadiana Company located in Cortes-Sines, Mértola, Alentejo, harvested in Canais do Guadiana. Collecting was performed in their respective flowering seasons, namely: everlasting and thyme – June; European pennyroyal – July; fennel – August. For the essential oil extraction tests, the entire aerial part of the plants was used, cut at about 15 cm from the ground to allow its renewal in the following year.

Extraction method – Aerial parts of the plants were manually cut into small pieces of 2 cm long and 100 g of each sample were extracted with 2 L of distilled water in a Clevenger type-apparatus, during 2 h. After the extraction, the essential oils' volumes were measured for yield determination.

Chemical characterization – A sample of each EO was prepared to gas chromatographic-mass spectral (GC-MS) analysis, dissolving 50  $\mu\text{L}$  of the EO in 2 mL of Dichloromethane. The EOs analyses [21] were performed using an Agilent 7890 CG coupled with an Agilent 5975 C inert XL mass selective detector (MSD) and an Agilent ChemStation data system. The MSD was operated under the following conditions: electron impact EI mode (with an electron energy of 70 eV, scan range of 50-500 amu and a scan rate of 3.99 scans/sec. The separation conditions included a DB-5MS fused silica capillary GC column, constituted by a (5% phenyl)-polymethylsiloxane stationary phase, film thickness of 0.25  $\mu\text{m}$  and an internal diameter of 0.25 mm. The carrier gas was helium, and the column head pressure was 48.7 kPa and a flow rate of 1.0 mL/min. Temperature profile: injector temperature – 250°C, oven initial temperature – 60°C for 5 minutes; temperature rise – 10°C/min until 250°C. The GC was operated with an injection volume of 1  $\mu\text{L}$  and a split ratio of 1:50. The identification of components was ascertained by comparison of the retention times and mass spectra with those of the pure standard compounds. All mass spectra were also compared with those of the data system library NIST and Wiley.

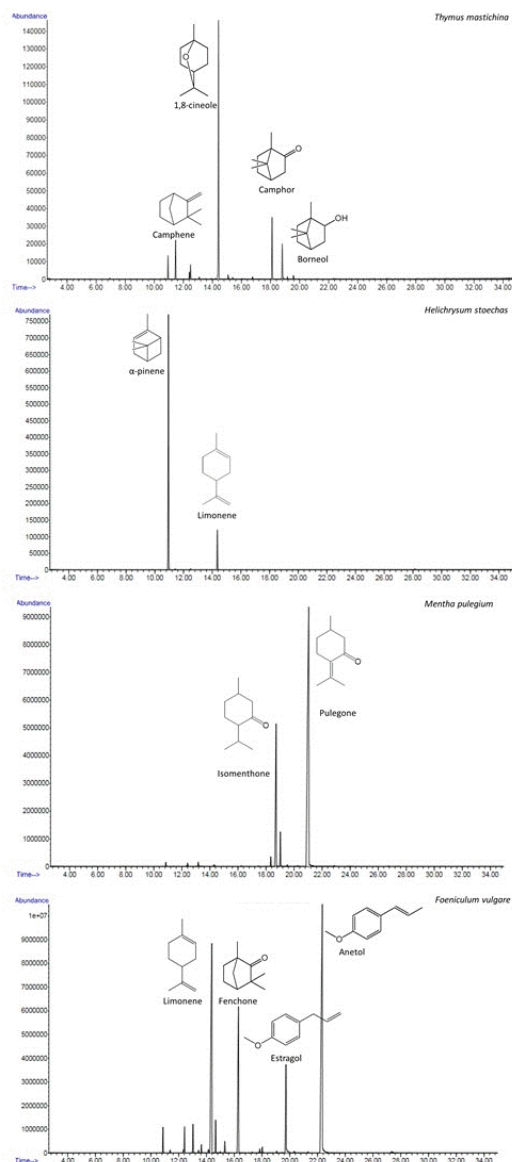
### 3. Results and discussion

The antimicrobial activity of essential oils is recognized, however, there aren't sufficient studies concerning their usage in the conservation of the cultural heritage. For this reason, the present study is dedicated to four Iberian aromatic plants and respective essential oils extracted by hydrodistillation. The yield of the essential oil obtained from *Helichrysum stoechas* was 0.99% (v/w). This value is higher than 0.04%, obtained by Vernin & Poite [22]. For *Thymus mastichina* the obtained yield was 3.76% (v/w) which is in accordance with the yields reported by other authors – from 0.4% to 6.90% (v/w) [23]. The yield obtained from *Mentha pulegium* was 4.27% (v/w). The present result is clearly above 1.6% reported for this species from Turkey [24] and 2.34% for aerial parts of European pennyroyal collected from Algeria [25]. The yield of the EOs of *Foeniculum vulgare* was 1.19% (v/w), which is in accordance with the results showed by Mota *et al.* [21], mainly regarding the species from de Center and South of the mainland. Other authors reported a yield of 2.5% (v/w) obtained also by hydrodistillation method [26].

The yield and chemical composition of the EOs can change from plant to plant due to many factors such as abiotic and biotic, location and growing conditions, postharvest treatment, conservation, and extraction conditions [18]. These factors could explain the results presented in this study.

The chemical characterization of the essential oils was performed by GC-MS analyses. The chromatograms are depicted in Figure 1 and the quantitative results are summarized in Table 1. A total of 14 components were identified in the EO of *Thymus mastichina*, which represented 98.13% of the total oil. The main component was 1,8-Cineole (55.72%). The other components present in significant percentage were Camphor (13.29%), Borneol (7.21%) and Camphene (6.19%). These four major compounds correspond to 82.41% of total oil. The chemical composition obtained for the EOs of the analysed thyme is identical to that mentioned by Rodrigues *et al.* [23], mainly for the leaves, leaves and flowers and aerial parts of the plants harvested in the flowering phase, at Algarve, where most of the samples exhibited 1,8-Cineole as the principal compound, representing about 50% of thyme EO composition.

In the case of EO of *Helichrysum stoechas*, three compounds were identified (99.43% of total oil). The main components were  $\alpha$ -Pinene (85.65%) and Limonene (13.62%) that constitutes approximately the entire oil (99.27%). These contents are similar to the reported by Vernin & Poite [22], where the major components were  $\alpha$ -Pinene and Limonene, despite the smallest amount reported for the former (63% and 14%, respectively) and the absence of compounds like  $\beta$ -Caryophyllene and  $\alpha$ -Humulene.



**Figure 1:** Chromatograms of the Essential Oils of the used aromatic plants.

In *Mentha pulegium* EO a total of 17 components were identified, fulfilling 99.48% of its oil. The main components found were Pulegone (70.02%) and Isomenthone (21.99%), followed by the isomers *trans*-Isopulegone (3.90%) and Menthone (1.10%). These four monoterpenes combined totalize 97.01% of the oil. Previous studies with the same plant harvested in other locations and continents reported Menthone and Pulegone as the major components of the essential oil, although some discrepancies in relative amounts. However, considering the results summarized in [27], there is some consistency between the present results and particular cases of European pennyroyal species of Portugal and Greece.

TABLE 1: Relative chemical composition (%) of Essential Oils from the used aromatic plants.

		<b>Thymus mastichina L.</b>	<b>Helichrysum stoechas (L.) Moench</b>	<b>Mentha pulegium L.</b>	<b>Foeniculum vulgare Mill.</b>
<b>RI *</b>	<b>Compound</b>	%	%	%	%
936	$\alpha$ -Pinene	4.78	85.65	0.44	1.70
950	Camphene	6.19	0.16		0.24
973	Sabinene	1.22		0.08	0.22
978	$\beta$ -Pinene	2.95		0.36	1.74
989	$\beta$ -Myrcene			0.04	1.88
993	Octan-3-ol			0.58	
1004	$\alpha$ -phellandrene				0.19
1011	$\delta$ -3-Carene				0.56
1022	m-Cymene				0.34
1030	Limonene		13.62	0.18	26.84
1032	1,8-Cineole	55.72		0.15	
1038	$\beta$ -cis-Ocimene				2.10
1048	$\beta$ -trans-Ocimene	1.29			
1060	$\gamma$ -Terpinene	1.21			0.82
1088	Fenchone			0.07	14.94
1099	Linalool	1.18			
1138	Limonene oxide				0.31
1143	Camphor	13.29			0.43
1151	Menthone			1.10	
1159	Isomenthone			21.99	
1166	Borneol	7.21			
1176	trans-Isopulegone			3.90	
1177	Terpinen-4-ol	1.18			0.16
1190	$\alpha$ -Terpineol	1.47		0.25	
1196	Estragol				7.22
1217	trans-Carveol				0.20
1234	Pulegone			70.02	
1284	Bornyl acetate	0.40			
1285	trans-Anethole				40.00
1289	Lavandulyl acetate	0.04			
1341	Piperitenone			0.15	
1420	trans-Caryophyllene			0.03	
1453	$\alpha$ -Humulene			0.08	
1480	$\gamma$ -Curcumene				0.09
1481	Germacrene D			0.06	

\* RI- retention index on GC column; blank spaces correspond to an absence or compound traces.

*Foeniculum vulgare* EO presented the higher number of constituents, totaling 19 different compounds and the amount of 99.98% of the oil. The compound with higher relative abundance is *trans*-Anethole (40.00%), followed by Limonene (26.84%), Fenchone (14.94%) and Estragol (7.22%). All the other constituents are presented in tiny amounts below 2.10%. These results are according to the composition detected by Mota *et al.* [21].

## 4. Conclusions

The essential oils yields were found to vary greatly in the range of 0.99 to 4.27% (v/w). The two major chemical constituents of the essential oils analysed by GC/ MS were as follows: thyme – 1,8-Cineole and Champhor; everlasting –  $\alpha$ -Pinene and Limonene; European pennyroyal – Pulegone and Isomenthone; fennel – *trans*-Anethole and Limonene. Overall yields, somehow differ from literature, allowing some room for in-depth research into the most adequate collection sites and later study of the viability of production and use. Considering the chemical compounds present in the essential oils of the four analysed plants, the existence of a bactericidal and fungicidal effect is predicted as described in Fidanza & Caneva [20]. For this reason, studies are being carried out to prove its antimicrobial activity in specific case studies. If the biocidal effect is verified, it may be considered that these natural pesticides (green biocides) hold potential as a valid alternative to the currently available methods for the biodeterioration control of cultural heritage.

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