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Hydrogels containing *Thymus vulgaris* essential oil as a novel approach for cleaning monument glazed tiles

Azam Aliasghari Veshareh^{1,2}, Parisa Mohammadi^{1,2}✉ & Shervin Ahmadi³

Preservation of ancient works is one of the most important concerns of those interested in cultural heritage. Restorers use various physicochemical and biological methods to preserve and/or clean ancient objects. One of the cleaning methods is using herbal essential oils (EOs). Since EOs are often volatile, their incorporation into polymers can mitigate this drawback. Here, the antimicrobial properties of *Thymus vulgaris* EO were evaluated through the disk diffusion method against microbial strains, which had been isolated from our previous work. To reduce its volatility, two commercial polymers, alginate and polyvinyl alcohol, were used, and their antimicrobial properties were evaluated. Next, the tests were done on tile models. The results showed that the EO was effective against all selected microbial strains. Furthermore, alginate hydrogel containing EO showed the best antimicrobial activity, suggesting its potential as an effective cleaning method. Further investigation is necessary to ensure that this hydrogel has no undesirable impacts on historical substrates.

Biodeterioration is a phenomenon that appears with microbial growth on the surfaces of cultural heritage and causes esthetic, physical, and chemical damage to cultural substrates¹. Microorganisms interact with their substrates causing destruction and changing them². Biodeterioration of building materials is a global problem that imposes a high cost on buildings³. Preventing and controlling the biodeteriorative agents is very important in the protection of cultural heritage¹. Due to esthetic issues and their impact on material durability, biofilms colonizing on substrates must be removed regularly³. The artwork cleaning process is one of the most critical as well as potentially damaging methods. High-efficiency cleaning systems are currently required⁴. The cleaning has always been an issue, as the treatment should completely remove the biological structures without damaging the substrate, and preventing early new biological colonization⁵.

Removing the biological colonization of stone substrates using biocides is one of the most widely used treatment methods, and limitations should be taken regarding their use due to adverse effects on humans and the environment². The chemical materials used in the treatment of cultural heritage must not be toxic and destructive⁶. A suitable biocide should not create an unwanted color /or change the material properties and should be compatible with the substrate⁷. Today, the use of natural products derived from plants is widespread and has some advantages, such as being effective, easily available, and cheap with low side effects. The usage of them in the conservation of cultural heritage material can be more proper than chemical compounds for human and environmental health⁸.

Several studies have investigated the antimicrobial activity of EOs against biodeteriogenic microorganisms of cultural assets^{9–11}. Recently, the method of encapsulating biocides in hydrogel matrices has been stated to be a suitable method to reduce the biocide concentration needed with similar efficiency¹².

Various natural and synthetic polymers were used to prepare the hydrogel. Alginate is a natural anionic polysaccharide composed of β -D-mannuronic acid and α -L-guluronic acid and linked together with 1 $\rightarrow$ 4 connection and forms a gel network with calcium cations as cross-linking agents. Alginate has many industrial applications due to its characteristics, such as biocompatibility, low toxicity, and low cost as well it can form hydrogel at room temperature without using any toxic solvents⁴. Therefore, given the aforementioned properties of alginate, it can be a suitable choice to conserve cultural heritage. This reduces health risks for conservators and visitors as well as prevents damage to the substrates. Moreover, it has been stated that alginate hydrogels do not change the color and capillary properties of substrate¹³. The PVA is a synthetic polymer with various applications in the fields of pharmaceutical, biomedical, industrial, and cultural heritage^{14,15}. This polymer which is biocompatible and modifiable has favorable viscoelastic properties, and can form hybrid hydrogels in combination with natural and synthetic polymers¹⁴. Furthermore, the PVA polymer has outstanding mechanical properties, such as strength and elasticity. This hydrogel has been revealed to be suitable for cultural heritage conservation. Moreover, this hydrogel can be removed from surfaces without any changes in the shape of substrate¹⁶.

¹Department of Microbiology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran. ²Research Center for Applied Microbiology and Microbial Biotechnology, Alzahra University, Tehran, Iran. ³Iran Polymer and Petrochemical Institute, Tehran, Iran. ✉e-mail: p.mohammadi@alzahra.ac.ir

This study aimed to determine the antimicrobial activity of thyme EO on deteriorogenic microorganisms isolated from *Masjed-e Jāmē* of Isfahan, and the preparation of hydrogel prepared with two mentioned polymers and EO. Since tile is one of the important building materials in the architecture of historical monuments of Iran, especially *Masjed-e Jāmē* of Isfahan, hydrogel effect on tile was evaluated before using this product in the main monument. It is the first experience to use hydrogel containing *Thymus* EO on cultural heritage tile.

Material and Methods

Microorganisms

Bacterial strains of *Kocuria rosea*, *Bacillus licheniformis*, and *Bacillus cereus*, and fungal strains of *Aspergillus niger*, *Penicillium polonicum*, *Cladosporium limoniforme*, *Penicillium chrysogenum* and *Parengyodontium album* used here, were isolated from deteriorated areas of brick, glazed tile and gypsum surfaces of *Masjed-e Jāmē* of Isfahan. They were identified using the molecular method which were recorded in the NCBI database with accession numbers: MW883714, MW879389, MW893688, MW767126, MW876234, MW729033, MW896114, and MW767610, respectively (<https://www.ncbi.nlm.nih.gov/nucleotide/>). These microorganisms showed biodeteriogenic properties, including acid production, CaCO_3 dissolution, and mineral production in *in vitro* studies¹⁷. Furthermore, they could attach, grow, and form crystals on the surfaces of the tile models¹⁸. They had the ability to adhere, grow, penetrate, and change the color of the brick model's surface [unpublished paper].

Essential oils

A plant-based product, *Thymus vulgaris* L. essential oil, was purchased from Barij Essence Pharmaceutical Company.

Chemical analysis of the EO

The Agilent 6890N Gas Chromatograph system was used to analyze the EOs. The chromatographic settings were as follows: injector in the splitless mode 1:50, HP5MS, Agilent 10091s-433, 0.25 mm*30 m*0.25μm columns; oven temperature program: initial temperature 60 °C, hold: 1 min, ramp: 20 °C/min, to 280 °C, hold: 5 min. The mass spectrometer was done with an electron ionization in the 32–550 *m/z* range *amu*. The deconvoluted peak spectra obtained by Agilent Masshunter software were matched against the Wiley library for tentative identification.

Disk diffusion method

A suspension of the fungal spores with a concentration of 10^5 spores per milliliter was counted using a Neubauer chamber. Then, 200 microliters of the spore suspension were cultured on potato dextrose agar (PDA, Merck, Germany). Bacterial suspensions with 1.5×10^8 concentration were prepared from fresh cultures of bacteria in nutrient broth (NB, Merck, Germany), and then were cultured on the nutrient agar (NA, Merck, Germany). After that, paper discs with a 6 mm diameter were soaked in 30 microliters of different concentrations of the EOs placed on the cultures. The purchased EOs were considered 100%. EO was diluted in 0.1% dimethyl sulfoxide (DMSO). DMSO 0.1% was also used as a control for assessing the antimicrobial effect of the solvent. The disk of Tetracycline (30 μg) was used as a positive control for bacteria and Cycloheximid (20 μg/ml) for fungi. Next, the plates were incubated at 28 °C for 2–7 days¹⁹. Finally, the diameter of the growth inhibition zone was measured. Antimicrobial activity was analyzed using one-way ANOVA and T-test in GraphPad software version 8.0.2 (263).

Preparation of different hydrogels containing EO

Alginate (FMC BioPolymer, USA) and PVA (98-99 degree of hydrolysis [mol%]) polymers were used for the preparation of hydrogel containing EO. First of all, the proper concentration of polymers was determined. The PVA and alginate hydrogels were prepared with different concentrations of 1%, 2%, 5%, and 8%. For this, alginate and PVA were placed in deionized water under stirring conditions until complete dissolution. Then, solutions of 5%

(V/V), 7% (V/V), and 10% (V/V) of the *Thymus* EO were added to achieve a homogeneous solution of PVA and alginate polymers.

Preparation of tiles for the cleaning test

Test modeling on the substrate similar to the monument is essential for evaluating the antimicrobial effect of biocides and assessing their impacts on the substrate before using in the monument. To investigate the effectiveness of hydrogel containing essential oil on tiles inoculated with fungi, chambers were designed to control the physicochemical conditions, such as humidity. Then, the tiles were sliced into 2.5×2.5 cm. Three of each with anthracite blue color were sterilized at 121 °C and 1.0 atm pressure for 20 minutes.

A. niger, *P. polonicum* and *P. album* fungi were selected to assess the antimicrobial properties of the alginate and PVA hydrogels, containing *Thymus* EO. From each of the fungal spores, a suspension of 10^5 cells/mL was prepared and then mixed. After that, 150 μl of this mixture was inoculated on the center of the tiles. All tiles were kept at 28–30 °C with 80–85% relative humidity for 3 months.

Assessment of the possibility of cleaning tiles using hydrogels containing EO

The hydrogels of alginate and PVA containing *T. vulgaris* EO were applied on the tile surfaces using a brush. It was necessary to mention a cotton gauze was placed between the tile and hydrogel and incubated overnight. Using cotton gauze made it easier to remove the dried hydrogel. Cotton gauze did not decrease the hydrogel antimicrobial effect. Then, the surfaces of the tiles were cleaned with distilled water. This was repeated three times. As a control, six colonized tiles were treated with alginate hydrogel and PVA⁴. Furthermore, in this study, the effect of alginate and PVA hydrogels containing EO, on the colonized tiles was evaluated.

SEM analysis

To investigate the removal of fungi from the tile surfaces, tile samples of the controls and the treated hydrogel containing EO were revealed with a WEGA3-TESCAN scanning electron microscope. SEM observations were performed after preparing the samples through the fixation with glutaraldehyde 3%, and then dehydration with serial concentration of ethanol (30, 50, 70, 80, 90, and 100%) and gold sputtering in 3–10 mm Hg²⁰. Fungal growth was evaluated for both control and treated groups.

Colorimetric experiments

The color changes of the glazed tile surfaces in all groups were measured by the CIE $L^*a^*b^*$ system coupled with the FRU WRIO colorimeter. L^* value, a^* value, and b^* value indicate lightness, alteration of red to green, and yellow to blue color, respectively. The total color variation (ΔE^*) was obtained according to the following equation.

$$\Delta E^* = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (1)$$

ΔL , Δa , and Δb indicated the differences between L^* , a^* , and b^* values for the reference and tested tiles.

Results

Results of GC analysis

The chemical compositions of *T. vulgaris* EO, determined by GC/MS are listed in Table 1. The major components are thymol (40.046%), γ -terpinene (20.278%), benzene (6.296%), methyl-cyclohexane (5.230%), trans-caryophyllene (4.244%), α -pinene (3.226%), and camphene (3.199%). GC-mass analyses were performed, and whose RT values were calculated from the n-alkane series and recorded.

Antimicrobial activity of EO

The quantitative results of the antimicrobial activity of *T. vulgaris* EO against different bacterial and fungal strains are presented in Table 2. The EO in 50% concentration could inhibit the growth of the tested

Table 1 | Chemical compositions of *T. vulgaris* EO

Number	RT	Area	Area (%)	Compound
1	3.620	9,655,294	0.056	Beta-Myrcene
2	3.740	99,658,032	0.577	Alpha-Pinene
3	3.843	457,728,951	2.649	Alpha-Pinene
4	3.883	4,723,777	0.027	Bicycloheptane
5	3.992	552,706,990	3.199	Camphene
6	4.089	7,400,086	0.043	Bicycloheptan
7	4.186	19,066,497	0.110	Bicycloheptan
8	4.221	19,147,936	0.111	Cyclohexane
9	4.278	39,1561,351	2.266	Bicycloheptan
10	4.323	17,127,209	0.099	Beta-Myrcene
11	4.438	85,349,594	0.494	Beta-Myrcene
12	4.563	903,746,461	5.230	Methyl-cyclohexane
13	4.689	2,513,378,951	14.545	Gamma-Terpinene
14	4.741	557,883,896	3.229	Alpha-Terpinene
15	4.935	1,088,000,917	6.296	Benzene
16	5.141	180,217,058	1.043	1,8-Cineole
17	5.226	990,647,010	5.733	Gamma-Terpinene
18	5.729	149,299,936	0.864	Alpha-Terpinolene
19	5.792	497,234,574	2.878	Linalool
20	5.947	572,836	0.003	Isoborneol
21	5.958	137,447,480	0.795	Borneol
22	5.998	23,574	0.000	Isoborneol
23	6.015	144,704,445	0.837	3-Cyclohexen
24	6.209	211,188,086	1.222	Delta.3-Carene
25	6.249	79,915,178	0.462	Alpha-Terpinolene
26	6.444	41,864,549	0.242	Beta-Citronellol
27	6.518	56,307,676	0.326	Neol 2,6-Octadien
28	6.855	6,919,695,151	40.046	Thymol
29	7.290	184,738,513	1.069	2,6-Octadien
30	7.364	29,494,303	0.171	Alpha-Copaene
31	7.707	733,314,650	4.244	Trans-Caryophyllene
32	7.907	109,000,975	0.631	Alpha-Humulene
33	8.730	86,642,752	0.501	Caryophyllene

microorganisms, except *B. cereus*. The diameter of the growth inhibition zone for this bacterium was 42 mm, which was higher than the positive control ($p < 0.05$). The EO up to 10% concentration inhibited the growth of *P. album*. No growth inhibition was seen at 0.5% concentration for all fungi, although the diameter of growth inhibition zone in this concentration was 10 mm for both *B. licheniformis* and *K. rosea*.

Antimicrobial activity of Alginate and PVA containing EO

The antimicrobial activity of alginate hydrogel, containing 5% of *T. vulgaris* EO significantly increased compared to 5% of *T. vulgaris* EO ($p < 0.05$). The exception was for *K. Rosea* that the diameter of inhibition zone decreased from 25 mm to 22 mm ($p < 0.05$). The antifungal activity of PVA containing 5% of *T. vulgaris* EO was significantly higher than 5% of *T. vulgaris* EO ($p < 0.05$). The exception was for *P. polonicum* where the diameter of the inhibition zone for both treatments was identical ($p > 0.05$). The antibacterial activity of PVA containing 5% of EO was lower than 5% of EO ($p < 0.05$). Furthermore, the antimicrobial effects of alginate and PVA were individually investigated. The results showed that both polymers without *T. vulgaris* EOs did not show any antimicrobial activity on the tested microorganisms. As shown in Table 3, the inhibition zone of alginate hydrogel, containing EO on *A. niger*, *P. polonicum*, and *P. chrysogenum* was 11, 52, and 24 mm, respectively. The inhibition zone of alginate hydrogel on *B. cereus*, *B. licheniformis*, and *K. rosea* was 25, 25, and 22 mm, respectively.

Alginate hydrogel containing 5% of *T. vulgaris* EO showed higher antimicrobial activity significantly than PVA hydrogels containing the same concentration of EO on the tested microorganisms ($p < 0.05$). In the case of *A. niger*, there was not observed any significant difference between PVA and alginate containing 5% of *T. vulgaris* EO ($p > 0.05$). Finally, the growth of *P. album* was inhibited in the presence of both polymers containing *T. vulgaris* EO.

SEM analysis

SEM images showed a significant removal of fungal growth after the treatment with 2% alginate containing *T. vulgaris* EO 10% (Fig. 1E), compared to control (Fig. 1B). As seen, no damages were observed on the treated tiles' surfaces compared to the control (Fig. 1A, E, F). Alginate containing *T. vulgaris* EO 5% also significantly caused fungal growth reduction on the surface of the tile (Fig. 1D), which was less than alginate containing EO 10% (Fig. 1E). Reduction of fungal growth was better observed in alginate 2% containing 5% EO (Fig. 1E) than alginate 3% containing 5% EO (Fig. 1F). The use of PVA containing *T. vulgaris* EO did not have an impact on removal of fungi, grown on the tile surface (Fig. 1C).

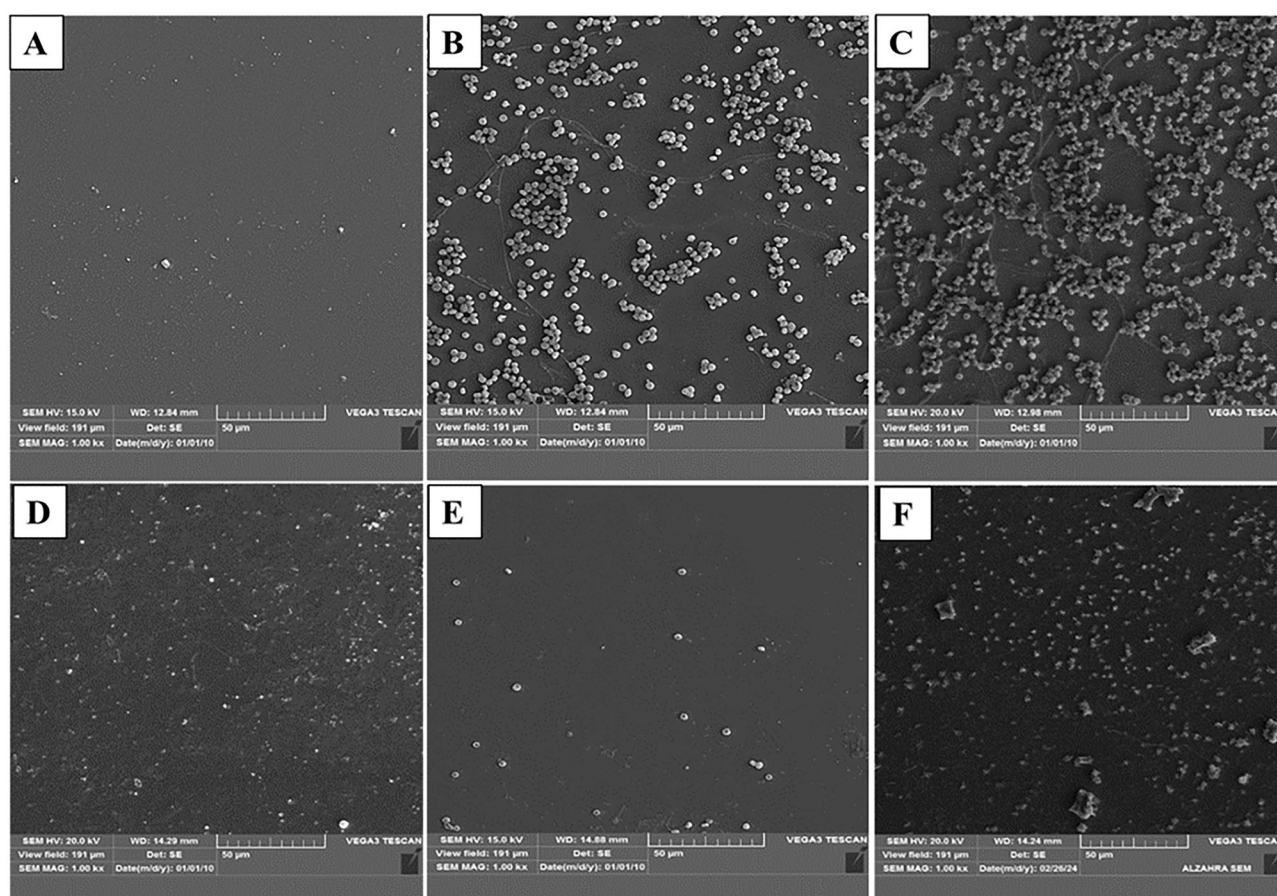
Table 2 | Diameter of growth inhibition zone by *T. vulgaris* EO (mm)

Microorganisms	Thyme Conc. (%)						Positive control	Solvent control
	50	25	10	5	2	0.5	Cycloheximide (20 µg/ml)	DMSO 0.1%
Fungi								
<i>Aspergillus niger</i>	TIG	33	13	0	0	0	10	0
<i>Cladosporium limoniform</i>	TIG	34	15	15	10	0	17	0
<i>Penicillium polonicum</i>	TIG	34	18	12	0	0	27	0
<i>Penicillium chrysogenum</i>	TIG	32	12	0	0	0	19	0
<i>Parengyodontium album</i>	TIG	TIG	TIG	30	15	0	8	0
Bacteria							Tetracycline (30 µg)	
<i>Bacillus cereus</i>	42	28	25	16	12	0	23	0
<i>Bacillus licheniformis</i>	TIG	35	32	16	14	10	40	0
<i>Kocuria rosea</i>	TIG	30	30	25	20	10	30	0

TIG Total inhibition of growth.

Table 3 | Diameter of growth inhibition zone (mm) of PVA and alginate hydrogels

Microorganisms	Alginate 2% + <i>T. vulgaris</i> EO 5%	PVA 2% + <i>T. vulgaris</i> EO 5%	Alginate 2%	PVA 2%
Fungi				
<i>Aspergillus niger</i>	11	12	0	0
<i>Cladosporium limoniform</i>	TIG	23	0	0
<i>Penicillium polonicum</i>	52	12	0	0
<i>Penicillium chrysogenum</i>	24	10	0	0
<i>Parengyodontium album</i>	TIG	TIG	0	0
Bacteria				
<i>Bacillus cereus</i>	25	7	0	0
<i>Bacillus licheniformis</i>	25	10	0	0
<i>Kocuria rosea</i>	22	11	0	0

**Fig. 1 | SEM images of hydrogel impact on the removal of fungi from tile substrate. A** Tile control, **B** Fungi control, **C** PVA containing 5% EO, **D** Alginate 2% containing 5% EO, **E** Alginate 2% containing 10% EO, **F** Alginate 3% containing 5% EO.

Colorimetric results

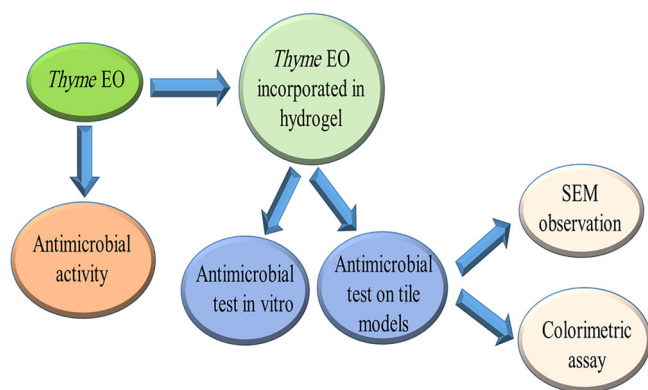
The results of the colorimetric measurements are presented in Table 4. This table shows the chromatic differences between the two colonized and the reference tiles (ΔE^* 8.396). After the treatment, the color change was decreased up to ΔE^* lower than 3.5–4, the limit of perception of the human eye. The lowest ΔE^* has been observed in the treated samples with alginate 2% containing EO 10% (1.181). ΔE^* was 2.35 for alginate 3% containing EO 5%, and 2.997 for alginate 2% containing EO 5%. The highest ΔE^* were for PVA 2% and PVA 2% containing EO 5% with ΔE^* of 13.47 and ΔE^* of 8.84, respectively (Fig. 2).

Discussion

Biocides such as benzalkonium chloride, are chemical compounds used for cleaning biodeteriorated surfaces, but they are potentially toxic for operators, visitors, and the environment, and they can also cause harmful outcomes on the substrate. Plant compounds can be a promising approach as a safe and environmentally friendly alternative compared to chemical biocides for cultural heritage substrates^{5,21}. Natural products derived from herbal materials, such as EOs have a wide range of secondary metabolites that are known as environment-compatible antimicrobials. Recently, researchers working on cultural heritage have been investigating the use of EOs as natural biocides to

Table 4 | Colorimetric results according to CIE L* a* b*

	L*	a*	b*	ΔE
Reference	34.91	3.27	-10.99	
Tile + Fungi	28.41	3.06	-5.31	8.396
Tile + Fungi + Alginate	32.63	4.05	-12.77	3.81
Tile + Fungi + Hydrogel alginate 2% containing EO 5%	32.02	4	-10.72	2.997
Tile + Fungi + Hydrogel alginate 2% containing EO 10%	34.14	4	-11.51	1.181
Tile + Fungi + PVA	24.8	3.89	-8.02	13.47
Tile + Fungi + PVA containing EO	27.73	0.6	-5.13	8.84
Tile + Fungi + Alginate 3%	31.57	4.09	-11.63	3.55
Tile + Fungi + Alginate 3% + EO 5%	33.08	4.29	-12.07	2.35

**Fig. 2 | Schematic diagram of experimental design.**

protect cultural heritage materials⁸. These natural compounds do not change the substrate nature and are especially non-toxic to human health and the environment¹¹. It has been shown that the antimicrobial activity of EOs is mostly due to the presence of active compounds, such as monoterpenes, sesquiterpenes, and other hydrocarbons, as well as phenolic compounds. Several studies have reported the effect of phenolic compounds on a wide range of microorganisms²². The major impact of EOs applied on microorganisms is related to aldehydes, alcohols, and phenols, which can induce alteration in the cellular membrane permeability or interference with the enzymatic activities¹². Furthermore, The antimicrobial effect of EO may be due to the lipophilic nature and low molecular weight of some their derivatives such terpenes/terpenoids, which cause disruption of cell membrane function, cause cell death, inhibition of fungal sporulation and its germination²³, disorder of membrane- embedded proteins, inhibition of cell respiration, and alteration of ion transport processes²⁴.

The *Thymus* genus is one of the genera in the *Lamiaceae* family. *Thymus* includes 250–350 species of wild and perennial herbs with herbaceous subshrubs. The *Thymus* EO has a broad range of antimicrobial compounds²². The EO of *T. vulgaris* is rich in phenolic compounds, such as carvacrol and thymol. Different compounds in thyme have been known, including linalool, borneol, geraniol, sabinene hydrate, thymol, and carvacrol. The most commercial antimicrobial agent of *T. vulgaris* EO is thymol²⁵. The chemical analysis of the used EO in this study showed that its most compound was thymol (40.046%) as in other studies have been reported^{25,26}. It has been shown there is a positive correlation between the thymol content of *Thymus* EOs and its fungicidal activity²⁵. Carvacrol was not detected in the *T. vulgaris* EO, used in this study, although in other studies have also been reported this component in low concentration^{25,26}. The absence of this compound in the present study could be attributed to used different strain of plant.

To evaluate EO efficiency in the field of cultural heritage, the antimicrobial activity of *T. vulgaris* EO was assayed *in vitro* against fungi and bacteria isolated from *Masjed-e Jāmē* of Isfahan by the disc diffusion agar method.

In a study, the antifungal activity of two EOs of *Origanum vulgare* and *T. vulgaris* on *Scopulariopsis* sp. and *Fusarium* sp., isolated from paper documents by microatmosphere method evaluated, and the results have been shown the volatile compounds of the EOs have the favorable antifungal activities²⁷. In another study *Oregano* and *Thymus* EOs inhibited the growth of bacterial and fungal colonies, isolated from the marble²⁶. In other research, *T. vulgaris* EO and its hydro-alcoholic (HA) solution had antimicrobial activity on microorganisms isolated from wooden surfaces of artwork. This material had the favorable effect in the removal of microbial patina. In 12.5% concentration of EO the diameter of inhibition zone for *Bacillus* sp., *Aspergillus* sp. and *Penicillium* sp. It was reported 21.5–22, 8.5–9, and 12–12.5 mm, respectively²⁸. In the present study, 10% concentration of *Thymus* EO inhibited the growth of two tested *Bacillus* sp., and the diameter of the growth inhibition zone was 25 and 32 mm. The diameter of growth inhibition for *Aspergillus niger* was 13 mm. This diameter was 18 and 12 mm for both tested *Penicillium* sp. The antimicrobial activity of *Thymus* EO in this study was higher than in other studies. In our study, *Thymus* EO showed proper antimicrobial activity on deteriorative bacteria and fungi up to 5% concentration. Different results were observed with different EO concentrations. This differences could be attributed to differences in cell wall structure, cytoplasmic membrane compositions, and physiological characteristics of used microorganisms²⁸.

The encapsulation of EOs could have an alternative method in applying EOs as natural biocides, causing a controlled release of EO²⁹.

The results of this study showed that the antimicrobial efficiency of encapsulated EO in hydrogel was increased compared to its same concentration when used alone. Then, the antimicrobial effect of the alginate and PVA hydrogels containing *T. vulgaris* EO was evaluated on the surfaces of the tiles in laboratory conditions. Our results showed the proper fungal removal from tile surfaces. The comparative assay exhibited that the alginate hydrogel containing EO, has been the most effective one. As shown in Fig. 1E, Alginate 2% could reduce the fungal growth more than Alginate 3%, because the release of EO in this concentration of polymer is better done.

In a study, two biocidal hydrogels containing *Thymus* EO and sodium dichloroisocyanurate were applied on mosaic surfaces of outdoor monuments. The photographic, colorimetric, and microscopic analyses were shown the hydrogels were effective in removing all microbial colonizers¹². In another study, different concentrations of *T. vulgaris* EO, including 0.25%, 0.5%, and 0.1% were encapsulated in the alginate hydrogel and applied to cyanobacterial biofilms. The results showed *T. vulgaris* EO had the most inhibitory effect on cyanobacterial cells²¹. This study results showed the proper fungal removal from tile surfaces. The comparative study exhibited that the alginate hydrogel containing EO has been the most effective one.

To obtain proper treatment without changing the surface color, the values of ΔE^* should be lower than the perception limit of the human eye¹². To analyze the possible color change, a threshold of $\Delta E^* 3$ was used as the limit created in the substrate as stated by some researchers²⁶.

In our study, ΔE^* of treatment with alginate hydrogel containing 5% and 10% EO was lower than the suggested threshold, showing no noticeable color change occurred on the tile with this treatment. The color change caused by the hydrogel containing 10% *Thymus* EO was much lower than the threshold limit, which is valuable.

In the present study, the antimicrobial activity of *T. vulgaris* EO and the hydrogels containing *T. vulgaris* EO in cleaning the biodeteriorated cultural heritage tiles were compared. As shown, when the *Thymus* EO was loaded in alginate hydrogel, its volatility was controlled. The hydrogel containing EO exhibited good antimicrobial effects on biodeteriogenic bacteria and fungi. These polymers, with easy preparation and their good antimicrobial properties, could be applied in cleaning of biodeteriorated cultural heritage. However, more studies are needed to investigate its stability and durability in environment, its interaction with different substrates, and its possible harmful effects in the long term.

Data availability

Not applicable.

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Author contributions

Azam Aliasghari Veshareh performed the experiments, collected the data, performed the analysis, and wrote the draft of manuscript. Parisa Mohammadi supervised the project, designed experiments, completed and edited the manuscript. Shervin Ahmadi helped to prepare polymers .

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to Parisa Mohammadi.

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