

EFFECT OF SOME MEDICINAL PLANTS EXTRACTS ON FUNGI INVADED LIMESTONE OF SOME MONUMENTAL BUILDINGS IN OLD CAIRO

Ayman Hassan HEGAB¹, Saad Azab AMIRA¹, Ayman Salah TAHA^{2*}

¹ Conservation & Restoration Center, National Library of Egypt.

² Conservation department, Faculty of Archaeology, Aswan University, 81528, Egypt.

Abstract

The present work was designed to pinpoint some medicinal plants as good candidates of antifungal activities. These medicinal plants were collected from Egyptian Herbalism, and identified. Extraction of the crude oil using steam distillation method. Isolation of different fungal species from contaminated three different historical buildings in Old Cairo (mosque of Tameem Alrassafy, madrasa of Azbak Al-Yusufi, and the mosque of Yusuf Agha Al-Heen), which are infected by biological deterioration on its limestone surface. The antifungal activity of clove, piper, and thymus crude oil extracts on these isolated fungi was examined. The potential Sterilization of these extracts on limestone samples was investigated. The effectiveness of the treatment using these extracts has been evaluated with laboratory tests. The results obtained seem to indicate that this type of treatment will be suitable for monumental stone conservation. This research is illustrated by a set of tables, figures, and pictures.

Keywords: *Antifungal activity; Clove; Biological Deterioration; Piper nigrum Linn.; Thymus; Steam distillation*

Introduction

Egypt has a huge historical heritage, which represent different historical eras pass through it. Starting from ancient ages Before Christ, pass through Pharonic period, Greek, Batolamic, Romans ending of the Islamic period, modern Egypt and nowadays by which Egypt takes important place between the country has a different monumental heritage. Cairo is the Capital of Egypt. So that, the old Cairo is considered as an open museum containing these different historical periods [1], because it contains all the old capitals preceding it (ex-capitals). This great history produced a huge archeological treasure immovable and movable. Immoveable archeological treasure like: temples, tombs, pyramids, mosques, buildings, etc. Movable archeological treasure like: Arts pieces which Egyptian museums full of it. The different these archeological treasures were made of different materials between organic materials like: wood, manuscripts, textiles, etc. Inorganic materials like: stones, metals etc.

Monumental buildings in old Cairo were constructed from limestone [1] and those in downtown are suffering from man effects, climate change of the last decade as a result, some fungi, algae, and bacteria attack the building blocks and enhance their decay. We have thought it may be worthwhile, we extend our approach for sterilizing buildings to limestone. Monumental buildings made from El-Mokattam quarry in Mamluk [2], Fatimic, and Khedive

* Corresponding author: Aymansalahtaha82@yahoo.com

Cairo. In this article we report simulation results of treating limestone blocks cut from El-Mokattam quarry treated in away similar to that now applied for paper documents, and manuscripts in the National Library of Egypt [3]. This research was done on three monumental buildings in Old Cairo City, which are: (i) Mosque of Tameem Al-Rassafy (Mamluk period), with monument No. 227, building period 1471 AD, 876 HD, was located in Tameem Al-Rassafy St. Sayeda Zainab, (ii) Madrasa of Azbak Al-Yusufi (Mamluk period), with monument No. 211, building period 1494 AD, 900 HD, was located in Azbak St. Sayeda Zainab, (iii) Mosque of Yusuf Agha Al-Heen (Ottoman period), with monument no. 196, building period 1625 AD, 1035 HD, was located in Port said St. Bab El-Khalk square, El-Darb Al-Ahmer.

These monumental buildings are exposed to the weathering action of several physical, chemical and biological factors, which cause changes in its physical, chemical, and mechanical properties, and color of stone surfaces [4, 5]. Fungi, the most damaging organisms attacking and even penetrating the surfaces of stone monuments [6, 7], have activities are enhanced by climatic conditions (high irradiation, extreme wetting and drying by humidity, temperature, and underground water etc.), other environmental factors such as air pollution and air eutrophication, and cause degradation of the physical, chemical, and mechanical properties of construction materials. The persistence of chemical corrosion by sulfation on the exposed materials [8, 9], along with the remarkable presence of fungal colonies on the surface of these building stones [10], points to a combined action of particulate pollutants and microbial colonization in the degradation and deterioration of stone. Microorganisms through their microbiological action cause initially, degradation, which change the chemical composition (alteration processes), leading to weakening of the mineral structures, then there is the secondary effect of physical-structural destruction of the surface to the inside: to pore size, cracking, changes in humidity ratio [11]. Although the inorganic composition of the stones was not favorable for the growth of these heterotrophic microorganisms, yet it was likely that organic residues from different sources on the stone surface were utilized as nutrients [12]. Light and sufficient warm temperature combined with rich nutrients of organic residues present on stone surface to create suitable conditions for reproduction of quiescent (dormant) fungal spores [13]. Those sensitive to humidity and heat emit hyphae that penetrate the substrate, with mechanical destructive effects [14]. Black pepper, *Piper nigrum* Linn. (Piperaceae), is frequently used in the curry recipes an ingredient in the prescriptions of folk medicine, Ayurveda and traditional medicinal systems, as aphrodisiac, carminative, analgesic, stomachic, antiseptic diuretic and for the treatment of cough, rheumatoid arthritis, peripheral neuropathy, melanoderma, and leprosy due to the presence of volatile compounds, tannins, phenols and other substances [15-19]. Antimicrobial activity of the volatile oils of black pepper against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisiae* [20].

The essential oil of common thyme (*Thymus vulgaris*) is made up of 20-54% thymol, which is the main active ingredient. The plant's actions are considered to be disinfectant, antiseptic, anodyne, anti-inflammatory, rubefacient, antitussive, aperitif, carminative, demulcent, depurative, diaphoretic, digestive, and diuretic, fungicide, nervine, sedative, stimulant, and vermifuge [21, 22]. It has also been shown to be effective against the fungus that commonly infects toenails, and also shown to have antimicrobial, insecticidal and fungicidal properties [23]. Assessment of the Larvicidal potentials of Thymol derivatives on Anopheles mosquitoes *I.R. Jack et al* [24], harnessed the structure of Thymol and prepared its o-methyl (2), o-ethyl (3), acetate (4) and benzyloxy (5) derivatives, with a view to determine their suitability or potentials as insecticides/larvicides [25].

Another bioactive product is clove oil which is extracted from *Eugenia caryophyllata* Thumb. It contains eugenol, which is useful as bactericides and lipoxxygenase inhibitor [26, 27]; also it inhibited the growth of microorganism in vitro [28-30]. Antifungal activity of essential

oil of clove was evaluated Chromatography and spectrometric analysis of cloves showed that eugenol is the major antioxidant [31-33], also its radical-scavenging activity may be responsible for its chemo preventive action, so it decreases the number of tumor-bearing animals [34,35]. Eugenol has antioxidant activity [36, 37] where it inhibited superoxide anion generation and also inhibited the generation of hydroxyl groups (-OH), generates $\cdot\text{OH}$ radicals [37]. Eugenol reduces the iron-induced hepatic damage by lowering lipid peroxidation [38, 39].

Materials and Methods

Sample Collection

Isolation of different fungal species from contaminated three different historical buildings in Old Cairo (mosque of Tameem Alrassafy, madrasa of Azbak Al-Yusufi, and the mosque of Yusuf Agha Al-Heen), which are infected by biological deterioration on its limestone surface. Mosque of Tameem Al-Rassafy (Mamluk period), with monument No. 227, building period 1471 AD, 876 HD, was located in Tameem Al-Rassafy St. Sayeda Zainab, Madrasa of Azbak Al-Yusufi (Mamluk period), with monument No. 211, building period 1494 AD, 900 HD, was located in Azbak St. Sayeda Zainab and Mosque of Yusuf Agha Al-Heen (Ottoman period), with monument no. 196, building period 1625 AD, 1035 HD, was located in Port said St. Bab El-Khalk square, El-Darb Al-Ahmer (Fig. 1).

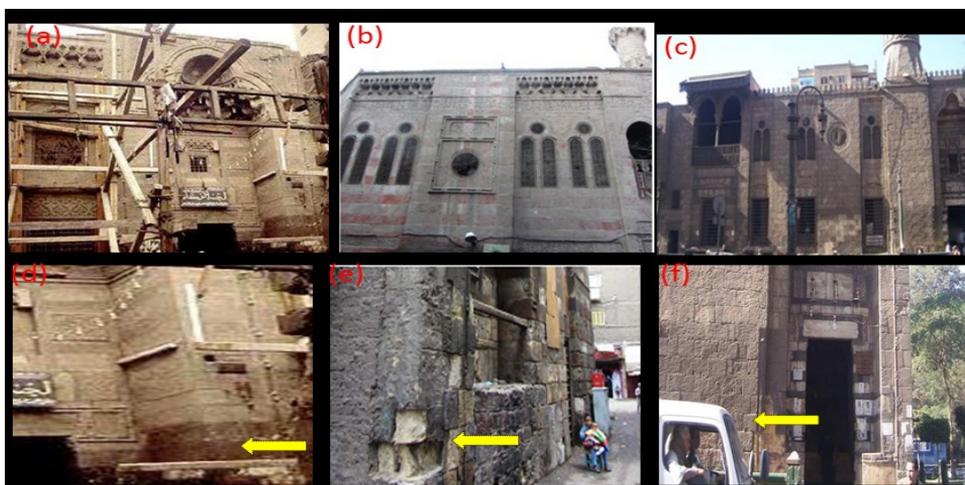


Fig. 1. Shows the fungal isolates location of the three buildings

Collection of different medicinal plants

Thymus vulgaris L., *Eugenia caryophyllata* Thumb is the collected. They were air dried, ground to powder and weighted.

Extraction of the crude essential oils

A quantity of 250g of powdered plant was used for extraction of crude essential oils by the steam distillation method.

Isolation of different fungal species

Isolates of different fungal species from contaminated samples from the three historic buildings (Mosque of Tameem Al-Rassafy, madrasa of Azbak Al-Yusufi, and the Mosque of Yusuf Agha Al-Heen), were performed on PDA media. After five days of incubation, separated fungal species were isolated and transferred to Cezapex – media at (25°C for 72h) to grow as single pure fungal species. Identification of these separated fungal species was carried out.

The antifungal activity of the crude oil extracts

The antifungal activity of crude essential oil extracts on the cellulose decomposing fungi was assayed using a concentration 500ppm.

Simulating experiment

Prepare cubic samples from limestone with dimension (5×5×5), and infected them by injection with these fungal isolates, then incubated for five days in 25°C, and weathering with water and sodium chloride 5% solution in the normal environmental conditions for one month.

Sterilization of the samples

Samples are sterilized by painting and spraying the stones and leave them in the normal polluted environment for two weeks, using a concentration 500ppm of each Clove, Piper, and Thymus extracts alone and mixtures.

Results and Discussion

Collection of different medicinal plants

Aerial shoot was taken from *Thymus vulgaris*, while Calyx and Petals from *Eugenia caryophyllata* Thumb, and Seeds from *Piper nigrum* Linn. Of the collected medicinal plants for *Eugenia* [40-42] and for *Thymus* [40, 43, 44].

Extraction of the crude oil from medicinal plants

The yield of crude essential oil extracts obtained was 0.072g/g (18g/250g dry aerial shoots) for *T. vulgaris*, 0.064 g/g (16g/250 g dry calyx and petals) for *E. caryophyllata*, and 0.088 g/g (22g/250 g dry seeds) for *P. nigrum*.

Isolation of different fungal species from samples

Fungi cause severe deterioration of historical buildings [11-14]. *A. Abdel Hameed et al* [45] and *A.H. Hegab* [46] reported that the most dominant fungi isolated from Egypt are *Aspergillus spp.* Eight different isolates from the three historic buildings which were characterized based on their morphology; they were identified as *Aspergillus flavus*, *Aspergillus flavus var. columuaris*, *Aspergillus parasiticus*, *Aspergillus terreus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Fusarium* and *Penicillium*. Four strains: *A. niger*, *A. flavus*, *Fusarium* and *Penicillium*, were consistently isolated in higher concentrations of all the three buildings as recorded in tables 1 and 2, and shown in Figure 2.

Table 1. Fungal isolates concentration

Building	Microorganism	CFu/cm²
Azbak Elyoussefy	<i>Aspergillus niger</i>	12
	<i>Aspergillus flavus</i>	10
	<i>Aspergillus terreus</i>	6
	<i>Fusarium spp.</i>	9
	<i>Penicillium spp.</i>	8
Youssef Agha Elheen	<i>Aspergillus niger</i>	6
	<i>Aspergillus flavus</i>	4
	<i>Aspergillus parasiticus</i>	3
	<i>Fusarium spp.</i>	5
	<i>Penicillium spp.</i>	2
Tameem Elrassafy	<i>Aspergillus niger</i>	12
	<i>Aspergillus flavus</i>	7
	<i>Aspergillus parasiticus</i>	2
	<i>Aspergillus terreus</i>	5
	<i>Aspergillus flavus var. columuaris</i>	3
	<i>Aspergillus fumigatus</i>	1
	<i>Fusarium spp.</i>	10
	<i>Penicillium spp.</i>	9

Table 2. Fungal isolates percentage.

Microorganism	CFu/cm ²	%
<i>Aspergillus niger</i>	30	26.3
<i>Fusarium spp.</i>	24	21.1
<i>Aspergillus flavus</i>	21	18.4
<i>Penicilium spp.</i>	19	16.7
<i>Aspergillus terreus</i>	11	9.6
<i>Aspergillus parasiticus</i>	5	4.4
<i>Aspergillus fumigatus</i>	3	2.6
<i>Aspergillus flavus var. columuaris</i>	1	0.9
Total	114	100%

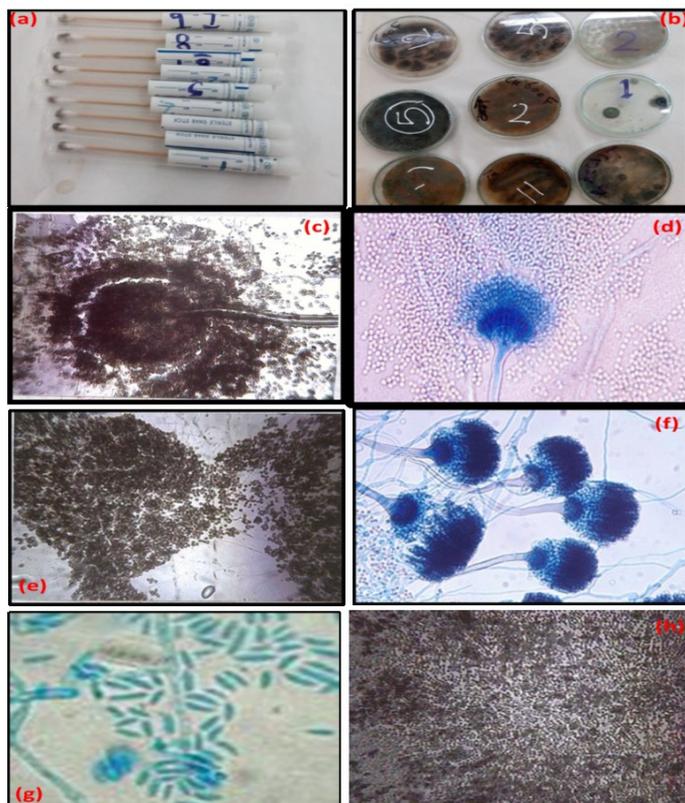


Fig. 2. Fungal isolates from the three historic buildings

Antifungal activity of the crude essential oil extracts

Antifungal activity of the isolated fungi was assayed using the crude extract at a concentration 500ppm by using Radial Growth method as illustrated in tables 3 and 4 and figure 2 to investigate the most effective one. This concentration is in agreement with other authors [44, 47].

It is noticeably from the previous tables that, the mixture of the crude oils is the most effective mixture, then the mixture from both clove oil and piper oil, the mixture from clove and thyme oil, the mixture piper and thyme oil, however the clove or piper or thyme itself alone is less active. This indicated that, the synergistic effect of these three crude oils extracted is higher than the crude oil alone, on other word we have a positive synergistic effect [3].

Table 3. The radial growth test

Fungi	<i>A. flavus</i>	<i>A. parasiticus</i>	<i>A. flavus var</i>	<i>A. terreus</i>	<i>A. fumigatus</i>	<i>A. niger</i>	<i>Penicillium</i>	<i>Fusarium</i>	Growth %
Control	8.0 cm	8 cm	7.8 cm	7 cm	6.5 cm	8.0cm	5.0 cm	7.0 cm	100%
Clove oil	0.5cm	1.0 cm	1.0 cm	0.25 cm	1.0 cm	0.5cm	1.0 cm	0.5 cm	7.7%
Thyme oil	1.0 cm	1.0 cm	0.75cm	1cm	0.0 cm	1.0cm	0.5 cm	1.0cm	8.3%
Piper oil	0.5cm	1.0 cm	1.0 cm	0.75 cm	1.0 cm	0.5cm	0.0 cm	0.0 cm	6.3%
Clove + Thyme	0.5cm	1.0 cm	1.0 cm	0.25 cm	1.0 cm	0.5cm	0.0 cm	0.0 cm	5.7%
Clove + Piper	0.25 cm	0.50 cm	0.25 cm	0.0 cm	1.0 cm	0.5cm	0.0 cm	0.0 cm	3.3%
Thyme + Piper	0.5cm	1.0 cm	1.0 cm	0.0 cm	1.0 cm	0.5cm	1.0cm	1.0 cm	8.0%
Clove + Thyme + Piper	0 cm	0 cm	0 cm	0.0 cm	0.0 cm	0 cm	0 cm	0.0 cm	0.0%

Table 4. The radial growth percentage

Herb	Growth (%)
Control	100
Thyme oil	8.30
Thyme + Piper	8.00
Clove oil	7.70
Piper oil	6.30
Clove + Thyme	5.70
Clove + Piper	3.30
Clove + Thyme + Piper	0.00%

Simulating experiment

Preparation of the cubic samples from limestone, due to most of the historical Islamic buildings in Old Cairo were built from limestone of El-Mokattam plateau, which is the main quarry in Cairo at this period of time [48]. Using NaCl, 5% solution due to, this salt is generally found in the soil of Old Cairo in addition to sulphur, and carbonic salts etc. [46] as seen in (Figs. 3 and 4).These limestones were infected by injection with the fungal isolates, then incubated for seven days in 25°C, and weathering with water and sodium chloride 5% solution in the normal environmental conditions for two months.

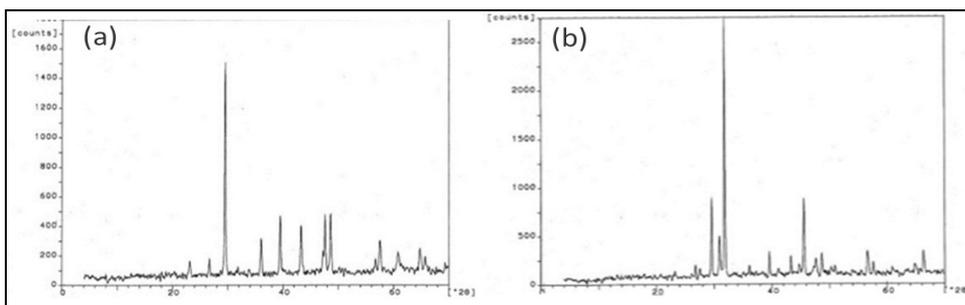


Fig. 3. (a) Displays X.R.D of stone samples [Calcite] and (b) displays X.R.D of stone samples [Calcite] and [Halite]

Sterilization of the samples

After sterilization of the previously infected cubic limestone samples by using the crude extracts, isolates from these sterilized samples have been taken and compared with the isolates before sterilization process. It was found that the samples before sterilization process contain these fungi, but after sterilization they became clear due to the disappearance of the fungal growths as shown in (Figs. 5 and 6).

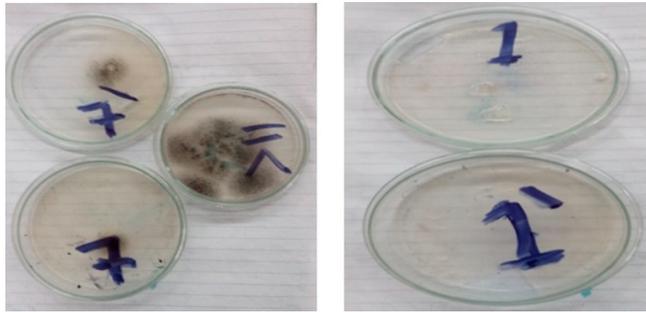


Fig. 4. Sterilization by: painting (1, 7), spraying (1',7'), and control (7'')



Fig. 5. Illustrates the difference between the cubic samples (a) before and (b) After fungal injection, and weathering.

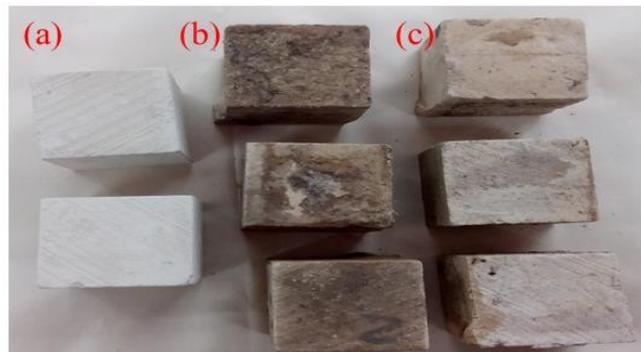


Fig. 6. Shows (a) limestone before fungal injection, (b) limestone after fungal injection, (c) Limestone after treatment by the mixture of crude oil extract.

Conclusion

- From the present study the following conclusions can be drawn:
- The used stone is limestone (Calcite - CaCO_3), and the salt found in the stone is Halite (NaCl).
 - We found and isolate eight fungi from three monumental buildings.
 - The mixture from the three crude extracts with concentration 500ppm is the most effective one, while the mixture of any two crude extracts with the same concentration is stronger than one extract alone with the same concentration as $3 > 2 > 1$.

- This method of treatment using these selected medicinal plants extracts mixtures could be affected by one hundred percent (100%) in sterilization of the surface of historical building's limestone.
- It is possible to sterilize limestone efficiently and less expensive than traditional Laser treatment in the light of the fact that treatment of all monumental stone buildings in Egypt, which have more than 60% of all the world monuments is rather than expensive utility of Laser and other techniques common with Roman and Greek monuments.

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