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Antifungal activity of essential oils isolated from Egyptian plants against wood decay fungi

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Abstract The essential oils of eighteen Egyptian plants were extracted by hydrodistillation and their chemical compositions were analyzed by GC-MS. The antifungal activity of the isolated oils was evaluated against two wood decay fungi *Hexagonia apiaria* and *Ganoderma lucidum* in vitro. The essential oil of *Artemisia monosperma* showed the highest inhibitory effect against *H. apiaria* (EC_{50} = 31 mg L^{-1}) and *G. lucidum* (EC_{50} = 53 mg L^{-1}). The results of in vitro tests indicated that the essential oils of *Cupressus sempervirens*, *Citrus limon*, *Thuja occidentalis*, *Schinus molle*, *A. monosperma* and *Pelargonium graveolens* were the most potent inhibitors against both fungi. These six oils caused significant reduction of wood mass loss of Scots pine sapwood after 6 weeks of fungal exposure. The oil of *C. limon* revealed the highest reduction of wood mass loss caused by *H. apiaria*, while *A. monosperma* oil displayed the highest reduction of wood loss caused by *G. lucidum*. These results support the potential use of essential oils for wood protection against decay fungi.

Keywords Essential oils · Chemical composition · Antifungal activity · Wood preservation · Wood decay fungi

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Introduction

A wide range of organic and inorganic fungicides is currently in use for wood preservation industry [1]. However, because of the health concerns associated with the use of these chemical treatments, the recent trend has been to develop environmentally benign approaches for the protection of wood. In this context, the search for simple bioactive compounds derived from plants that can be used against wood decay fungi has been a research direction for ecologically safe products [2]. For instance, essential oils are known to contain a natural mixture of monoterpenes, sesquiterpenes, diterpenes, and hydrocarbons, with a variety of functional groups, giving them antibacterial, antifungal, antitermite, and insecticidal activities. To protect wood from fungal and other biological damage, natural preservatives such as essential oils could be an attractive alternative compared to the use of highly toxic traditional wood preservatives [3]. However, essential oils have a wide use in pharmaceutical and food industry as antimicrobial agents, but their use as wood preservatives has not been fully explored. In this study, the chemical composition of essential oils from eighteen Egyptian plants was described using gas chromatography-mass spectroscopy (GC-MS), and their antifungal activities against two common tropical wood decay fungi were investigated.

Materials and methods

Test fungi

Two tropical wood decay fungi (provided from Laboratoire d'Etudes et de Recherche sur le Matériau Bois, LERMAB, Nancy University, France) *Ganoderma lucidum* and

Hexagonia apiaria were used in this study. These two fungi are common for causing wood decay and white rot and are used in European standard methods (EN 113) for evaluating wood decay. Fungal cultures were grown and maintained in 90-mm Petri dishes containing 20 mL of Malt Extract Agar medium (MEA); 4 % malt extract, 2 % agar at 27 °C and 75 % relative humidity (R.H).

The two tested fungi are among the many fungi that cause wood decay and white rot.

Plant materials

Various parts of eighteen plant species: *Artemisia judaica* L. (aerial parts), *Artemisia monosperma* Del. (leaves), *Callistemon viminals* (Sol. ex Gaertn.) G. Don (leaves), *Citrus aurantifolia* (Christm.) Swingle (fruit peels), *Citrus limon* (L.) Burm.f. (fruit peels), *Citrus paradisi* Macfad (fruit peels), *Citrus sinensis* (L.) Osbeck (fruit peels), *Cupressus macrocarpa* Hartw. ex Gordon (leaves), *Cupressus sempervirens* L. (leaves), *Myrtus communis* L. (leaves), *Origanum vulgare* L. (aerial parts), *Pelargonium graveolens* L'Her (leaves) *Rosmarinus officinalis* L. (leaves), *Syzygium cumini* L. Skeels (leaves), *Schinus molle* L. (leaves), *Schinus terebinthifolius* Raddi (leaves) *Thuja occidentalis* L. (leaves) and *Vitex agnus-castus* L. (leaves) were collected during the flowering stage from different locations of Alexandria, Behira and Matrouh Governorates, Egypt, in August 2010–April 2011. These plants are common ornamental trees and wild-grown herbs in Alexandria region north Egypt. The plant materials were identified by Prof. FathAllah Zaitoon of Plant Pathology Department, Faculty of Agriculture, Alexandria University. Voucher specimens have been deposited in Department of Pesticide Chemistry, Faculty of Agriculture, Alexandria University.

Isolation of essential oils

The aerial plant parts were partially dried at room temperature (26 ± 1 °C) for 5 days and the fruit peels were used fresh. Essential oils were extracted by hydrodistillation in a Clevenger-type apparatus for 3 h. The oils were dried over anhydrous sodium sulfate, and stored at 4 °C until used for GC-MS analysis and biological activity tests.

Analysis of essential oils

Essential oils were diluted in diethyl ether and 0.5 µl was injected into the gas chromatography (Hewlett Packard 5890)/mass spectrometry (Hewlett Packard 5989B) (GC-MS) apparatus. The GC column was a 30 m (0.25 mm i.d., film thickness 0.25 µm) HP-5MS (5 % diphenyl) dimethylpolysiloxane capillary column. The GC conditions were as follows: injector temperature, 240 °C; column temperature, isothermal at 70 °C for 2 min, then programmed to 280 °C at 6 °C/min and held at this temperature for 2 min; ion source temperature, 200 °C; detector temperature, 300 °C. Helium was used as the carrier gas at the rate of 1 mL/min. The effluent of the GC column was introduced directly into the ion source of the MS. Spectra were obtained in the EI mode with 70 eV ionization energy. The sector mass analyzer was set to scan from 40 to 400 amu for 5 s. The oil components were identified by comparison of their retention indices and mass spectra with the NIST Mass Spectral Library.

In vitro antifungal assay

Antifungal assessment of the isolated essential oils was conducted using a mycelial radial growth inhibition technique against *G. lucidum* and *H. apiaria*. The oils were diluted with acetone and added to sterilized MEA medium before pouring in 9-cm Petri dishes. The oils were tested at concentrations ranged from 10 to 1000 mg L^{-1}. The discs of mycelial culture (0.5 cm diameter) of fungi, taken from 8-day-old cultures, were transferred aseptically to the center of the Petri dishes. The plates were incubated at 27 °C in the dark. Three replicates were setup for all concentrations and control. Colony growth diameter was measured after the fungal growth in the control treatments had completely covered the Petri dishes. Inhibition percentage of mycelial growth was calculated as follows:

$$\text{Mycelial growth inhibition (\%)} = \left[\frac{(\text{DC} - \text{DT})}{\text{DC}}\right] \times 100$$

where DC and DT are average diameters of fungal growth of control and treatment, respectively. Effective concentration that caused 50 % inhibition of mycelial growth (EC_{50}) and its corresponding 95 % confidence limits were estimated by probit analysis [4].

In vivo antifungal assay

Based on the obtained data from the in vitro antifungal assays, six plant essential oils (*C. sempervirens*, *C. limon*, *T. occidentalis*, *S. molle*, *A. monosperma* and *P. graveolens*) were used for the in vivo antifungal assays. The assay was carried out as described by Mohareb et al. [5]. Mini-block specimens (5 × 10 × 50 mm, radial × tangential × longitudinal) from Scots pine sapwood (*Pinus sylvestris*) are used. Four replicates (12 blocks) for each treatment were oven dried at 103 °C, and weighed, then impregnated with the respective treating solutions of the different essential oils at three levels of concentrations, which varied based on the obtained EC_{50} values in the

in vitro assessment. The tested concentrations were EC_{50} value that obtained from the in vitro assessment, two- and fourfold of EC_{50} [6]. The samples were then wiped with a paper tissue before the weight was recorded for control of uptake. The treated wood specimens were then conditioned by transferring them into air-tight containers for 1 week to allow any potential fixation to occur. Upon drying they were then packaged and sterilized by exposure to ethylene oxide gas, and exposed to an active growth of the test fungi (*G. lucidum* and *H. apiaria*) inoculated 2 weeks prior to the placement of the blocks in Petri dishes. Three specimens were placed in each test plate. A perforated plastic mat was placed between the fungi growing on malt agar and the test block to prevent contact of the blocks with the agar medium. The blocks were then exposed to the tested fungi at 27 °C and 75 % R.H. After 6 weeks of fungal exposure, the blocks were removed from the incubation, brushed carefully to remove any adhering mycelium and placed on racks to air-dry (for 3–4 days). Once air-dried, blocks were oven dried at 103 °C, after which all the blocks were weighed and percentages of mass losses were calculated.

Statistical analysis

Statistical analysis was performed using the SPSS 16.0 software program (Statistical Package for Social Sciences, USA). The log concentration-response lines allowed determination of the EC_{50} values for the fungal bioassay according to the probit analysis [4]. The 95 % confidence limits for the range of EC_{50} values were determined by the least-square regression analysis of the relative growth rate (% control) against the logarithm of the compound concentration. The data of the in vivo experiments were analyzed by one-way analysis of variance (ANOVA). Mean separations were performed by Student–Newman–Keuls (SNK) test and differences at $P < 0.05$ were considered as significant (Cohort software Inc. 1985).

Results and discussion

Chemical composition of the isolated essential oils

The chemical composition of the eighteen essential oils obtained by hydrodistillation was analyzed using GC-MS. The major constituents of the essential oils and their percentages are given in Table 1. Some major components were prevalent in many of the tested plants, such as dl-limonene, α-pinene, β-pinene l-linalool, 1,8-cineole, sabinene, 4-terpineol and γ-terpinene but others were specific to the plant species. The major constituents of the essential oils mainly belonged to four chemical groups: oxygenated monoterpenes (i.e., α-thujone, β-thujone, chrysanthenone, 4-terpineol, l-linalool, d-pulegone, α-citral, β-citronellol

Table 1 Oil yield and major constituents of the essential oils isolated from Egyptian plants

Plant name	Plant part	Oil yield (%), (V/W)	Major compounds (%)
Artemisia judaica	Aerial parts	0.2	β-Thujone (49.83), chrysanthenone (10.88), α-thujone (8.21)
Artemisia monosperma	Leaves	0.8	1,2-Dihydroacenaphthylene (36.86), 2,4-pentadiynylbenzene (14.68), γ-terpinene (12.46)
Callistemon viminals	Leaves	0.5	1,8-Cineole (71.77), α-pinene (11.47), α-terpineol (3.18)
Citrus aurantifolia	Fruit peels	0.75	dl-Limonene (40.19), β-pinene (19.65), α-citral (8.14)
Citrus limon	Fruit peels	0.2	dl-Limonene (56.30), β-pinene (8.81), γ-terpinene (6.42)
Citrus paradisi	Fruit peels	0.12	dl-Limonene (74.29), *l*-linalool (4.61), linalool oxide (4.18)
Citrus sinensis	Fruit peels	0.7	dl-Limonene (89.23), linalool (2.98)
Cupressus macrocarpa	Leaves	0.45	4-Terpineol (20.29), sabinene (18.67), β-citronellol (13.01)
Cupressus sempervirens	Leaves	0.14	α-Pinene (37.88), δ-Carene (20.05), α-terpinolene (6.91)
Myrtus communis	Leaves	0.2	α-Pinene (26.16), 1,8-cineole (16.45), *l*-linalool (11.23)
Origanum vulgare	Aerial parts	0.5	d-Pulegone (77.45), Menthone (4.86), *cis*-isopulegone (2.22)
Pelargonium graveolens	Leaves	0.09	β-Citronellol (35.92), *trans*-geraniol (11.66), citronellyl formate (11.40)
Rosmarinus officinalis	Leaves	0.33	1,8-Cineole (19.60), Camphor (17.01), α-pinene (15.12)
Syzygium cumini	Leaves	0.08	α-Pinene (17.26), (+)-α-terpineol (13.88), β-pinene (11.28)
Schinus molle	Leaves	0.88	α-Phellandrene (29.87), β-phellandrene (21.08), elemol (13.00)
Schinus terebinthifolius	Leaves	0.25	Sabinene (14.93), γ-elemene (13.18), β-elemene (6.63)
Thuja occidentalis	Leaves	0.25	α-Pinene (35.49), β-carene (25.42), α-cedrol (9.05)
Vitex agnus-castus	Leaves	0.16	*trans*-Caryophyllene (15.19), 1,8-cineole (13.04), bicyclogermacrene (7.30)

camphor and linalool oxide), monoterpene hydrocarbons (i.e., dl-limonene, sabinene, γ-terpinene, β-pinene, β-carene, phellandrene and α-pinene), sesquiterpene hydrocarbons (i.e., bicyclogermacrene, α-elemene, β-elemene and *trans*-caryophyllene), and oxygenated sesquiterpenes (i.e., cedrol and elemol).

The chemical compositions of the isolated essential oils from *C. aurantifolia*, *C. paradise*, *C. limon*, *C. sinensis*, *C. viminals*, *C. sempervirens*, *S. molle*, *C. macrocarpa*, *P. graveolens*, *R. officinals* and *M. communis* are in accordance with those previously reported [7–13]. On the other hand, the major constituents of the essential oils isolated from *A. monosperma*, *O. vulgare T. occidentalis* and *A. judaica* were completely different with those previously reported on the chemistry of these oils [14–17]. Some of the major constituents of the essential oils of *V. aguns*, *S. terebinthifolius* and *S. cumini* were similar to those previously reported for the oils isolated from plants growing in Egypt and other countries around the world [18–20]. However, the percentages of constituents are differed. The differences in essential oil compositions could be due to several factors, such as geographical location, season, environmental conditions, nutritional status of the plants and other factors [21].

In vitro antifungal activity of essential oils

The inhibitory effects of 18 essential oils isolated from Egyptian plants were evaluated against two wood decay fungi, *G. lucidum* and *H. apiaria*. The essential oils revealed various mycelial growth inhibitions against the tested fungi depending on fungal species and tested oil. The essential oil of *A. monosperma* exhibited the highest inhibitory effect against *H. apiaria* with EC_{50} value of 31 mg L^{-1}, while the oil of *V. aguns* was the less effective one (Table 2). The essential oils of *A. monosperma*, *T. occidentalis*, *C. sempervirens*, *S. molle* and *C. limon* showed promising antifungal activity against *H. apiaria* as their EC_{50} values were less than 100 mg L^{-1}. On the other hand, the oils of *C. paradisi*, *A. judaica*, *C. sinensis*, *S. cumini* and *P. graveolens* revealed pronounced antifungal activity against this fungus as EC_{50} values were less than 200 mg L^{-1}. The oil of *A. monosperma* caused the highest inhibitory effect against *G. lucidum* with EC_{50} value of 53 mg L^{-1}, followed by the oils *P. graveolens*, *S. molle*, *C. limon* and *C. sempervirens*, while the oil of *M. communis* had the weakest antifungal effect (Table 3). In general, all the tested oils showed higher inhibitory effect against *H. apiaria* than *G. lucidum*.

Table 2 In vitro antifungal activity of essential oils against *Hexagonia apiaria* using mycelial growth inhibition method

Oil	EC_{50}[a] (mg L^{-1})	95 % confidence limits (mg L^{-1})		Slope[b] ± SE	Intercept[c] ± SE	(χ^2)[d]
		Lower	Upper			
Artemisia judaica	154	102	200	1.29 ± 0.26	−2.83 ± 0.63	0.01
Artemisia monosperma	31	20	47	2.70 ± 0.20	−4.05 ± 0.32	28.34
Callistemon viminals	446	377	546	1.81 ± 0.22	−4.79 ± 0.57	2.57
Citrus aurantifolia	374	293	498	1.20 ± 0.20	−3.09 ± 0.51	3.34
Citrus limon	98	72	134	2.27 ± 0.14	−4.53 ± 0.28	16.48
Citrus paradisi	169	137	200	2.09 ± 0.28	−4.65 ± 0.66	1.06
Citrus sinensis	176	121	231	1.24 ± 0.26	−2.78 ± 0.62	0.01
Cupressus macrocarpa	251	204	301	1.63 ± 0.21	−3.93 ± 0.52	2.82
Cupressus sempervirens	56	30	95	1.36 ± 0.09	−2.38 ± 0.09	22.95
Myrtus communis	358	273	471	1.08 ± 0.20	−2.75 ± 0.50	2.59
Origanum vulgare	295	108	628	2.73 ± 0.24	−6.75 ± 0.61	8.41
Pelargonium graveolens	113	70	181	2.42 ± 0.15	−4.98 ± 0.32	35.89
Rosmarinus officinalis	265	86	518	3.77 ± 0.30	−9.15 ± 0.74	11.29
Syzygium cumini	157	126	187	2.06 ± 0.28	−4.54 ± 0.66	0.72
Schinus molle	96	63	144	1.74 ± 0.11	−3.45 ± 0.22	19.92
Schinus terebinthifolius	450	326	738	0.89 ± 0.20	−2.37 ± 0.50	2.06
Thuja occidentalis	39	22	61	1.36 ± 0.10	−2.18 ± 0.19	16.89
Vitex agnus-castus	890	598	2069	0.96 ± 0.21	−2.85 ± 0.53	0.95

[a] The concentration causing 50 % mycelial growth inhibition

[b] Slope of the concentration–inhibition regression line ± standard error

[c] Intercept of the regression line ± standard error

[d] Chi-square value

Table 3 In vitro antifungal activity of essential oils against *Ganoderma lucidum* using mycelial growth inhibition method

Oil	EC_{50}^{a} (mg L^{-1})	95 % confidence limits (mg L^{-1})		Slope[b] ± SE	Intercept[c] ± SE	$(\chi^2)^{d}$
		Lower	Upper			
Artemisia judaica	376	307	501	1.79 ± 0.28	−4.61 ± 0.68	1.16
Artemisia monosperma	53	41	68	2.74 ± 0.19	−4.74 ± 0.33	12.29
Callistemon viminals	617	491	863	1.49 ± 0.23	−4.16 ± 0.58	0.94
Citrus aurantifolia	388	342	443	2.72 ± 0.25	−6.54 ± 0.64	2.25
Citrus limon	262	133	518	2.46 ± 0.20	−5.94 ± 0.51	19.36
Citrus paradisi	494	364	884	1.30 ± 0.27	−3.52 ± 0.56	0.05
Citrus sinensis	417	361	487	2.18 ± 0.21	−5.73 ± 0.54	3.42
Cupressus macrocarpa	376	332	427	2.59 ± 0.25	−6.68 ± 0.64	3.76
Cupressus sempervirens	267	220	319	1.68 ± 0.28	−3.38 ± 0.63	3.66
Myrtus communis	>1000	–	–	–	–	–
Origanum vulgare	441	345	652	1.59 ± 0.28	−4.20 ± 0.68	0.91
Pelargonium graveolens	123	70	219	1.98 ± 0.12	−4.14 ± 0.26	40.23
Rosmarinus officinalis	517	433	652	1.78 ± 0.23	−4.83 ± 0.60	0.33
Syzygium cumini	355	257	520	0.92 ± 0.20	−2.34 ± 0.50	0.24
Schinus molle	252	204	304	1.59 ± 0.21	−3.82 ± 0.52	1.71
Schinus terebinthifolius	754	643	954	2.69 ± 0.37	−7.76 ± 1.00	2.35
Thuja occidentalis	318	135	555	2.67 ± 0.21	−6.68 ± 0.54	22.37
Vitex agnus-castus	530	423	729	1.38 ± 0.22	−3.78 ± 0.55	1.08

[a] The concentration causing 50 % mycelial growth inhibition

[b] Slope of the concentration–inhibition regression line ± standard error

[c] Intercept of the regression line ± standard error

[d] Chi-square value

To the best of our knowledge, there were no reported studies investigating the effectiveness of the tested essential oils against wood decay fungi, *H. apiaria* and *G. lucidum*. However, the antifungal activity of some of these oils against human pathogenic, food spoilage and plant pathogenic fungi was described. For example, the essential oils of *C. limon*, *C. paradise*, *C. aurantium*, *M. communis* and *C. sinensis* possessed antifungal activity against yeasts and food spoilage fungi [22, 23] and plant pathogenic fungi [24, 25]. In addition, the oil of *O. vulgare* showed effectiveness to inhibit the growth of food spoiling yeasts *Candida albicans* and *C. krusei* [26], and human pathogens *Malassezia furfur*, *Trichophyton rubrum*, and *Trichosporon beigelii* [25]. The antifungal activity of the oil *S. terebinthifolius* against *Aspergillus flavus, Candida albicans, A. niger*, *Colletotrichum gloeosporioides*, *Penicillium digitatum*, *Trichoderma* sp. and *Helminthosporium oryzae* was described [19, 27]. The oils of *C. sempervirens* and *R. officinalis* possessed antifungal against several fungi and yeasts species [25, 27, 28].

In vivo antifungal activity of essential oils

The results of the in vivo inhibitory effects of the most potent essential oils against wood decay fungi, *H. apiaria* and *G. lucidum* are presented in Table 4. Six essential oils were evaluated as wood preservatives against the tested fungi in the presence of wood blocks of Scots pine sapwood. The results are shown as wood mass loss that caused by fungi after 6 weeks of the essential oils treatment at the three tested concentrations (EC_{50}, two- and fourfold of the EC_{50}) compared with the control (Table 4). In general, the reduction of wood mass loss was concentration dependent and all the tested oils significantly decreased the wood mass loss compared with the control. The oil of *C. limon* revealed the highest reduction of wood loss caused by *H.apiaria* (1.90, 1.11 and 0.99 % at concentrations of EC_{50}, two- and fourfold of the EC_{50} compared with 7.87 % loss in the control). However, the oil of *A. monosperma* displayed the weakest reduction with wood loss percentages of 4.06, 3.68 and 1.97 % at the concentration of EC_{50}, two- and fourfold of the EC_{50}, respectively.

In the case of *G. lucidum* fungus, the oil of *A. monosperma* caused the highest reduction in wood mass loss at concentration of the EC_{50} value, while the oil of *P. graveolens* caused the lowest reduction. The oil of *C. sempervirens* was the most effective in the reduction of wood loss at concentration of fourfold of the EC_{50} (2.35 % compared with 8.94 % loss in the control). It can

Table 4 Mass losses of pine blocks treated with different essential oils at EC_{50}, two- and fourfold of the EC_{50} after 6 weeks exposure to *Hexagonia apiaria* and *Ganoderma lucidum*

Oil	Conc. (mg L^{-1})	Loss (% ± SE)	
		H. apiaria	*G. lucidum*
Control	0	$7.87^{a} \pm 0.04$	$8.94^{a} \pm 1.04$
A. monosperma	EC_{50}	$4.06^{b} \pm 0.48$	$3.64^{defg} \pm 0.40$
	$2 \times EC_{50}$	$3.68^{b} \pm 0.23$	$3.24^{efg} \pm 0.31$
	$4 \times EC_{50}$	$1.97^{cdef} \pm 0.45$	$2.98^{fg} \pm 0.11$
C. limon	EC_{50}	$1.90^{cdef} \pm 0.78$	$3.69^{defg} \pm 0.38$
	$2 \times EC_{50}$	$1.11^{ef} \pm 0.43$	$3.45^{defg} \pm 0.24$
	$4 \times EC_{50}$	$0.99^{f} \pm 0.66$	$2.96^{fg} \pm 0.54$
C. sempervirens	EC_{50}	$3.66^{b} \pm 0.20$	$3.78^{defg} \pm 0.30$
	$2 \times EC_{50}$	$2.13^{cdef} \pm 0.57$	$3.49^{defg} \pm 0.42$
	$4 \times EC_{50}$	$2.12^{cdef} \pm 0.40$	$2.35^{g} \pm 0.37$
P. *graveolens*	EC_{50}	$3.16^{bc} \pm 0.35$	$5.68^{b} \pm 0.54$
	$2 \times EC_{50}$	$2.56^{cd} \pm 0.68$	$4.18^{cdef} \pm 0.58$
	$4 \times EC_{50}$	$2.33^{cde} \pm 0.45$	$3.06^{fg} \pm 0.12$
S. molle	EC_{50}	$2.52^{cd} \pm 0.41$	$5.01^{bc} \pm 0.58$
	$2 \times EC_{50}$	$2.29^{cde} \pm 0.03$	$4.70^{bcd} \pm 0.43$
	$4 \times EC_{50}$	$1.77^{def} \pm 0.13$	$4.56^{bcde} \pm 0.43$
T. occidentalis	EC_{50}	$2.31^{cde} \pm 0.16$	$5.61^{b} \pm 0.07$
	$2 \times EC_{50}$	$2.08^{cdef} \pm 0.41$	$3.57^{defg} \pm 0.46$

Results are expressed as mean ± standard error ($n = 9$). Values followed by the same letter within a column are not significantly different ($P \leq 0.05$) according to Student–Newman–Keuls (SNK) test

be notice that the wood mass loss is higher than that found in the case *H. apiaria* fungus. This result is in agreement with the present in vitro results in which the oils showed higher inhibitory effect against *H. apiaria* than *G. lucidum*. It has been reported that some of essential oils had a potential wood protection against wood decay fungi [29]. On the other hand, the inhibitory effect of essential oils against wood decay fungi was described [30–33].

The mechanism of antifungal action of essential oils is not completely understood. However, some authors gave several assumptions according to their observations. A majority of the studies on the mechanism of action of essential oils are accentuated on the interference of oxygenated monoterpenes with certain enzymatic reactions evolved in the cell wall synthesis. Further, the mode of antifungal action of the essential oils could be dependent on two different mechanisms. Some oil components may irreversibly disrupt the cell membrane structure by cross-linking reactions, causing a leakage of electrolytes and subsequent depletion of amino acids and sugars, while others may selectively be inserted into the lipid-rich portion of the cell membrane, thereby disturbing membrane function [34–36].

Conclusion

In summary, this study demonstrated the in vitro and in vivo antifungal activity of essential oils against wood decay fungi and potential use of essential oils, particularly the oils of *C. sempervirens*, *C. limon*, *T. occidentalis*, *S. molle*, *A. monosperma* and *P. graveolens* as preservatives for the control of wood decay caused by *H. apiaria* and *G. lucidum*. However, for the use of essential oils as alternatives of synthetic wood preservatives, further studies are required to evaluate toxicity and the effectiveness of treatment for long term on wood.

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References

1. Barnes HM, Murphy RJ (1995) Wood preservation and the new age. For Prod J 45:16–26
2. Sen-Sung C, Huang-Yuan L, Shang-Tzen C (2005) Chemical composition and antifungal activity of essential oils from different tissues of Japanese Cedar (*Cryptomeria japonica*). J Agric Food Chem 53:614–619
3. Kartal SN, Hwang W, Imamura Y, Sekine Y (2006) Effect of essential oil compounds and plant extracts on decay and termite resistance of wood. Holz als Roh-und Werkstoff 64:455–461
4. Finney DJ (1971) Probit analysis, 3rd edn. Cambridge University Press, London, p 318
5. Mohareb H, Thevenon MF, Wozniak E, Gerardin P (2011) Effects of polyvinyl alcohol on leachability and efficacy of boron wood preservatives against fungal decay and termite attack. Wood Sci Technol 45:407–417
6. Lek-Uthai U, Rattanapreechachai P, Chowanadisai L (2011) Bioassay and effective concentration of Temephos against *Aedes aegypti* larvae and the adverse effect upon indigenous predators: *Toxorhynchites splendens* and *Micronecta* sp. Asia J Public Health 2:67–77
7. Chanegriha N, Baaliouamer A, Meklati B-Y, Chretien JR, Keravis G (1997) GC and GC/MS leaf oil analysis of four Algerian cypress species. J Essent Oil Res 9:555–559
8. Malizia RA, Cardell DA, Molli JS, González S, Guerra PE, Grau RJ (2000) Volatile constituents of leaf oils from the Cupressaceae family: part I. *Cupressus macrocarpa* Hartw., *C. arizonica* Greene and *C. torulosa* Don species growing in Argentina. J Essent Oil Res 12:59–63
9. Lota M-L, De Rocca Serra D, Jacquemon C, Tomi F, Casanova J (2001) Chemical variability of peel and leaf essential oils of sour orange. Biochem Syst Ecol 16:89–96
10. Srivastava SK, Ahmad A, Syamsunder KV, Aggarwal KK, Khanuja SPS (2003) Essential oil composition of *Callistemon viminalis* leaves from India. Flavour Fragr J 18:361–363
11. Tuberoso CIG, Barra A, Angioni A, Sarritzu E, Pirisi FM (2006) Chemical composition of volatiles in Sardinian Myrtle (*Myrtus communis* L.) alcoholic extracts and essential oils. J Agric Food Chem 54:1420–1426
12. Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Álvarez JA (2009) Chemical composition of mandarin (*C. reticulata* L.), grapefruit (*C. paradisi* L.), lemon (*C. limon* L.) and

orange (*C. sinensis* L.) essential oils. J Essent Oil Bear Plants 12:236–243
13. Bendaoud H, Romdhane M, Souchard JP, Cazaux S, Bouajila J (2010) Chemical composition and anticancer and antioxidant activities of *Schinus molle* L. and *Schin*us *terebinthifolius* Raddi aeries essential oils. J Food Sci 75:466–472
14. Şahin F, Güllüce M, Daferera D, Sökmen A, Sökmen M, Polissiou M, Agar G, Özer H (2004) Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. *vulgare* in the Eastern Anatolia region of Turkey. Food Cont 15:549–557
15. Mohamed MIE, Abdelgaleil SAM (2008) Chemical composition and insecticidal potential of the essential oils from Egyptian plants against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). J Appl Entomol Zool 43:599–607
16. Tsiri D, Graikou K, Pobłocka-Olech L, Krauze-Baranowska M, Spyropoulos C, Chinou I (2009) Chemosystematic value of the essential oil composition of *Thuja* species cultivated in Poland-antimicrobial activity. Molecules 14:4707–4715
17. Khan M, Mousa AA, Syamasundar KV, Alkhathlan HZ (2012) Determination of chemical constituents of leaf and stem essential oils of *Artemisia monosperma* from central Saudi Arabia. Nat Prod Commun 7:1079–1082
18. Singab AB (2003) Essential oils and lipids content of *Pituranthos* species growing in Egypt. Bull Fac Pharm Cairo Univ 41:213–217
19. Gundidza M, Gweru N, Magwa ML, Mmbengwa V, Samie A (2009) The chemical composition and biological activities of essential oil from the fresh leaves of *Schinus terebinthifolius* from Zimbabwe. Afr J Biotechnol 8:7164–7169
20. Stojković D, Soković M, Glamočlija J, Džamić A, Ćirić A, Ristić M, Grubišić M (2011) Chemical composition and antimicrobial activity of *Vitex agnus-castus* L. fruits and leaves essential oils. Food Chem 128:1017–1022
21. Perry NB, Anderson RE, Brennan NJ, Douglas MH, Heaney AJ, Mcgrimpsey JA, Smallfield BM (1999) Essential oil from Dalmation sage (*Salvia officinalis* L.), variations among individuals, plant parts, seasons and sites. J Agric Food Chem 47:2048–2054
22. Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Álvarez J (2008) Antifungal activity of lemon (*Citrus limon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.) and orange (*Citrus sinensis* L.) essential oils. Food Cont 19:1130–1138
23. Mahboubi M, Bidgoli FG (2010) In vitro synergistic efficacy of combination of amphotericin B with *Myrtus communis* essential oil against clinical isolates of *Candida albicans*. Phytomed 17:771–774
24. Wilson CL, Solar JM, El Ghaouth A, Wisniewski ME (1997) Rapid Evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. Plant Dis 81:204–210
25. Sokovic M, Van Griensven LJLD (2006) Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, *Agaricus bisporus*. Eur J Plant Pathol 116:211–224
26. Souza EL, Stamford TLM, Lima EO, Trajano VN (2007) Effectiveness of *Origanum vulgare* L. essential oil to inhibit the growth of food spoiling yeasts. Food Cont 18:409–413
27. Siddiqui RR, Ahma H, Shakoor CS, Ehteshamuddin AFM, Shireen S (1996) Antimicrobial activity of essential oils. Part II. Pak J Sci Ind Res 39:43–47
28. Mazari K, Bendimerad N, Bekhechi C, Fernandez X (2010) Chemical composition and antimicrobial activity of essential oils isolated from Algerian *Juniperus phoenicea* L. and *Cupressus sempervirens* L. J Med Plants Res 4:959–964
29. Yang VW, Clausen CA (2007) Inhibitory effect of essential oils on decay fungi and mold growth on wood. Proc Am Wood Protect Soc Birmingham AL 103:62–70
30. Wang S-Y, Chen P-F, Chang S-T (2005) Antifungal activities of essential oils and their constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) leaves against wood decay fungi. Biores Technol 96:813–818
31. Su Y-C, Ho C-L, Wang EI-C, Chang S-T (2006) Antifungal activities and chemical compositions of essential oils from leaves of four *Eucalypts*. Taiwan J For Sci 21:49–61
32. Zyani M, Mortabit D, El Abed S, Remmal A, Koraichi SI (2011) Antifungal activity of five plant essential oils against wood decay fungi isolated from an old house at the Medina of Fez. Int Res J Microbiol 2:104–108
33. Barkat M, Bouguerra A (2012) Study of the antifungal activity of essential oil extracted from seeds of *Foeniculum vulgare* Mill. for its use as food conservative. Afr J Food Sci 6:239–244
34. Chalchat JC, Garry PR, Menut C, Lamaty G, Malhuret R, Chopineau J (1997) Correlation between chemical composition and antimicrobial activity. VI. Activity of some African essential oils. J Essent Oil Res 9:67–75
35. Inouye S, Tsuruoka T, Watanabe M, Takeo K, Akao M, Nishiyama Y, Yamaguchi H (2000) Inhibitory effect of essential oils on apical growth of *Aspergillus fumigatus* by vapour contact. Mycoses 43:17–23
36. Carmo ES, Lima EDO, De Souza EL (2008) The potential of *Origanum vulgare* L. (Lamiaceae) essential oil in inhibiting the growth of some food-related *Aspergillus* species. Bra J Microbiol 39:362–367