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Research Article

Antimicrobial Efficacy of Three Essential Oils against Decaying Cedar Wood Isolates

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Abstract

Background and Objective: The use of traditional methods as synthetic chemical products to protect cedar wood raises concerns because of the potential negative impact of these products on the public health and environment. The aim of this study was to investigate the chemical composition of three essential oils: *Mentha pulegium*, *Rosmarinus officinalis* and *Cananga odorata* and their antimicrobial effect against six fungi and two bacteria causing degradation of cedar wood. **Materials and Methods:** Determination of the chemical composition of essential oils was conducted using gas chromatography-mass spectrometry (GC-MS) (Trace GC Ultra). The minimal inhibitory concentrations and minimal bactericidal/fungicidal concentrations were also determined using the broth microdilution assays. **Results:** The GC/MS analysis of oils studied showed that menthone and pulegone were the major components of *Mentha pulegium* essential oil, camphene and α -humulene were the major ones in *Rosmarinus officinalis*, whereas linalool and geranyl acetate were the major components for *Cananga odorata*. Results also showed potent antifungal activity against all fungi tested, with MICs ranging from 0.0625-0.25% for *Mentha pulegium*, 0.25-1% for *Rosmarinus officinalis* and from 0.5-1% for *Cananga odorata*. An important antibacterial activity was shown against the two bacteria tested with MICs ranging from 0.125-2%. **Conclusion:** This study suggested that these essential oils may be used as an alternative of traditional methods used for wood protection.

Key words: Essential oils, chemical composition, antibacterial activity, antifungal activity, cedar wood microorganisms

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Wood is a complex biopolymer composed of cellulose, hemicelluloses and lignin. It is used as raw material for paper industries but also as material in built constructions. In Morocco, cedar wood was used for building historical monuments of different imperial cities including the old medina of Fez. However, moisture effect promotes its deterioration with bacteria and fungi and caps its outdoor applications¹. The growth of various wood degrading biological organisms is frequently associated with aesthetical degradation^{2,3}, biodeterioration and reduction of wood durability^{4,5} and thus lead to a health risk by fungal mycotoxins⁶. Different techniques were used previously to improve the lifetime of cedar wood and boost its resistance to decay including the use of biological, physical and chemical means⁷. These traditional methods as the use of toxic chemicals provide certainly a decay resistance but have environmental effects. Recently, environmental concerns required the use of non-biocidal solutions for wood protection. Thus, the search to develop solutions with good environmental profile and antimicrobial assets is undeniably the priority for the researchers of material sciences. Among natural resources that can be used to prevent wood biodeterioration, essential oils and medicinal and aromatic plant extracts known by their important antimicrobial activity for one hand and for the other hand for their wealth in eco-friendly natural molecules.

Several studies have reported the strong antimicrobial activity of essential oils and their components against a broad range of pathogenic microorganisms⁸⁻¹⁰. However, few studies reported the antibacterial and antifungal activities against bacterial and fungal strains causing degradation of cedar wood¹¹⁻¹³. Thus, the purpose of this research was to investigate firstly the chemical composition of three essential oils (*Mentha pulegium*, *Rosmarinus officinalis* and *Cananga odorata*) and secondly, to examine their antimicrobial activity against bacteria and fungi associated with deterioration of historical wood.

MATERIALS AND METHODS

The present study was conducted during the year 2016-2017. The essential oil extraction, the composition of essential oils and the antimicrobial activities have taken six months.

Plant material and extraction of essential oils: The leaves of *Mentha pulegium* and *Rosmarinus officinalis* were harvested in the region of Sefrou located in the Middle Atlas (Morocco)

and the flowers of *Cananga odorata* (generally known as Ylang-Ylang) were harvested from the Comoros Islands. The plants were shade dried at room temperature with ventilation then subjected to hydrodistillation with Clevenger-type apparatus. The obtained oils were dried with anhydrous sodium sulfate and stored in a refrigerator at 4°C.

Essential oils analysis: Chemical composition of tested oils was determined using gas chromatography-mass spectrometry (GC-MS) (Trace GC Ultra). The analysis were carried out using a Trace GC Ultra gas chromatograph equipped with anapolar capillary TR-5 column (60 m × 0.32 mm ID, 0.25 µm film thickness) coupled to a mass detector (MS Quadrupole). Helium was used as carrier gas at a (1.2 mL min⁻¹). Oven temperature was held at 300°C and the set conditions were: initial temperature of 40°C/2 min, then increasing (5°C min⁻¹) until 280°C and held for 10 min; a 10:1 split ratio mode and an injector temperature of 220°C. The ionization energy was 70 eV. Retention Indices (RI) were calculated using a homologous series of n-alkanes C₈-C₁₈ (Sigma-Aldrich, St Louis, MO). Compounds were identified by comparing their RI and mass spectra with those of main components from literature and the NIST (National Institute of Standards and Technology) Library.

Microbial cultures: The following microbial strains were selected for their ability to deteriorate cedar wood: Two Gram-positive bacteria of the genus *Bacillus* (*B. safensis* and *B. subtilis*) and six fungi (*Penicillium commune* (PDLd¹¹), *Penicillium expansum*, *Penicillium crustosum*, *Penicillium commune* (PDLd10), *Thielavia hyalocarpa* and *Aspergillus niger*). The strains were isolated from cedar wood decayed from an old house in the old medina of Fez and identified in our laboratory^{12,14,15}. They were stored at -18°C in 20% glycerol and subcultured as follows: Bacteria were subcultured in Luria-Bertani (LB) agar and incubated at 37°C for 24 h and fungi in Malt-Extract (EM) agar medium at 25°C for 10 days. After the incubation period, bacterial inoculum was prepared in physiologic saline solution and adjusted to a final concentration of 2.10⁶ Colony-Forming Unit mL⁻¹ (CFU mL⁻¹) for each bacterium under study. Likewise, the fungal spores were collected by scraping the culture surface using sterile physiologic saline solution with 1% Dimethylsulfoxide (DMSO). The spore suspensions were concentrated by centrifugation at 7000 rpm for 15 min at 4°C to a final concentration of 2.10⁴ spores mL⁻¹ ¹⁶.

Antimicrobial activity tests: The Minimum Inhibitory Concentration (MIC) corresponds to the lowest concentration of antibacterial agent that inhibits the bacterial growth¹⁷. It

was determined using the broth microdilution assay^{18,19} with some modifications. The 96-well plate was prepared by dispensing into each well (from the second to the 12th well) 50 μ L of Mueller Hinton Broth (MHB) for antibacterial assay and Malt Extract Broth (MEB) for antifungal activity supplemented with bacteriological agar (0.15% w/v). Essential oils were dissolved in the suitable culture medium with 0.15% of agar according to the test carried out. A serial dilution of these latter was realized until the 11th well in such a way that the final concentration was between 8-0.00781% (v/v) for *Mentha pulegium* and *Rosmarinus officinalis* and between 4-0.0081% (v/v) for *Cananga odorata*. The 12th well was considered as growth control. At the end, 50 μ L of bacterial/spores suspension previously prepared were added to each well. Plates were incubated at 37°C for 20 h for antibacterial tests and for 48 h at 25°C for antifungal tests. A further incubation for 2 h was realized after addition of 5 μ L of resazurin to determine the MIC of essential oils against bacteria tested¹⁸. Bacterial growth is revealed by reduction of blue dye resazurin to pink resorufin. The minimum bactericidal/fungicidal concentration (MBC/MFC) is defined as being the lowest concentration of the essential oil giving negative subcultures after incubation for 24 h at 37°C for bacterial strains and at 28°C for 72 h for fungi. It was determined by spot inoculating 5 μ L from negative wells on LB/EM plates.

RESULTS AND DISCUSSION

Essential oils composition: Total 100% of the volatiles compounds of *Mentha pulegium* essential oil were identified as shown in Table 1.94.43% being oxygenated monoterpenes, principally represented by menthone and pulegone representing 60.56 and 15.77%, respectively of the total essential oil. About 1.53% of the constituents were represented by monoterpene hydrocarbons with a single compound which is the α -pinene. Sesquiterpenes hydrocarbons were represented by β -caryophyllene with a percentage of 4.04%. The chemical composition of *Mentha pulegium* essential oil was the subject of many investigations in different countries like Portugal with menthone as major component²⁰, Morocco²¹⁻²⁴, Tunisia²⁵, Algeria²⁶, India²⁷, Spain²⁸ and Iran²⁹. The major constituent was pulegone in all these studies. However, the quantities were different from one to another study because of the geographical source collection and seasons growing conditions³⁰.

For *Rosmarinus officinalis* essential oil, the identified volatiles were 99.55% and major constituents were

represented by sesquiterpene hydrocarbons (39.06%) principally α -humulene which represents 25% followed by monoterpene hydrocarbons (28.11%), the main volatile constituent being camphene presents 21.22%. Oxygenated monoterpenes were 23.87% and oxygenated sesquiterpenes only 3%. Similar statements were reported in other studies. However, the amount achieved is greater than those found³¹⁻³³. The chemical composition of the essential oil varies according to the plants origin. Derwich *et al.*³⁴ have shown α -pinene (18.25%) as the major component of *Rosmarinus officinalis* essential oil collected from the region of Atlas median (Tafersoust), Morocco. The 1,8-cineol was the major constituent of *Rosmarinus officinalis* essential oil collected from Crete (Greece) with a percentage of 88.9%³⁵. Pintore *et al.*³⁶ reported the presence of α -pinene, verbenone and bornyl acetate of rosemary oils from Sardinia and Corsica and Mata *et al.*³⁷ reported the presence of verbenone (35.4%) of rosemary oil from Portugal.

The oxygenated fraction of *Cananga odorata* essential oil represents 67.71% of the total identified volatiles, principally constituted by 1,8-cineol, linalool, geranyl acetate, cinnamyl acetate, isoeugenol, methyleugenol, caryophyllene oxide, γ -cadinol, δ -cadinol, δ -dodecalactone, benzyl benzoate and benzyl salicylate. Geranyl acetate and linalool were shown to be main components present in oxygenated fraction with a percentage of 14.51 and 13.81%, respectively that are responsible for the floral smell of Ylang-Ylang. The hydrocarbon fraction of Ylang-Ylang oil represented 30.77% of the volatiles identified and consisted mainly of sesquiterpenes and monoterpenes. Current results were comparable with those found by Stashenko *et al.*³⁸ for Colombian Ylang-Ylang flower sessential oil with an appearance of linalool in higher concentration, which is more than twice our reported value from Comoros Ylang-Ylang essential oil. Stashenko *et al.*^{38,39} have shown also the presence of 1,8-cineole, geranyl acetate, cinnamyl acetate, α -pinene, α -ylangene, β -caryophyllene and other compounds with different concentrations. These variations could be explained by the extraction method, extraction time, the origin and the flower maturity. Other studies have shown the chemical composition of leaves of *Cananga odorata* grown in Australia (β -caryophyllene 34.2%, sabinene 19.6%, α -humulene 6.8%, α -pinene 6%) and Cameroon (β -caryophyllene 26.3%, α -pinene 14%, germacrene D 11.7%)^{40,41}.

Antibacterial activity: The MICs obtained in the antimicrobial test are shown in Table 2. The data showed that the three essential oils possess a strong antimicrobial activity against bacterial strains studied since MIC values were between 2 and

Table 1: Chemical composition of *Mentha pulegium*, *Rosmarinus officinalis* and *Cananga odorata* essential oils

N°	Compounds	RI	<i>M. pulegium</i> (%)	<i>R. officinalis</i> (%)	<i>C. odorata</i> (%)
1	α-pinene	930	1.53	4.77	9.48
2	Camphene	938	-	21.22	-
3	1,8-cineol	1015	-	4.97	5.64
4	γ-terpinene	1035	-	2.12	-
5	Fenchol	1065	-	0.91	-
6	Linalool	1084	-	-	13.81
7	Camphor	1095	-	0.72	-
8	Borneol	1134	-	8.42	-
9	A-terpinenol	1159	-	1.76	-
10	Menthone	1163	60.56	-	-
11	Bornylacetate	1180	-	1.62	-
12	α-terpineol	1189	2.09	-	-
13	Pulegone	1241	15.77	-	-
14	Piperitone	1247	4.88	-	-
15	2-Ethyl-4,5-Dimethylphenol	1305	-	3.18	-
16	Menthylacetate	1323	4.4	-	-
17	Eugenol	1327	-	1.42	-
18	Piperitenone	1347	2.62	-	-
19	Nerylacetate	1359	0.7	-	-
20	Geranylacetate	1379	3.41	-	14.51
21	α-Ylangene	1382	-	-	0.59
22	β-Caryophyllene	1414	4.04	8.12	9.83
23	Cinnamylacetate	1418	-	-	3.09
24	Isoeugenol	1429	-	-	7.07
25	Geranylacetone	1453	-	5.47	-
26	α-Humulene	1455	-	25	9.16
27	Methyleugenol	1472	-	-	2.76
28	γ-Murolene	1475	-	3.41	-
29	γ-Cadinene	1512	-	2.53	0.7
30	δ-Cadinene	1515	-	-	1.01
32	Caryophyllene oxide	1583	-	1.76	4.04
33	Humulene 1,2-epoxide	1611	-	1.24	-
34	γ-Cadinol	1627	-	-	0.82
35	δ-Cadinol	1639	-	-	7.8
36	Methyljasmonate	1642	-	0.91	-
37	δ-Dodecalactone	1706	-	-	2.72
38	Benzyl benzoate	1724	-	-	4.29
39	Benzyl salicylate	1828	-	-	1.16
	Hydrocarbon monoterpenes		1.53	28.11	9.48
	Oxygenated monoterpenes		94.43	23.87	33.96
	Hydrocarbon sesquiterpenes		4.04	39.06	21.29
	Oxygenated sesquiterpenes		-	3	12.66
	Others		-	5.51	21.09
	Total identified compounds		100	99.55	98.48

RI: Retention index

Table 2: Determination of MIC values of *Mentha pulegium*, *Rosmarinus officinalis* and *Cananga odorata* essential oils against bacterial strains tested

Concentration (% v/v)	<i>B. safensis</i>			<i>B. subtilis</i>		
	MPE	ROE	COE	MPE	ROE	COE
8	-	-	*	-	-	*
4	-	-	-	-	-	-
2	-	-	-	-	-	-
1	-	+	+	-	-	-
0.5	-	+	+	-	+	-
0.25	-	+	+	-	+	-
0.125	-	+	+	+	+	+
0.0625	+	+	+	+	+	+
0.03125	+	+	+	+	+	+
0.01562	+	+	+	+	+	+
0.00781	+	+	+	+	+	+

MPE: *Mentha pulegium* essential oil, ROE: *Rosmarinus officinalis* essential oil, COE: *Cananga odorata* essential oil, +: Presence of growth, -: Absence of growth, *Not done; positive control: Bacterial suspensions and Mueller-Hinton Broth supplemented with agar (0.15% w/v)

0.125%. The antibacterial effect of *Mentha pulegium* essential oil is eight times higher than that obtained for the essential oil of *Rosmarinus officinalis* and six times higher than that obtained for *Cananga odorata* essential oil with a MIC values lie between 0.125 and 0.25%, thus presenting an interesting potential as antimicrobial substance in cedar wood preservation. *Mentha pulegium* essential oil exhibits a bacteriostatic effect against *B. subtilis* with a MFC value of 8% and a ratio MBC/MIC value of 32 higher than four⁴².

As expected, bacterial strains tested were found susceptible to the three essential oils. These bacteria (Gram-positive) contain a layer of lipids in their cell wall, which help the passage of essential oils. Indeed, *B. safensis* showed a similar susceptibility for *Rosmarinus officinalis* and *Cananga odorata* essential oils. The Minimum Bactericidal Concentration (MBC) against *B. safensis* and *B. subtilis* was higher than 8% for *Rosmarinus officinalis* and *Cananga odorata* essential oils.

The essential oils evaluated in this study displayed a variable degree of antibacterial activity against the two bacterial strains tested. Regarding this concern, a number of studies on *M. pulegium* essential oil have already been published^{20,22-26,43}. According to these reports, the latter has shown a high bacteriostatic and bactericidal activity against pathogenic microorganisms: MIC < 0.5 $\mu\text{L mL}^{-1}$ and MBC = 0.5 $\mu\text{L mL}^{-1}$ for *Enterococcus faecium*²², Cherrat *et al.*²³ have also reported it to have a strong antimicrobial activity against *B. subtilis* with value of MIC = 1 $\mu\text{L mL}^{-1}$ and MBC = 8 $\mu\text{L mL}^{-1}$. Hajlaoui *et al.*²⁵ have also concluded that *M. pulegium* essential oil has a broader spectrum of antimicrobial activity (MIC = 0.62 $\mu\text{L mL}^{-1}$ and MBC > 0.5 $\mu\text{L mL}^{-1}$ for *Bacillus cereus*, MIC = 2.5 $\mu\text{L mL}^{-1}$ and MBC = 0.312 $\mu\text{L mL}^{-1}$ for *Enterococcus faecalis*).

Pintore *et al.*³¹ and Zaouali *et al.*³⁶ have revealed a low to moderate antimicrobial activity for *Rosmarinus officinalis* essential oil against the range of bacteria tested with values of MIC lie between 2.4 and 4 mg mL^{-1} and between 1.25-10 $\mu\text{L mL}^{-1}$, respectively. For *Cananga odorata* essential oil, few studies have been conducted on its antibacterial activity. However, a good antimicrobial activity has been reported against *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*^{41,44}.

The antibacterial activity is attributed not only to the major components of essential oil but also to the minor components that may have a synergistic effect. Gill *et al.*⁴⁵ and Mourey and Canillac⁴⁶ have shown that the whole essential

oils have better antibacterial activity than the major components mixed. Interestingly, it can be seen from Table 1 that oxygenated monoterpenes present a high percentage for *M. pulegium* essential oil (94.43%) therefore an important antimicrobial activity compared to *R. officinalis* and *C. odorata* essential oil (23.87 and 33.96%, respectively). In addition, several studies have shown that the essential oils possessing the strongest antibacterial properties were rich in phenolic compounds^{47,48}. So, the presence of phenolic compounds especially hydroxyl groups position played an important role in antimicrobial activity⁴⁹.

Antifungal activity: The three essential oils studied show an antifungal activity as reflected by the MIC values obtained. The MICs determined for all fungal strains tested in this study oscillated between 0.0625 and 1% (v/v) (Table 3). Strong antifungal activity was recorded for *Mentha pulegium* essential oil with a MIC values lie between 0.0625 and 0.25% (v/v), followed by those of *Rosmarinus officinalis* and *Cananga odorata* essential oil. *Mentha pulegium* essential oil has a fungicidal activity against all fungal strains studied with a MFC values ranged from 2 and 4% (Table 4). Thus, a similar susceptibility was shown for *P. commune* (PDLd^o), *P. commune* (PDLd10) and *A. niger* with a MIC value of 0.125% (v/v). *Rosmarinus officinalis* and *Cananga odorata* essential oil presented the same effect against *P. commune* (PDLd^o) (MIC of 1% (v/v)), *P. expansum* and *P. crustosum* (MIC of 0.5% (v/v)). Similar studies have shown the antifungal activity of some essential oils including that of Zyani *et al.*⁵⁰ who reported the important activity of *Origanum compactum*, *Eugenia caryophyllata* and *Ocimum basilicum* essential oils against *Thielavia hyalocarpa*, *Penicillium commune*, *Penicillium chrysogenum*, *Penicillium expansum* and *Cladosporium cladosporioides*. Soidrou *et al.*¹¹ have found that Comorian essential oils isolated from *Piper capense*, *Piper borbonense* and *Vetiveria zizanioides* have a strong fungicidal activity against fungi decay wood (*Gloeophyllum trabeum*, *Poria placenta*, *Coniophora puteana* and *Coriolor versicolor*).

Several authors have attributed the antifungal activity of essential oils to their major phenolic components⁹. Hassan *et al.*⁵¹ have shown the important antifungal activity of the carvacrol against *P. expansum* in a lower concentration (MIC = 0.625% (v/v)). The antifungal activity of the same component against *A. niger*, *A. flavus*, *P. citrinum* and *P. chrysogenum* was studied (MIC = 50, 100, 150 and 125 $\mu\text{g mL}^{-1}$, respectively)⁵². Menthone, borneol and other

Table 3: Determination of MIC values of *Mentha pulegium*, *Rosmarinus officinalis* and *Cananga odorata* essential oils against fungal strains tested

Concentration (%, v/v)	<i>P. commune</i> (PDLd ⁿ)			<i>P. expansum</i>			<i>P. crustosum</i>			<i>P. commune</i> (PDLd10)			<i>T. hyalocarpa</i>			<i>A. niger</i>		
	MP	RO	CO	MP	RO	CO	MP	RO	CO	MP	RO	CO	MP	RO	CO	MP	RO	CO
8	-	-	*	-	-	*	-	-	*	-	-	*	-	-	*	-	-	*
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	-	+	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+
0.25	-	+	+	-	+	-	-	+	+	-	-	+	-	-	+	-	+	+
0.125	-	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+
0.0625	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
0.03125	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.01562	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.00781	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

MP: *Mentha pulegium* essential oil, RO: *Rosmarinus officinalis* essential oil, CO: *Cananga odorata* essential oil, +: Presence of growth, -: Absence of growth, *Not done, positive control: Bacterial suspensions and Malt Extract Broth supplemented with agar (0.15% w/v)

Table 4: Determination of MFC values of *Mentha pulegium*, *Rosmarinus officinalis* and *Cananga odorata* essential oils against fungal strains tested

Fungal strains	<i>P. commune</i> (PDLd ⁿ)			<i>P. expansum</i>			<i>P. crustosum</i>			<i>P. commune</i> (PDLd10)			<i>T. hyalocarpa</i>			<i>A. niger</i>		
	MP	RO	CO	MP	RO	CO	MP	RO	CO	MP	RO	CO	MP	RO	CO	MP	RO	CO
MFC	4	>8	≥8	2	>8	≥8	2	>8	2	2	>8	≥8	2	>8	4	2	>8	≥8

MP: *Mentha pulegium* essential oil, RO: *Rosmarinus officinalis* essential oil, CO: *Cananga odorata* essential oil

components have exhibited a good antifungal activity against *Botrytis cinerea*⁵³ and linalool exhibited an effect on *S. cepivorum* and *F. oxysporum*⁵⁴.

The essential oils studied could be used as a non-biocidal solution to be applied on cedar wood surface to ensure better protection against fungi and bacteria causing its degradation and thus a conservation of our cultural heritage while respecting the environment.

CONCLUSION

Mentha pulegium essential oil showed a strong antibacterial activity followed by *Cananga odorata* and *Rosmarinus officinalis*. It presented also an important antifungal activity against all fungal strains studied which was five times higher than that obtained for *Cananga odorata* essential oil and three times higher than that obtained for the essential oil of *Rosmarinus officinalis*. These results supported the use of these essential oils as natural product to prevent wood biodeterioration, thus providing an alternative to the use of synthetic chemical products.

SIGNIFICANCE STATEMENTS

This study discovers the antimicrobial effect of *Mentha pulegium*, *Rosmarinus officinalis* and *Cananga odorata* essential oils that can be beneficial for the protection of cedar

wood against microorganisms causing its degradation and thus the preservation of our cultural heritage. This study will help the researcher to uncover the critical areas of essential oils efficacy against decaying cedar wood microorganisms that many researchers were not able to explore. Thus, a new theory on the antibacterial, antifungal and preservative effects of these biological molecules may be arrived at.

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