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Essential oils from *Origanum compactum* as an alternative active ingredient against wood decay fungi

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ABSTRACT

In this study, we evaluated the antifungal activity of essential oils from two provenances of *O. compactum*, from two Moroccan forests, against wood-decay fungi responsible for most of the damage to the timber service. Essential oils of the aerial parts of *O. compactum* were extracted by hydrodistillation, analyzed by gas chromatography-mass spectrometry (GC-MS) and tested against four fungi (*Trametes versicolor*, *Coniophora puteana*, *Gloeophyllum trabeum* and *Poria placenta*). Our results showed that thymol (20.49%, 27.49%), carvacrol (16.68%, 25.31%), γ-terpinene (26.11%, 22.65%) and o-cimene (20.68%, 9.76 %) were the main constituents present in essential oils of both *O. compactum* provenances. Bioactivity experiment revealed that the two essential oils tested had a strong antifungal activity against all of the fungi studied, which is very likely due to the high content of thymol and carvacrol. This finding suggests the use of these essential oils as an environmentally friendly preservative against white- and brown-rot fungi.

KEYWORDS

Origanum compactum;
Essential oils;
Chemical composition;
Wood-decay;
Fungi.

INTRODUCTION

Wood products are extensively used for indoor and outdoor applications because of their diversity, aesthetic appearance, low density, low thermal expansion and good mechanical strength. However, due to its biological origin, wood is susceptible to degradation caused by many xylophageous organisms, especially fungi. Thus, when timber is in service these degradations can result in tremendous economic and resource losses[1].

As a matter of fact, the white- and brown-rot Basidiomycete fungi cause most of the damage to the timber in service. Brown-rot fungi depolymerize and metabolize wood polysaccharides, leaving behind a brown lignin residue; i.e., wood polysaccharides are extensively degraded while lignin is modified or slightly depolymerized[2]. Brown-rot is a common and destructive type of decay in wooden structures in the northern hemisphere. Conversely, white-rot fungi cause the simultaneous degradation of lignin, cellulose and hemicelluloses[3,4]. For instance, T*rametes versicolor*, one of the most common wood-degrading basidiomycetes, produces di-

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verse extracellular enzymes, among which laccase, peroxidases and cellobiose dehydrogenase are the most important in the lignocellulolytic process[5].

To extend their service life of the timber that are not naturally durable enough, wood products are treated with preservatives. A major problem of these wood preservatives such as creosote, pentachlorophenol and inorganic arsenicals, e.g. chromium, copper and arsenic, is that they pose a serious threat to the environment and human health[1,6-8]. Indeed, while arsenic is strictly banned in the USA and Europe since 2004, creosote and pentachlorophenol are restricted to used only for specific uses(such as poles and historic monuments respectively)[9-11]. Since some years now, there has been a huge effort from the researcher and industrial communities to set up alternatives to these banned or restricted wood preservatives due to the public concern about their effect on health and theirimpact on the environment[11-14]. Because of this situation, environmentally friendly organic alternative for wood protection are urgently needed[1].

On the other hand, natural compounds present in essential oils extracted from various woods, as well as aromatic and medicinal plants have been proven to have antifungal properties. Examples of such compounds are cinnamaldehyde[15,16], α-cadinol[17], carvacrol[18], thymol[19], cryptomeridiol[20] and tropolones[21]. The use of these compounds and other natural antifungal ones can be an alternative to classical wood preservative active ingredients. Actually, essential oils from aromatic and medicinal plants and their constituents have a long history of application as antimicrobial agents. Applications are found in the areas of food preservation, as well as medicinal antimicrobial agents and disinfectants manufacturing. As an example, it has been demonstrated that eugenol, thymol and carvacrol, well-known phenolic representatives found in clove, thyme and oregano essential oils, have an inhibitory activity against both bacteria[22,23] and fungi[24-28]. The phenolic toxicity mechanism of such compounds towards fungi is based on the inhibition of fungal enzymes containing thiol groups (–SH) in their active sites[29,30].

Among the aromatic and medicinal plants, the Lamiaceae family is of a great ecological and economic interest, since it is a global source of spices and essential oils rich in constituents with high antimicrobial and antioxidant properties. In Morocco, this family is represented by more than 30 genera and 225 species (about 90 of which are endemics)[31]. In particular, of the five species of the genus Origanum, *Origanum compactum* has a very widespread use. It is highly appreciated in local cuisine, and in folk medicine, as it exhibits particular properties in relieving respiratory and digestive disorders[31-33].

The aim of this study was to determine the chemical composition of essential oils from *O. compactum* collected from two Northeast Moroccan forests and to evaluate their antifungal activity against four wood-decay Basidiomycete fungi in order to assess the potential use of these natural products as wood preservative active ingredients.

RESULTS AND DISCUSSION

Yields and chemical constituents of essential oils

Based on the statistical analysis using the student's t-test, the two provenances of the *O. compactum* from Tanaqoub and Talambot forests provided the same yield of essential oils (the p-value equaled 0.081 using an α of 0.05). Their calculated yields, expressed as mean ± standard deviation, were 2.25±0.05 % and 2.4±0.1 % respectively (see TABLE 1) and allow to conclude that there was no effect of the provenance on the yield of essential oils. In addition, our results showed that *O. compactum* productivity of essential oils was high compared with other aromatic and medicinal plants: e.g. 0.47 ± 0.02 % for *Cladanthus mixtus*[34], 1.2 ± 0.05 % for *Thymus ciliates*[35] and 0.3 ± 0.07 % for *Thymus algeriensis*[35], which is very beneficial from many points of view.

The analysis of *O. compactum* essential oils by GC-MS revealed 28 constituents for the Tanaqoub provenance and 22 constituents for the Talambot one, representing 98.72% and 99.02% of their total essential oils respectively (see TABLE 1). The monoterpens constitute the predominant class of constituents in both Tanaqoub and Talambot *O. compactum* essential oils (98.13% and 97.11% re-

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TABLE 1 : Chemical composition of essential oils of two provenances of *origanum compactum*

KI	Constituents	Area %	
		A	B
931	α-thujene	1,05	1,32
939	α-pinene	0,44	0,57
948	Camphene	0,10	0,14
973	Sabinene	0,14	0,15
980	β-pinene	0,12	0,15
983	Cis-pinene	0,63	0,93
987	2-octanone	0,58	0,86
991	Myrcene	2,12	2,21
999	δ-2-carene	-	0,09
1005	α-Phellandrene	0,26	0,28
1011	δ-3-carene	-	0,08
1018	α-terpinene	2,44	2,79
1023	o-cymene	9,76	20,68
1026	p-cymene	0,52	0,59
1050	β-E-ocimene	-	0,09
1062	γ-terpinene	22,65	26,11
1067	Cis-hydrate sabinene	0,35	0,33
1080	m-cymenene	-	0,14
1087	Terpinolene	-	0,13
1098	Linalool	0,83	1,40
1165	Borneol	0,27	0,30
1177	terpin-4-ol	0,47	0,54
1184	ρ -cymen-8-ol	-	0,09
1189	α-terpineol	1,56	0,99
1290	Thymol	27,49	20,49
1298	Carvacrol	25,31	16,68
1418	E-caryophyllene	2,48	1,36
1513	γ–cadinene	0,35	-
1590	Longiborneol	-	0,13
	Total	99,02	98,72
	Yield[a] (%)	2.40 ± 0.1	2.25 ± 0.05

KI: *Kováts indices*; A: *O. compactum* fromTalambot; B: *O. compactum* from Tanaqoub; -: *none;* ±: standard deviation. [a] mean +/- standard deviation, Calculated on a dry weight basis.

spectively). Essential oils of Tanaqoub *O. compactum* contained γ-terpinene (26.11%), o-cymene (20.68 %), thymol (20.49 %) and carvacrol (16.68%) as main constituents whereas the major ones in Talambot *O. compactum* oil were thymol (27.49%), carvacrol (25.31%), γ-terpinene (22.65%) and o-cymene (9.76%). The chemical composition of the two *O. compactum* essential oils analyzed seemed qualitatively very similar, but they differed as to the quantitative content of the major constituents. In particular, the carvacrol and thymol contents of Tanaqoub *O. compactum* (16.68% and 20.49% respectively) was lower than those of Talambot (25.31% and 27.49% respectively); conversely, its o-cymene *content* (20.68 %) was obviously higher than that of Talambot (9.76%). Less significant differences between the minor components from the two essential oils were also found.

In a previous work, Van Den Broucke and Lemli[36] (1980), who analyzed essential oils from

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various Moroccan provenances of *O. compactum*, evidenced that the content of constituents such as thymol, carvacrol and p-cymene varied within very wide ranges: 0-43.4%, 3.8-71% and 0-25.4% respectively. This is obviously corroborated by the very differences observed among the essential oils compositions reported by the research community. In this sense, Bouhdid et al.[37] reported that, for commercial essential oils of Moroccan *O. compactum*, carvacrol (30.53%), thymol (27.50%) and γ-terpinene (18.20%) were the major constituents. This composition is certainly relatively similar to that of our essential oils but differ significantly from that reported by Bouchra et al.[38]: carvacrol (58.1%), thymol (9.0%) and γ-terpinene (7.1%). These results show that the genus *Origanum* is very diverse both morphologically and chemically and that many transitional forms occur worldwide; phenomenon that was also observed for *Artemisia herba-alba*[39].

Antifungal activity of essential oils

The results concerning the antifungal activities of essential oils, obtained by a screening test with the agar dilution method, are presented in TABLE 2. The recorded minimum inhibition concentration (MIC) showed that the *Coniophora puteana*, *Poria placenta* and *Gloeophyllum trabeum* fungi are more sensitive than *Trametes versicolor* to both *O. compactum* oils. Their growth was completely inhibited at a MIC of 0.05% for the two essential oils tested while *Trametes versicolor* resisted up to a MIC of 0.1%.

It appears that the two essential oils of *O. compactum* have strong antifungal activity against all the wood-decay fungi tested. This is very likely due to the high contents of constituents such as thymol (20.49%, 27.49%) and carvacrol (16.68%, 25.31%). The correlation between the intensity of the antifungal activity and the content of these two constituents was already reported by El Ajjouri et al.[40] in a study on the antifungal activity of essential oils of *Thymus capitatus* (carvacrol 70.92%) and *Thymus bleicherianus* (thymol 23.95%) against the same fungi. The strong activity of thymol and carvacrol phenols against *T. versicolor, C. puteana, P. placenta* and *G. trabeum* was also confirmed by Karmen et al.[19] and Panek et al.[28].

These phenolic compounds are among the most efficient plant antimicrobials agents known to date[41]. They have a very large spectrum of antimicrobial activity and are present in the essential oils of most species of oregano and thyme[25,42-43]. The synergetic effect between these two phenols was also observed in several studies[42,44]. However, it should be noticed that major constituents of the essential oils are not necessarily the only ones responsible for the total activity; the involvement of less abundant constituents should also be considered[4].

On the other hand, *T. versicolor* showed a higher resistance compared to the 3 brown rots to both provenances of the essential oils studied. This can be attributed to the fact that *T. versicolor* produces extracellular laccase that catalyzes the oxidation of the added phenolic compounds, resulting in its inactivation. Other ligninolytic enzymes excreted by white-rot fungi such as *T. versicolor*, including manganese peroxidases (MnP) and lignin peroxidases (LiP), have been demonstrated to oxidize both phenolic and non-phenolic aromatic compounds, even though the binding sites for these reducing substrates have not been confirmed for any of these enzymes so far[46,47].

TABLE 2 : Antifungal activity of essential oils from two provenances of *Origanum compactum*

	Concentrations															
	1 %		0.4 %		0.2 %		0.1 %		0.05 %		0.033 %		0.02 %		T	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
TV	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
GT	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
PP	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
CP	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+

A: *O. compactum* fromTalambot; B: *O. compactum* fromTanaqoub; TV : *Trametes versicolor* ; C P : *Coniophora puteana* ; G T : *Gloeophyllum trabeum* ; P P : *Poria placenta* ; T : *control* ; - *inhibition* ; + : *growth*.

EXPERIMENTAL

Plant materials

Fresh aerial parts (stems, leaves and flowers) of *O. compactum* were collected from Tanaqoub and Talambot forests (Northeast of Morocco) in March 2012. The plants were taxonomically identified at the Forestry Research Center in Rabat (Laboratory of Botany).

Essential oil isolation

Plant material samples (200 g) of the two *O. compactum* provenances were subjected to hydrodistillation using a Clevenger-type apparatus for two hours[48]. Three replicates were carried out for each *O. compactum* provenance. The yields (w/w) of essential oils were determined based on the dry weight of the plant materials (see TABLE 1). Essential oils were dried over anhydrous sodium sulfate and stored under refrigeration (4°C).

Analysis of essential oils

Gas chromatography (GC) analysis was performed using a Hewlett Packard Gas Chromatographer (HP 6890) with electronic pressure control, equipped with a HP-5MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm), a FID detector set at 250 °C and fed with a H_2/Air mixture, and a split splitless injector set at 250 °C. The injection mode was split (1:50) and the injected volume was 1 µl. Nitrogen was used as carrier gas with a flow rate of 1.7 ml.min^{-1}. The column temperature was programmed from 50 to 200 °C at a heating rate of 4 °C.min^{-1}. The apparatus was controlled by a "Chemstation" computer system.

Gas chromatography/mass spectrometry (GC/MS) analysis was performed using a Hewlett-Packard Gas Chromatographer (HP 6890) coupled with a mass spectrometer (HP 5973). Fragmentation was performed by electron impact at 70 ev. The column used was HP-5MS (30 m x 0.25 mm, film thickness 0.25 µm). The injection mode was split (1:50). The column temperature was programmed from 50 to 200 °C at a heating rate of 4°C.min^{-1}. The components of the essential oils were identified based on Kováts retention indices and mass spectral database (NIST 98 library).

Fungal strains

The four wood decay fungi used in this study are the most important wood-destroying fungi, and are foundabove and in-ground contact(buildings,bridges, poles and railway sleepers). They were chosen for the considerable damage they cause to wood and timber products. The following Basidiomycetes were used: *Gloeophyllum trabeum* (Persoon ex Fries) Murril, *Poria placenta* (Fries) Cooke sensu J. Eriksson, *Coniophora puteana* (Schumacher ex Fries) Karsten as brown rots, and *Trametes versicolor* (L.) Quélet as white rot.

Determination of antifungal activity

Minimum inhibitory concentrations (MIC) of essential oils were determined according to the method reported by Remmal & al.[49] and Satrani & al.[50]. Due to the immiscibility of essential oils in water and hence in the culture medium, emulsification was obtained by using a solution of 0.2% (v/v) agar to promote contact between the mycelium and the compound. Dilutions were prepared at 1/10th, 1/25th, 1/50th, 1/100th, 1/200th, 1/300th and 1/500th in the agar solution. Each test tube contained 9 ml of agar medium in 2% malt extract. The samples were autoclaved for 20 min at 121 °C and cooled to 45 °C. Aliquots (1 ml) of each dilution were then added to obtain final concentrations of 1/100, 1/250, 1/500, 1/1.000, 1/2.000, 1/3.000, 1/5.000 (v/v), and the tubes were stirred well before the solution was poured into Petri dishes. Negative controls containing only the culture medium and the 0.2% agar solution were also prepared.

Inoculation of the wood-decay fungi was performed by depositing fragments (1 cm in diameter) taken from the periphery of a mycelium cultured for 7 days in PDA. Samples were incubated in the dark for 24 h at 25 °C. Each test was repeated three times. Minimum inhibition concentration (MIC) was determined as the lowest concentration of oil able to inhibit visible growth of each microorganism on the agar plate.

CONCLUSIONS

In this work, the antifungal activity *of e*ssential

oils obtained from two provenances of *O. compactum*, collected from Talambot and Tanaqoub forests in Northeast of Morocco, was assessed. The two provenances studied gave approximately the same yield of the extracted essential oils, which were constituted essentially by thymol (20.49%, 27.49%), carvacrol (16.68%, 25.31%), γ-terpinene (26.11%, 22.65%) and o-cimene (20.68%, 9.76 %).

Both of the two provenances showed significant antifungal activity against *Trametes versicolor*, *Coniophora puteana*, *Poria placenta* and *Gloeophyllum trabeum*. The minimum inhibitory concentration was of 0.1% for the fibrous/white rot and 0.05% for the cubic/brow rots. From this result, it seems that there is a positive correlation between the antifungal activity of the essential oils studied and their content of thymol and carvacrol, well-known for their anti-fungalactivity.

From a practical point of view, essential oils from *O. compactum* plants do have a wide spectrum of applications as antimicrobial agents. Based on the results above, these essential oils could be suggested as alternative fungicides for wood preservation. The development of such natural wood preservatives would help to decrease the negative impact of synthetic agents for they may be effective, selective, biodegradable and more environmentally acceptable.

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