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Simultaneous Hydrodistillation of *Cedrus atlantica* Manetti and *Salvia rosmarinus* Spenn: Optimization of Anti-Wood-Decay Fungal Activity Using Mixture Design Methodology

Published as part of the ACS Omega virtual special issue "Phytochemistry".

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Cite This: *ACS Omega* 2023, 8, 27030−27043

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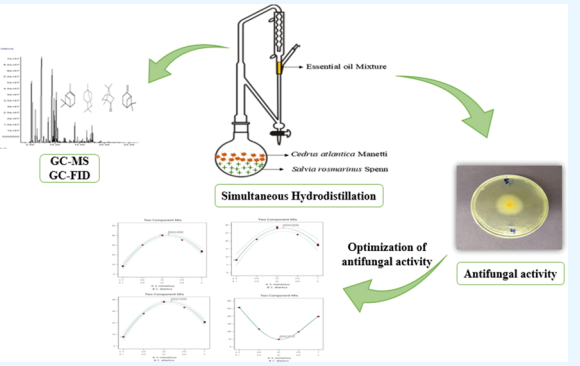
ABSTRACT: Chemical fungicides are often harmful to people and the environment because of their toxicity. The wood protection industry places a high priority on replacing them with natural products. Therefore, this investigation focused on developing a formulation of a binary combination of *Salvia rosmarinus* Spenn and *Cedrus atlantica* Manetti obtained by Simultaneous hydrodistillation to protect the wood from decay using a mixture design methodology. The chemical composition of essential oil was identified by gas chromatography coupled with mass spectrometry (GC/MS), and their anti-wood-decay fungal activity was assessed using the macrodilution method against four fungi responsible for wood decay: *Coniophora puteana*, *Coriolus versicolor*, *Gloeophyllum trabeum*, and *Poria placenta*. The results of GC/MS identified myrtenal as a new component appearing in all binary combinations. The optimum anti-wood-decay fungal activity was observed in a combination of 60% *S. rosmarinus* and 40% *C. atlantica* essential oils, providing an effective concentration for 50% of maximal effect (EC_{50}) value of 9.91 ± 1.91 and 9.28 ± 1.55 μg/mL for *C. puteana* and *C. versicolor*, respectively. The highest anti-wood-decay fungal activity for *G. trabeum* and *P. placenta* was found in the combination of 55% of *S. rosmarinus* and 45% of *C. atlantica* essential oils, with EC_{50} values of 11.48 ± 3.73 and 22.619 ± 3.79 μg/mL, respectively. Combined simultaneous hydrodistillation improved the antifungal effect of these essential oils. These results could be used to improve antifungal activity and protect wood against wood-decay fungi.



1. INTRODUCTION

Although wood is an organic material, it is often damaged by microorganisms, including fungi, especially in hot, humid areas. Fungi decay significantly impacts the construction industry because of the considerable damage caused to wood in use and storage.[1] Preserving wood to prevent decay and other biodegradation processes is essential during storage, transportation, and use. The two types of wood-decay fungi are white-rot fungi and brown-rot fungi.[2,3] White-rot fungi attack lignin, cellulose, and hemicellulose, while brown-rot fungi degrade cellulose and the hemicellulose of cell walls.

Sometimes, wood-decay fungi demethylate, ionize, or weakly depolymerize lignin.[4] Most wood products are treated with chemical fungicides, such as benzimidazoles, copper acid chromate, copper chromate arsenate, and copper zinc arsenate, to preserve their functionality and extend their lives.[5,6] However, chemical fungicides are often harmful to people and the environment because of their toxicity.[7] Therefore, this research focused on developing effective, nontoxic, lower-cost, easy-to-use wood preservatives to protect wood from decay.[8] Several scientists have suggested the utilization of aromatic and medicinal plants as agents to prevent wood-decay fungi.[9−12] Using volatile formulations derived from aromatic and medicinal plants could offer many advantages over current synthetic products. Indeed, essential oils are only mildly toxic to the environment and can exhibit higher biocidal activity.[13]

Wood extracts constitute an important class of secondary metabolites.[14] Several wood species seem indebted to contain chemical compounds.[15,16] These compounds make trees resistant to insect attacks.[17] One of these trees is the Atlas cedar, one of the most economically and ecologically important

Received: March 23, 2023
Accepted: June 23, 2023
Published: July 12, 2023

species in the mountains of Morocco. *Cedrus atlantica* Manetti (*C. atlantica*), known as the Atlas cedar, belongs to the Pinaceae family and is considered the oldest tree after Pinusgenus.[18] Cedarwood essential oil is antiseptic, anti-inflammatory,[19] antifungal, purifying, and relaxing.[20] Cedarwood essential oils are helpful in perfumery and cosmetics[21] because of the himachalenes they contain, such as β-hemachalene, α-hemachalene, and γ-hemachalene.[22] It is also used in perfumery and cosmetology. Moreover, it is prescribed for dermatitis and skin inflammation.

The Mediterranean region is characterized by *Salvia rosmarinus* Spenn (*S. rosmarinus*), commonly known as rosemary, which belongs to the Lamiaceae family. One of the most widely used medicinal plants worldwide, rosemary, is Morocco's most important wild species.[23] The essential oil *S. rosmarinus* is widely used for treating various diseases due to its pharmacological properties.[24] Several studies have shown that the essential oil *S. rosmarinus* presents antioxidant,[25] antimicrobial,[26] antifungal,[27] anti-inflammatory,[28] insecticidal,[29] and antiparasitic[30] activities. It can act against fungal phytopathogens[31] due to its richness in monoterpene such as 1,8-cineole, camphor, and α-pinene.[32]

The antifungal activity of the studied essential oils (EOs) against wood-decay fungi has been previously reported for *S. rosmarinus*[33] and *C. atlantica*[34] Yet no study has examined the synergistic effects of binary combinations in achieving effective antifungal activity at sufficiently low concentrations. This combination was chosen to facilitate sesquiterpene extraction using monoterpenes as a green solvent, thus reducing the extraction duration of sesquiterpenes by improving biological activity. This study aimed to develop a formulation of the two essential oils obtained by a simultaneous hydrodistillation of *S. rosmarinus* and *C. atlantica* to optimize the anti-wood-decay fungal activity against four wood-decay fungi using the methodology of experimental design.

2. MATERIALS AND METHODS

2.1. Plant Material. The aerial parts of *S. rosmarinus* were harvested from the Talsint area (eastern Morocco) (latitude 32°32′18.29″N, longitude 3°26′32.143″W). *C. atlantica* sawdust was collected from a cedarwood sawmill in Azrou (the Middle Atlas Mountains in Morocco) (latitude 33°26′9.226″N, longitude 5°13′21.104″W). All plants were collected during the same period—in April—because it is the best time to exploit rosemary.[35] The herb should be dried in a shady place at an ambient temperature.

2.2. Essential Oils Extraction. The aerial parts of *S. rosmarinus*, the wood sawdust of *C. atlantica*, and the binary mixtures of the two plants were distilled by hydrodistillation and simultaneous hydrodistillation, using a Clevenger-type apparatus, as performed by El Kharraf et al.[36] El Kharraf et al.,[37] and El Kharraf et al.[38] The two plants were placed in two layers, with *S. rosmarinus* at the bottom of the distillation flask and the wood sawdust of *C. atlantica* at the top (Figure 1). The ratio of the plants in the mixture varied according to the experience matrix of the mixture design (Table 1). A total of 200 g of dried plants was put in a distillation flask containing 1 L of distilled water, and the mixture was heated for 4 h to boil, except for *S. rosmarinus* and *C. atlantica* alone, which were heated for 3 and 8 h to boil, respectively. The essential oils were collected, dried with anhydrous sodium sulfate, and stored at 4 °C in an opaque green flask until used. The experiments were performed in triplicate.

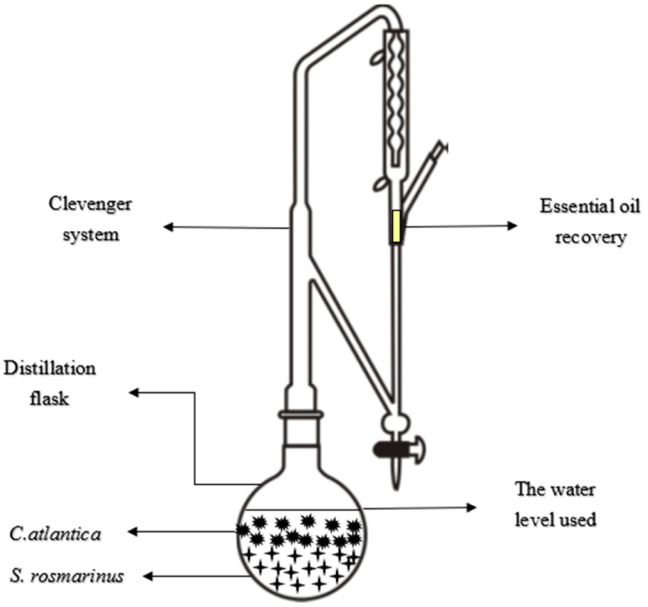


Figure 1. Combined simultaneous hydrodistillation of *S. rosmarinus* and *C. atlantica*.

Table 1. Experience Matrix of Simplex-Lattice Design for Two Components

exp	*S. rosmarinus* (g)	*C. atlantica* (g)
1	200	0
2	150	50
3	100	100
4	100	100
5	100	100
6	50	150
7	0	200

2.3. Chromatographic Analysis. The essential oils were chemically analyzed employing gas chromatography coupled to mass spectrometry (GC/MS) and flame ionization detection (GC-FID). GC-FID analysis was performed for component quantification, whereas GC/MS analysis was carried out for identification.

All of the samples were analyzed with gas chromatography using a capillary column equipped with HP-5 (30 m × 0.25 mm, film thickness 0.25 μm), an FID detector, and an injector set at 275 °C. After 5 min, the oven temperature was increased from 50 to 250 °C at a rate of 4 °C/min. Nitrogen (1.8 mL/min) was used as the carrier gas. Samples were diluted 1/50 in methanol and injected in a 1 μL volume, utilizing a split mode at a 1/50 ratio and a flow rate of 72.1 mL/min. The component proportions of the EOs were given as percentages evaluated applying peak area normalization. The retention indices (RIs) on the HP-5 MS column were calculated employing homologous series (C8−C28) alkanes.

The gas chromatography/mass spectrometry (GC/MS) analysis was conducted using a Hewlett-Packard gas chromatographer (HP 6890) and a mass spectrometer (HP and stationary syringe 5973). The employed column was HP-5MS (30 m × 0.25 mm, 0.25 μm film thickness). The column temperature was set at 50 °C and increased to 250 °C at a rate of 2 °C/min. The carrier gas was helium (99.995% purity), the flow rate was 1.5 mL/min, the split ratio was 1/74.7, and the flow rate was 112 mL/min. The MS identities of the

https://doi.org/10.1021/acsomega.3c01970
ACS Omega 2023, 8, 27030−27043

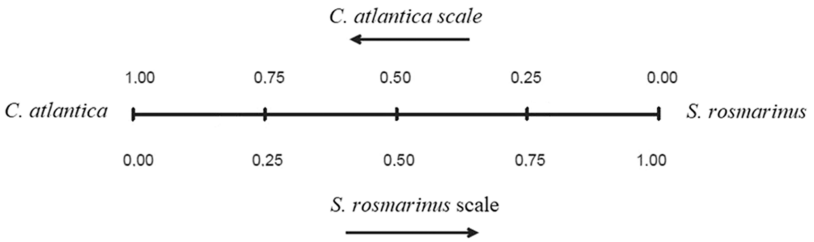


Figure 2. Segment of *S. rosmarinus* and *C. atlantica* binary mixture design.

components were approved employing the NIST 98 spectral library. The ionization voltage was 70 eV, the ion source temperature was 230 °C, and the scan mass range was 35−450m/z. Component identification was verified by checking the elution order of the compounds with the relative retention indices reported in the literature. All of the experiments were conducted in triplicate.

2.4. Anti-Wood-Decay Fungal Activity. The anti-wood-decay fungal activities of the essential oils were evaluated against four wood-decay fungi using the methods of Remmal et al.[39] and Satrani et al.,[40] with slight modification. The wood-decay fungal strains were one white-rot fungi (*Coriolus versicolor* [Linnaeus] Quélet, [ATCC 12679]) and tree brown-rot fungi (*Gloeophyllum trabeum* [Persoon ex Fries] Murril [ATCC 11539]), (*Coniophora puteana* [Schumacher ex Fries] Karsten [ATCC 9351]), and (*Poria placenta* [Fries] Cooke sensu J. Eriksson [ATCC 9891]). These fungi were taken from the Culture Collections of the Mycotheque of Microbiology and Mycology Laboratory at the Forest Research Center in Rabat, Morocco.

EOs diluted serially in a sterile agar solution at 0.2% 20 mL of solid medium malt extract were kept in sterilized Petri dishes to obtain final concentrations of 7.81, 15.63, 31.25, 62.5, 125, 250, 500, and 1000 μg/L (w/v). A negative control was prepared by substituting the EOs with an agar solution of 0.2%. One fragment of 1 cm^3 diameter was put in the center of the Petri dishes. Each Petri dish was closed with parafilm and incubated at 25 °C for seven days. Anti-wood-decay fungal assays were carried out in triplicate. The anti-wood-decay fungal activity was expressed as EC_{50} (the effective concentration for 50% of maximal effect). EC_{50} values (concentrations that are 50% inhibitory of mycelium growth) were calculated by the following measurement of the inhibition (percentage) of mycelial growth

$$\text{mycelial growth inhibition}(\%) = \frac{\text{DC} - \text{DT}}{\text{DC}} \times 100 \quad (1)$$

where DC and DT are the average diameters (mm) of mycelial growth zone in the control and the test, respectively, the positive control was Nystatin. The EC_{50} values were calculated for each investigated EO using probit analysis.[41]

2.5. Experimental Mixture Design. The mixture design was performed to optimize the anti-wood-decay fungal activity (EC_{50}) against four wood-decay fungi with minimal experiments. This investigation was tested to determine an optimal formulation using a combination of two plants: *S. rosmarinus* and *C. atlantica*.

A simplex-lattice design of experiments (DoE) was performed to investigate the formulation of two components, *S. rosmarinus* and *C. atlantica*, for the EC_{50} of four wood-decay fungi, *Coniophora puteana*, *Coriolus versicolor*, *Gloeophyllum trabeum*, and *Poria placenta*, to minimize the response values. The experimental design comprised five experiments (Table 1), which included two pure components in the vertices of the segment (experiments 1 and 7), the central point 0.5:0.5 (experiment 3), and two axial points 0.75:0.25 and 0.25:0.75 (experiments 2 and 6) (Figure 2). The sum of the components of the mixture was 100%.

$$\sum_{i=1}^{n} x_i = 100\% \quad (2)$$

The following Scheffe quadratic model was applied to the mixture design in eq 3

$$Y = a_1X_1 + a_2X_2 + a_{12}X_1X_2 + \varepsilon \quad (3)$$

where Y is the measured response variable (the EC_{50} of four wood-decay fungi); a_1, a_2, and a_{12} represent the linear terms and the interaction term coefficients; X_i represents the proportion of components; and ε represents the error related with the experiments.

An analysis of variance (ANOVA) was conducted to test the fitted models, and the F-value was carried out to determine whether the models were statistically significant. The determination coefficients (R^2 and *adjusted* R^2) were performed to ensure the quality of the adopted models and their predictions.[42,43]

Finally, the significance of the regression coefficients in each component was determined using the Student t-test.[44] All of the tests were conducted at a 95% confidence level.

DESIGN EXPERT software version 13 (Stat-Ease, Minneapolis, Minnesota) was used in the DoE to treat all experimental design treatments.

2.6. Statistical Analysis. Experiments were carried out through an analysis of variance based on Tukey's test of $p < 0.05$ to determine the significance of the means of the essential oil yield of the mixture design using Origin 2021 software (OriginLab Corporation, Northampton, Massachusetts).

3. RESULTS AND DISCUSSION

3.1. Essential Oils Yield. The results for the essential oil yields were calculated to evaluate the effect of hydrodistillation and simultaneous hydrodistillation (Figure 3). The yield of the essential oil varied from 2.25 to 3.20%. The highest yield (3.20%) was found in *C. atlantica* essential oil obtained by hydrodistillation. The binary mixture of *S. rosmarinus*: *C. atlantica* (0.25:0.75) presented with a value of 2.96%, followed by *S. rosmarinus*: *C. atlantica* (0.5:0.5) mixture (2.54%), and the *S. rosmarinus* and *C. atlantica* mixture (0.75:0.25) (2.41%), were obtained by simultaneous hydrodistillation. The lowest yield (2.25%) was observed in *S. rosmarinus* essential oil obtained by hydrodistillation. The plant ratio, in combination with simultaneous hydrodistillation, significantly affects EO

https://doi.org/10.1021/acsomega.3c01970
ACS Omega 2023, 8, 27030−27043

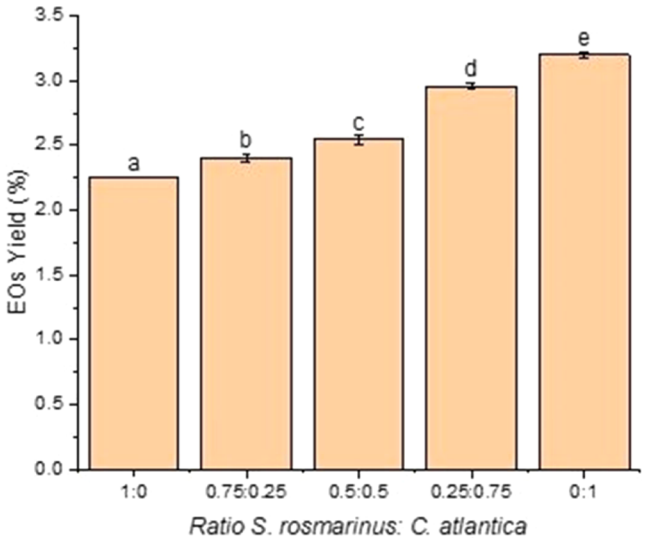


Figure 3. Average yield of the essential oils as a function of the plant's ratio. Different letters (a−e) listed in each column illustrate a significant difference ($p < 0.05$) according to the Tukey test.

yields. The results showed that the yield increases with an increase in the proportion of *C. atlantica*, which explains why *C. atlantica* contains more essential oil than *S. rosmarinus*. Our results differ from those observed by Kharraf et al.,[37] who found that the EO yields of the plant combinations were significantly higher than those of individual plants.

3.2. Chemical Composition of Essential Oil. The analyses of the essential oils of *S. rosmarinus*, *C. atlantica*, and their binary combination by gas chromatography coupled to mass spectroscopy allowed for the identification of 66 compounds representing 99.34−100% of total oils. Table 2 shows the retention indices, percentage composition, and identification methods.

As Table 2 indicates, the chemical analysis of *S. rosmarinus* EOs identified 26 components (100%) that were main volatile constituents, namely, 1,8-cineole (50.86%), camphor (18.81%), α-pinene (9.36%), α-terpineol (4.40%), β-pinene (1.48%), borneol (3.90%), camphene (3.04%), p-cymene (2.21%), linalool (1.42%), and terpinen-4-ol (1.03%). The oxygenated monoterpenes represented the dominant class in *S. rosmarinus* EO (81.64 ± 3.02%), followed by hydrocarbon monoterpene (17.63 ± 1.41%), whereas sesquiterpenes were detected in much lower amounts. This composition is similar to that found by Annemer et al.[35] and Sabbahi et al.,[45] who also harvested in the Province of Figuig, the Oriental region of Morocco. The *S. rosmarinus* Eos from the Province of Ouezzan, in the north of Morocco, was composed of 1,8-cineole (23.70%), camphor (18.70%), borneol (15.50%), and α-pinene (14.10%).[46] The rosemary from Loukkos had the following composition: camphor (21.30%), 1,8-cineole (17.0%), α-pinene (9.20%), β-pinene (8.60%), camphene (7.40%), terpinen-4-ol (2.80%), borneol (4.80%), and p-cymene (2.40%). However, the major components of *S. rosmarinus* EO from the Middle Atlas Mountains of Morocco were 1,8-cineole (46.2%), camphor (17.3%), borneol (6.80%), α-terpineol (5.30%), α-pinene (5.60%), camphene (2.60%), and terpinen-4-ol (2.2%).[47] This Moroccan EO predominantly comprised 1,8-cineole and camphor.

Thirty-nine components were identified in *C. atlantica* EO with a mean percentage of 100%. It was dominated by oxygenated sesquiterpenes (67.2 ± 2.05%) and sesquiterpene hydrocarbons (31.6 ± 0.89%). However, the quantities of oxygenated monoterpenes and monoterpenes hydrocarbons nearly ceased to exist. The *C. atlantica* EO consisted of β-himachalene (11.46%), (E)-α-atlantone (10.87%), deodarnone (10.74%), Himachalol (10.52%), α-himachalene (7.55%), E(E)-γ-atlantone (6.96%), (Z)-α-atlantone (5.56%), and γ-himachalene (3.66%). Chemical analysis showed that the chemical composition of *C. atlantica* sawdust essential oil was similar to that indicated by Zrira et al.,[20] who identified the main compounds as α-(E)-atlantone (19.3%), β-himachalene (15.1%), 8-Cedren-13-ol, (13.1%), α-himachalene (5.1%), cedroxyde (4.6%), and deodarone (4.6%). According to Bennouna et al.,[48] γ-himachalane (4.05%), β-himachalane (7.23%), γ-calamenene (7.77%), δ-cadinene (7.34%), isocedranol (13.78%), cedranone (19.35%), cedrol (4.44%), and caryophyllene oxide (8.73%) were the major compounds of Moroccan *C. atlantica* located in Azrou in the Middle Atlas region. In contrast, Fidah et al.[34] found that E-γ-Atlantone (19.73%), E-α-Atlantone (16,86%), isocedranol (11.68%), 9-iso-Thujopsanone (4.45%), Cedranone (4.13%), and Z α-Atlantone (4.02%) were the main components in Moroccan Cedarwood oil. Then, Ainane et al.[22] determined that α-himachalene (15.63%), β-himachalene (31.24%), and γ-himachalene (14.46%) were the most abundant compounds in *C. atlantica*. The essential oil of *C. atlantica* from the Khemisset region (located in the central plains of Morocco) was found to consist of atlantones (19.73%), 5-isocedranol (11.70%), 9-iso-thujopsanone (4.45%), and cedranone (4.30%).[49]

Forty-three components were identified in a binary combination of 75% of *S. rosmarinus* and 25% of *C. atlantica* (99.53%). The EO contained a significant proportion of oxygenated monoterpenes (72.97 ± 2.28%) and monoterpene hydrocarbons (15.18 ± 1.62%), but low proportions of the oxygenated sesquiterpene (7.23 ± 1.09%) and hydrocarbon sesquiterpenes (4.15 ± 0.98%). The major compounds were 1,8-cineole (44.85%), camphor (16.78%), α-pinene (9.36%), myrtenal (4.04%), and borneol (3.60%).

A total of 44 compounds were identified in a binary combination of 50% of *S. rosmarinus* and 50% of *C. atlantica* (99.34%). The essential oils were featured by a substantial amount of oxygenated monoterpenes (62.07 ± 1.52%) and oxygenated sesquiterpenes (15.32 ± 1.61%). Conversely, low amounts of monoterpenes hydrocarbons (13.78 ± 1.21%) and sesquiterpenes hydrocarbons (8.17 ± 1.74%) were noted. The main constituants were 1,8-cineole (38.51%), camphor (14.09%), α-pinene (7.50%), myrtenal (3.41%), β-himachalene (3.39%), and borneol (3.19%).

The total number of identified compounds determined in a binary combination of 25% of *S. rosmarinus* and 75% of *C. atlantica*, with a total of 99.98%, was 26. The oxygenated monoterpene and oxygenated sesquiterpenes (37.15 ± 2.53% and 35.49 ± 1.19%, respectively) were found at higher levels than the monoterpene hydrocarbons and sesquiterpenes hydrocarbons (19.02 ± 1.56% and 8.32 ± 1.01%, respectively) in the essential oil. The EO was dominated by 1,8-cineole (22.66%), camphor (8.11%), β-himachalene (6.96%), deodarnone (6.52%), (E)-α-atlantone (5.76%), Himachalol (5.08%), α-himachalene (4.71%), α-pinene (4.35%), and E(E)-γ-atlantone (4.13%).

These terpenes were previously found in the individual essential oils while in different amounts, notably 1,8-cineole,

https://doi.org/10.1021/acsomega.3c01970
ACS Omega 2023, 8, 27030−27043

Table 2. Chemical Composition of *S. rosmarinus*, *C. atlantica* Eos, and Their Binary Combination

				% relative peak area				
no.[a]	compounds[b]	RI[c]	RI lit.[d]	*S. rosmarinus* (100%)	*S. rosmarinus*: *C. atlantica* (75−25%)	*S. rosmarinus*: *C. atlantica* (50−50%)	*S. rosmarinus*: *C. atlantica* (25−75%)	*C. atlantica* (100%)
1	α-pinene	936	938	9.36 ± 2.18	8.10 ± 1.10	7.50 ± 1.25	4.35 ± 0.51	
2	camphene	952	952	3.04 ± 1.15	2.68 ± 0.64	2.44 ± 1.79	1.46 ± 0.31	
3	β-pinene	981	980	1.48 ± 1.21	1.34 ± 0.42	1.20 ± 0.32	0.68 ± 0.11	
4	myrcene	991	993	0.87 ± 0.19	0.74 ± 0.09	0.65 ± 0.21	0.37 ± 0.01	
5	δ-3-carene	1007	1011	0.09 ± 0.01				
6	α-terpinene	1019	1018	0.31 ± 0.18	0.14 ± 0.02	0.12 ± 0.04	0.08 ± 0.01	
7	p-cymene	1026	1026	2.21 ± 1.65	1.89 ± 0.61	1.68 ± 0.21	1.01 ± 0.22	
8	4-acetyl-1-methylcyclohexene	1035	1031					0.33 ± 0.04
9	1,8-cineole	1037	1033	50.86 ± 3.5	44.85 ± 2.30	38.51 ± 1.76	22.66 ± 1.54	
10	γ-terpinene	1059	1062	0.08 ± 0.02	0.09 ± 0.01			
11	terpinolene	1088	1089	0.19 ± 0.08	0.2 ± 0.51	0.19 ± 0.65	0.18 ± 0.31	
12	linalool	1095	1098	1.42 ± 0.34	1.23 ± 0.33	1.03 ± 0.46	0.58 ± 0.11	
13	fenchol	1116	1113	0.09 ± 0.06	0.09 ± 0.10			
14	trans rose oxyde	1130	1127				0.16 ± 0.02	0.87 ± 0.02
15	camphor	1149	1144	18.81 ± 2.1	16.78 ± 1.21	14.09 ± 1.45	8.11 ± 0.99	
16	pinocarvone	1164	1163	0.28 ± 0.08	0.22 ± 0.07			
17	borneol	1170	1166	3.90 ± 1.14	3.60 ± 0.86	3.19 ± 1.12	1.79 ± 1.58	
18	terpinen-4-ol	1179	1178	1.03 ± 0.12	0.95 ± 0.74	0.79 ± 0.11	0.43 ± 0.51	
19	α-terpineol	1184	1189	4.40 ± 1.5	0.27 ± 0.02	0.44 ± 0.22	0.76 ± 0.41	
20	myrtenal[e]	1192	1194		4.04 ± 1.01	3.41 ± 0.52	2.02 ± 1.90	
21	dodecane[e]	1204	1200				0.19 ± 0.01	
22	verbenone	1213	1205	0.49 ± 0.09	0.52 ± 0.32	0.38 ± 0.28	0.24 ± 0.10	
23	carvone	1241	1243	0.1 ± 0.01	0.07 ± 0.01	0.08 ± 0.02	0.05 ± 0.01	
24	bornyl acetate	1280	1286	0.08 ± 0.01	0.1 ± 0.03		0.12 ± 0.18	
25	thymol	1286	1290	0.07 ± 0.00	0.09 ± 0.01		0.13 ± 0.09	
26	carvacrol	1297	1293	0.11 ± 0.04	0.16 ± 0.01	0.15 ± 0.02	0.10 ± 0.01	
27	longifolene	1391	1387				0.27 ± 0.14	0.55 ± 0.04
28	tetradecane	1402	1399				0.38 ± 0.08	0.74 ± 0.09
29	himachala-2,4-diene	1409	1424				0.18 ± 0.09	0.24 ± 0.01
30	β-caryophyllene	1431	1419	0.31 ± 0.06	0.24 ± 0.12	0.22 ± 0.10	0.06 ± 0.01	
31	α-himachalene	1446	1447		1.15 ± 1.03	1.84 ± 0.99	4.71 ± 2.69	7.55 ± 1.1
32	thujopsadiene	1463	1460		0.17 ± 0.18	0.25 ± 0.11	0.24 ± 0.21	0.44 ± 0.03
33	8,9-dehydro neoisolongifoléne	1471	1469				0.19 ± 0.02	0.23 ± 0.01
34	γ-himachalene	1477	1476		0.36 ± 0.12	0.75 ± 0.05	2.23 ± 1.15	3.66 ± 1.5
35	γ-curcumene	1483	1480				0.11 ± 0.07	0.36 ± 0.03
36	(E)-β-ionone	1490	1485				0.28 ± 0.03	0.29 ± 0.01
37	β-himachalene	1499	1499		1.63 ± 1.25	3.39 ± 1.41	6.96 ± 2.90	11.46 ± 2.3
38	cuparène	1502	1502				0.08 ± 0.01	0.15 ± 0.10
39	α-deshydro-ar-himachlene	1510	1511		0.09 ± 0.02	0.27 ± 012	0.14 ± 0.09	0.34 ± 0.03
40	δ-cadinene	1520	1524		0.07 ± 0.00	0.1 ± 0.01	0.76 ± 0.12	0.85 ± 0.1
41	γ-dehydro-ar-himachalene	1528	1529		0.16 ± 0.01	0.22 ± 0.02	0.7 ± 0.20	1.14 ± 1.6
42	α-calacorene	1540	1542		0.18 ± 0.07	0.2 ± 0.03	0.38 ± 0.01	1.38 ± 1.2
43	β-calacorene	1561	1563			0.77 ± 0.08	1.45 ± 1.01	1.97 ± 0.5
44	oxydo himachalene	1570	1574				1.03 ± 0.05	1.35 ± 0.07
45	turmoil	1587	1578			0.25 ± 0.09	0.64 ± 0.02	1.38 ± 0.8
46	carotol	1591	1594				0.56 ± 0.04	0.71 ± 0.01
47	caryophyllene oxide	1599	1581	0.06 ± 0.00				
48	cedrol	1603	1605			0.19 ± 0.01	0.55 ± 0.10	0.98 ± 0.08
49	β-himachalene oxyde	1610	1611		0.52 ± 0.12	1.04 ± 0.91	1.21 ± 1.00	2.77 ± 0.7
50	cedranone	1618	1620		0.28 ± 0.01	0.32 ± 0.19	-	2.7 ± 1.2
51	1-epi-cubenol	1628	1628		0.33 ± 0.15	0.67 ± 0.02	1.73 ± 1.10	2.52 ± 2.6
52	3-iso-thujopsanone	1638	1637		0.65 ± 0.10	1.36 ± 0.78	1.63 ± 0.34	2.65 ± 2.1
53	α-cadinol[e]	1655	1653				0.14 ± 0.01	
54	Himachalol	1647	1647		1.06 ± 1.01	1.42 ± 0.59	5.08 ± 1.39	10.52 ± 2.5
55	isocedranol	1661	1661			0.23 ± 0.10	0.28 ± 023	1.00 ± 1.4
56	cadalene	1667	1674		0.1 ± 0.02	0.16 ± 0.01	0.18 ± 0.01	0.54 ± 1.6
57	β-bisabolol	1670	1673	0.23 ± 0.03				
58	acorenone	1682	1685	0.13 ± 0.02	0.17 ± 0.10	0.19 ± 0.09	0.49 ± 0.45	0.69 ± 0.1
59	deodarnone	1694	1694		1.43 ± 0.31	2.50 ± 0.16	6.52 ± 1.10	10.74 ± 3.3
60	E(E)-γ-atlantone	1707	1704		1.00 ± 0.40	2.32 ± 0.17	4.13 ± 1.73	6.96 ± 2.1

https://doi.org/10.1021/acsomega.3c01970
ACS Omega 2023, 8, 27030−27043

Table 2. continued

no.[a]	compounds[b]	RI[c]	RI lit.[d]	% relative peak area				
				S. rosmarinus (100%)	*S. rosmarinus*: *C. atlantica* (75−25%)	*S. rosmarinus*: *C. atlantica* (50−50%)	*S. rosmarinus*: *C. atlantica* (25−75%)	*C. atlantica* (100%)
61	(Z)-α-atlantone	1719	1717		0.64 ± 0.76	1.66 ± 0.55	2.96 ± 1.23	5.56 ± 2.5
62	khusimol	1735	1736			0.24 ± 0.01	0.35 ± 0.11	0.76 ± 0.10
63	benzyl benzoate	1763	1762			0.50 ± 0.44	1.12 ± 1.24	2.44 ± 0.2
64	(E)-α-atlantone	1783	1773		1.15 ± 1.40	2.43 ± 1.52	5.76 ± 0.13	10.87 ± 2.3
65	4-hydroxy-muurolene	1795	1775				0.54 ± 0.82	1.25 ± 0.22
66	14 hydroxy-δ-cadinene	1808	1799				0.49 ± 0.68	1.06 ± 0.8
monoterpene hydrocarbons			17.63 ± 1.41	15.18 ± 1.62	13.78 ± 1.21	8.32 ± 1.01		
oxygenated monoterpenes			81.64 ± 3.02	72.97 ± 2.28	62.07 ± 1.52	37.15 ± 2.53	1.2 ± 0.81	
sesquiterpene hydrocarbons			0.31 ± 0.02	4.15 ± 0.98	8.17 ± 1.74	19.02 ± 1.56	31.6 ± 0.89	
oxygenated sesquiterpenes			0.42 ± 0.12	7.23 ± 1.09	15.32 ± 1.61	35.49 ± 1.19	67.2 ± 2.05	
total identified (%)			100 ± 0.00	99.53 ± 0.32	99.34 ± 0.45	99.98 ± 0.01	100 ± 0.00	

[a]In order of elution in an HP-5 apolar column. [b]Compounds are identified by GC-FID, GC/MS. [c]Calculated retention indices relative to n-alkanes (C8−C28) on the HP-5 MS column. [d]Retention indices from refs 50, 51. Volatile compounds and their proportions were identified from the chromatograms obtained on the HP-5 MS column; −: absence. Data expressed as mean ± standard deviation of triplicates. [e]A new compound appeared in binary combination.

camphor, α-pinene, borneol, camphene, p-cymene, β-himachalene, deodarnone, β-pinene, (E)-α-atlantone, α-himachalene, himachalol, and E(E)-γ-atlantone. When the quantity of *C. atlantica* in the binary combination was high, the amount of sesquiterpene increased. Yet when the quantity of *S. rosmarinus* in the binary combination was increased, the amounts of monoterpene were elevated. During the hydrodistillation of the binary combinations of *S. rosmarinus* and *C. atlantica*, a new component appeared, namely, the myrtenal compound. The latter component was assumed to have a high value at a binary combination of 75% of *S. rosmarinus*−25% *C. atlantica* (4.04%), followed by 50% of *S. rosmarinus*−50% *C. atlantica* (3.41%), and 25% of *S. rosmarinus*−75% *C. atlantica* (2.02%).

3.3. Antifungal Activities of the Tested Binary Combinations. The effective concentrations of 50% of the maximal effect (EC_{50}) values obtained for each essential oil and chemical fungicide (Nystatin) are indicated in Table 3. The anti-wood-decay fungal activities of *S. rosmarinus* and *C. atlantica* EOs and their binary combination of 50% *S. rosmarinus*−50% *C. atlantica* against *Poria placenta* at different concentrations are indicated in Figure 4.

The antifungal properties of *C. atlantica* and *S. rosmarinus* EOs have been recently reported in the literature.[52,53] Nevertheless, no study has examined the antifungal properties of their combination. In this study, *C. atlantica* EO showed antifungal activity against *C. puteana*, *C. versicolor*, *G. trabeum*, and *P. placenta*, with EC_{50} values of 71.03, 60.00, 103.46, and 130.44 μg/mL, respectively. Our data are superior to those found by Rhafouri et al.,[54] who reported that *C. atlantica* EO inhibited the growth of *G. trabeum* and *P. placenta* at a concentration of 3560 μg/mL and *C. versicolor* at a concentration of 1780 μg/mL.

S. rosmarinus EO inhibited the growth of mycelium (*C. puteana*, *C. versicolor*, *G. trabeum*, and *P. placenta*, with EC_{50} values of 31.62, 29.42, 67.51, and 85.93 μg/mL, respectively). Another study revealed that *S. rosmarinus* EO exhibited antifungal activity against *Aspergillus flavus*, with a minimum inhibitory concentration (MIC) of 250 μg/mL.[55] Khanjani et al.[56] reported that *S. rosmarinus* EO exhibited antifungal activity against *Fusarium oxysporum*, with a minimum inhibitory concentration (MIC) of 963 μg/mL.

It shows that the mixture of 50% *S. rosmarinus*−50% *C. atlantica* and Nystatin presented the lowest EC_{50} values of all of the studied fungi (9.98−26.22, 3. 07−12.25 μg/L), followed by the 75% *S. rosmarinus*−25% *C. atlantica* mixture (16.92−37.62 μg/L), the 25% *S. rosmarinus*−75% *C. atlantica* mixture (22.33−40.45 μg/L), and the *S. rosmarinus* EO (29.42−85.93 μg/L) mixture. The EC_{50} values were higher than those of *C. atlantica* EO against white and brown wood rot fungi (60.00−130.44 μg/L).

C. versicolor was the most susceptible fungus in all treatments. A low concentration of 50% *S. rosmarinus*−50% *C. atlantica* EO mixture (9.98 ± 0.61 μg/mL) was considered sufficient to inhibit 50% of the mycelium growth of this fungus. *P. placenta* was the most resistant fungus to all treatments. The 50% *S. rosmarinus*−50% *C. atlantica* mixture EO inhibited 50% of the *P. placenta*, with an EC_{50} value of 26.22 μg/mL.

As is clear from these results, the synergistic effects of the binary combinations provide effective antifungal activity at sufficiently low concentrations, which may be due to the simultaneous hydrodistillation and synergistic effect of the compounds of *S. rosmarinus* (monoterpene) on those of *C. atlantica* (sesquiterpene).

3.4. Establishment of Response Prediction Models. The results of the seven tests obtained by the binary mixture design are presented in Table 4. Before proceeding to the analysis by mixture design, the results show that the 50% of *S. rosmarinus*−50% *C. atlantica* mixture indicated the best anti-wood-decay fungal activity against the four wood-decay fungi.

The results posted in the analysis of the variance (Table 5) show that the main effect of the regression is significant since the probability of *p*-value risk significance is less than 0.05. Based on the table, the model does not show a lack of fit since the probability of risk significance (*p*-value) is greater than 0.05. The coefficient of determination values (R^2 and *adjusted* R^2) for the four models was close to 1 (between 0.95 and 0.98). These values demonstrate good correlation between the

https://doi.org/10.1021/acsomega.3c01970
ACS Omega 2023, 8, 27030−27043

Table 3. Effective Concentration Values (EC_{50}) of Different Mixtures of Essential Oils (EO) of *S. rosmarinus* and *C. atlantica* against Wood Rot Fungi[a]

	mixture						
wood-decay fungi	*S. rosmarinus* (%)	*C. atlantica* (%)	EC_{50} (μg/mL) (95% confidence intervals)	slope ± SE	intercept ± SE	R^2	*p*-value
C. puteana	100	0	31.62 (31.21−32.03)	1.34 ± 0.20	2.94 ± 0.45	0.91	0.00
	75	25	19.19 (18.19−20.19)	1.49 ± 0.22	3.09 ± 0.53	0.93	0.00
	50	50	11.28 (10.67−11.89)	1.47 ± 0.23	3.43 ± 0.54	0.92	0.00
	50	50	11.00 (10.45−11.55)	1.62 ± 0.22	2.65 ± 0.54	0.91	0.00
	50	50	10.89 (9.88−11.09)	1.61 ± 0.22	2.65 ± 0.56	0.92	0.00
	25	75	27.26 (26.04−28.48)	1.63 ± 0.23	2.66 ± 0.55	0.93	0.00
	0	100	71.03 (67.41−74.65)	0.96 ± 0.11	3.23 ± 0.26	0.97	0.00
	Nystatin		4.26 (3.40−5.12)	1.75 ± 0.19	2.55 ± 0.40	0.95	0.00
C. versicolor	100	0	29.42 (28.21−30.61)	1.39 ± 0.25	2.96 ± 0.59	0.91	0.00
	75	25	16.92 (15.97−17.87)	1.29 ± 0.25	3.26 ± 0.61	0.90	0.00
	50	50	10.67 (9.72−11.48)	1.26 ± 0.19	3.69 ± 0.46	0.92	0.00
	50	50	10.34 (9.41−11.27)	1.24 ± 0.18	3.70 ± 0.48	0.92	0.00
	50	50	9.98 (9.37−10.59)	1.27 ± 0.20	3.68 ± 0.47	0.91	0.00
	25	75	22.33 (20.88−23.78)	0.81 ± 0.16	3.93 ± 0.38	0.90	0.00
	0	100	60.00 (56.76−63.24)	0.73 ± 0.07	3.89 ± 0.16	0.97	0.00
	Nystatin		3.07 (2.21−3.93)	1.05 ± 0.27	3.72 ± 0.39	0.94	0.00
G. trabeum	100	0	67.51 (65.12−69.89)	1.69 ± 0.29	1.91 ± 0.69	0.90	0.00
	75	25	21.32 (20.37−22.27)	1.54 ± 0.20	2.95 ± 0.50	0.93	0.00
	50	50	15.97 (14.97−16.97)	1.55 ± 0.26	3.13 ± 0.62	0.91	0.00
	50	50	15.76 (14.84−16.63)	1.54 ± 0.25	3.12 ± 0.61	0.91	0.00
	50	50	15.69 (14.89−16.49)	1.55 ± 0.24	3.12 ± 0.63	0.92	0.00
	25	75	24.81 (23.37−26.25)	1.56 ± 0.27	2.83 ± 0.65	0.90	0.00
	0	100	103.46 (99.29−107.53)	0.93 ± 0.08	3.12 ± 0.20	0.96	0.00
	Nystatin		6.77 (5.78−7.76)	1.85 ± 0.25	3.01 ± 0.62	0.92	0.00
P. placenta	100	0	85.93 (81.49−90.37)	1.18 ± 0.19	2.71 ± 0.46	0.91	0.00
	75	25	37.62 (35.61−39.63)	1.25 ± 0.22	3.03 ± 0.53	0.90	0.00
	50	50	26.42 (25.66−27.18)	1.41 ± 0.20	2.99 ± 0.49	0.92	0.00
	50	50	26.32 (25.41−27.23)	1.42 ± 0.21	3.01 ± 0.50	0.93	0.00
	50	50	26.22 (25.21−27.23)	1.41 ± 0.21	3.02 ± 0.52	0.92	0.00
	25	75	40.45 (37.13−43.77)	1.13 ± 0.18	3.11 ± 0.43	0.91	0.00
	0	100	130.44 (125.71−135.17)	1.27 ± 0.20	2.31 ± 0.49	0.91	0.00
	Nystatin		12.25 (11.23−13.27)	1.34 ± 0.19	3.50 ± 0.45	0.94	0.00

[a]EC_{50}: effective concentration of 50% of maximal effect. Slope ± SE: slope of the concentration/ inhibition regression line ± standard error. Intercept ± SE: intercept of the concentration/inhibition regression line ± standard error. Df: degrees of freedom (Total). Significant effect at *p*-value < 0.05.

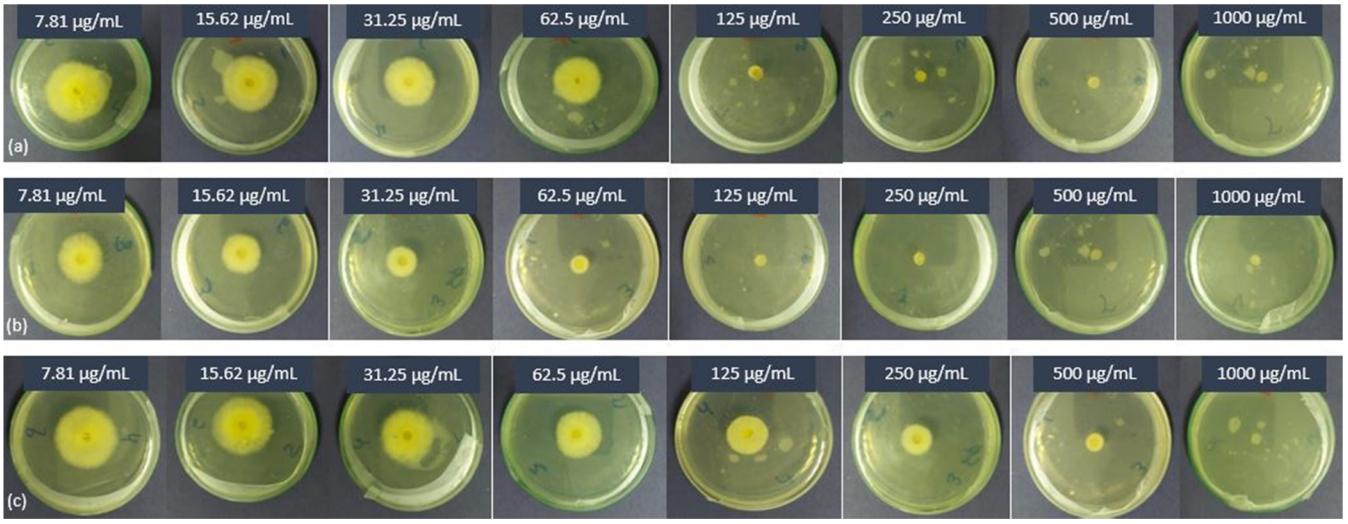


Figure 4. Anti-wood-decay fungal activities of (a) *S. rosmarinus*, (c) *C. atlantica* EOs, and (b) their binary combination 50%−50% against wood-decay fungi growth of *Coniophora puteana* after incubation at 25 ± 1 °C.

https://doi.org/10.1021/acsomega.3c01970
ACS Omega 2023, 8, 27030−27043

Table 4. Experimental Design and Observed Response Values of EC_{50} of Anti-Wood-Decay Fungal Using a Simplex-Lattice DoE with 7 Essays, Including Three Replicates of Central Points[a]

	mixture		observed response values[b]			
essay	*S. rosmarinus* (%)	*C. atlantica* (%)	CP EC_{50} (μg/mL)	CV EC_{50} (μg/mL)	GT EC_{50} (μg/mL)	PP EC_{50} (μg/mL)
1	100	0	31.62 ± 0.41	29.42 ± 1.20	67.51 ± 2.38	85.93 ± 4.44
2	75	25	19.19 ± 1.00	16.92 ± 0.95	21.32 ± 0.95	37.62 ± 2.01
3	50	50	11.28 ± 0.61	10.67 ± 0.88	15.97 ± 1.00	26.42 ± 0.67
4	50	50	11.00 ± 0.55	10.34 ± 0.93	15.76 ± 0.62	26.32 ± 0.91
5	50	50	10.89 ± 1.01	9.98 ± 0.61	15.69 ± 0.80	26.22 ± 1.01
6	25	75	27.26 ± 1.22	22.33 ± 1.45	24.81 ± 1.44	40.45 ± 3.32
7	0	100	71.03 ± 3.62	60.00 ± 3.24	103.46 ± 4.12	130.44 ± 4.73

[a]EC_{50}: effective concentration for 50% of maximal effect. CP: *Coniophora puteana*, CV: *Coriolus versicolor*, GT; *Gloeophyllum trabeum*, and PP: *Poria placenta*. [b]The observed value of three replicates is given with standard deviation.

Table 5. Coefficients of Determination R^2, the Adjusted Coefficient of Determination R_{adj}^2, and the Analysis of Variance of CP EC_{50}, CV EC_{50}, GT EC_{50}, and PP EC_{50}

	source	degrees of freedom	sum of squares	*F*-value	*p*-value
CP EC_{50}	model	2	1388.64	13 758.35	<0.0001[a]
	error	4	0.4037		
	total	6	2777.69		
	lack of fit	2	0.3229	3.99	0.2003
	pure error	2	0.0809		
	R^2	0.99			
	R_{adj}^2	0.99			
CV EC_{50}	model	2	4183.03	1881.54	<0.0001[a]
	error	4	4.45		
	total	6	4187.48		
	lack of fit	2	4.21	17.67	0.0536
	pure error	2	0.238		
	R^2	0.99			
	R_{adj}^2	0.98			
GT EC_{50}	model	2	6818.94	18 017.91	<0.0001[a]
	error	4	0.7569		
	total	6	6819.69		
	lack of fit	2	0.7144	16.82	0.0561
	pure error	2	0.0425		
	R^2	0.99			
	R_{adj}^2	0.98			
PP EC_{50}	model	2	9517.64	49 628.37	<0.0001[a]
	error	4	0.3836		
	total	6	9518.03		
	lack of fit	2	0.3636	18.18	0.0521
	pure error	2	0.02		
	R^2	0.99			
	R_{adj}^2	0.98			

[a]Statistically significant at $p < 0.05$. EC_{50}: effective concentration for 50% of maximal effect. CP: *Coniophora puteana*, CV: *Coriolus versicolor*, GT; *Gloeophyllum trabeum*, and PP: *Poria placenta*.

experimental and predicted values of the fitted models and their ability to predict the four responses.

The graph (Figure 5) shows that most points are on a straight line, indicating that the curve of the observed values versus the predicted values will appear perfectly straight. This graph is in agreement with the coefficient of determination presented in Table 5.

The effects of the two studied components were determined using the Student *t*-test, with a significance level of 95% reported in Table 6. According to this table, all coefficients are statistically significant, with a *p*-value of less than 0.05. Therefore, the proposed model had to include all coefficients.

The mathematical models adopted for anti-wood-decay fungal activity against four wood-decay fungi are as follows

$$\text{CP } EC_{50} = 34.73(S.\ rosmarinus) + 69.09(C.\ atlantica) - 162.741(S.\ rosmarinus{*}C.\ atlantica) + \varepsilon \quad (4)$$

$$\text{CV } EC_{50} = 44.86(S.\ rosmarinus) + 80.28(C.\ atlantica) - 210.08(S.\ rosmarinus{*}C.\ atlantica) + \varepsilon \quad (5)$$

$$\text{GT } EC_{50} = 67.29(S.\ rosmarinus) + 103.45(C.\ atlantica) - 279.17(S.\ rosmarinus{*}C.\ atlantica) + \varepsilon \quad (6)$$

$$\text{PP } EC_{50} = 87.14(S.\ rosmarinus) + 130.28(C.\ atlantica) - 329.37(S.\ rosmarinus{*}C.\ atlantica) + \varepsilon \quad (7)$$

After the models were validated, the next step consisted of a search for the optimal proportions that led to a minimal value of EC_{50}. Figure 6 illustrates that the desirable anti-wood-decay fungal activity against the four wood-decay fungi was in the compromise zone between 50% of *S. rosmarinus*−50% *C. atlantica* and 75% of *S. rosmarinus*−25% *C. atlantica*.

The desirability function might help determine the best compromise among the various options for the optimum value of the studied response. Figure 7 indicates the desirability plots for the responses of CP EC_{50}, CV EC_{50}, GT EC_{50}, and PP EC_{50} obtained by the validated model. Figure 7 shows that desirability achieved a maximum value of 0.99 for a CP EC_{50} of 9.91 μg/mL and a CV EC_{50} of 9.28 μg/mL when the proportion was 60% of *S. rosmarinus* and 40% of *C. atlantica*. The proportion should be 55% of *S. rosmarinus* and 45% of *C. atlantica* to attain a GT EC_{50} of 11.48 μg/mL and a PP EC_{50} of 22.62 μg/mL, with a desirability level of 0.99.

Through experimental design methodology, which involves employing mixture design models, several studies have investigated the applications of experiment design for antimicrobial properties.[57−60] Nonetheless, no studies have

https://doi.org/10.1021/acsomega.3c01970
ACS Omega 2023, 8, 27030−27043

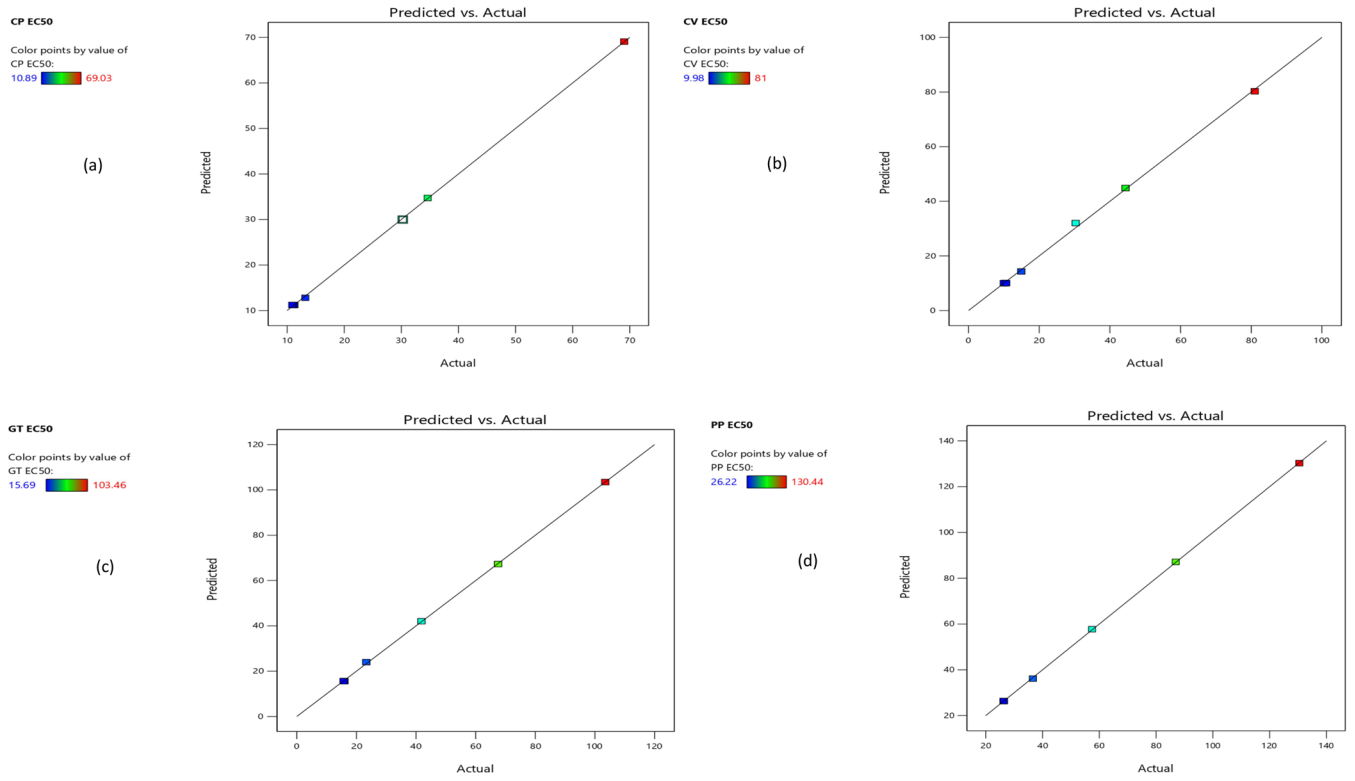


Figure 5. Observed vs predicted values curve of (a) CP EC_{50}, (b) CV EC_{50}, (c) GT EC_{50}, and (d) PP EC_{50}. EC_{50}: effective concentration for 50% of maximal effect. CP: *Coniophora puteana*, CV: *Coriolus versicolor*, GT: *Gloeophyllum trabeum*, and PP: *Poria placenta*.

Table 6. Estimated Values of the Regression Coefficients of Postulated Models for CP EC_{50}, CV EC_{50}, GT EC_{50}, and PP EC_{50}

	terms	coefficient	standard error	*t*-student	*p*-value
CP EC_{50}	*S. rosmarinus*	34.73	0.29	9.42	0.0007[a]
	C. atlantica	69.09	0.29	18.97	<0.0001[a]
	S. rosmarinus[a] *C. atlantica*	−162.74	1.14	−11.40	0.0003[a]
CV EC_{50}	*S. rosmarinus*	44.86	0.99	10.64	0.0004[a]
	C. atlantica	80.28	0.99	19.63	<0.0001[a]
	S. rosmarinus[a] *C. atlantica*	−210.08	3.80	−12.03	0.0003[a]
GT EC_{50}	*S. rosmarinus*	67.29	0.41	9.71	0.0006[a]
	C. atlantica	103.45	0.41	13.97	0.0002[a]
	S. rosmarinus[a] *C. atlantica*	−279.17	1.57	−10.51	0.0005[a]
PP EC_{50}	*S. rosmarinus*	87.14	0.29	10.43	0.0005[a]
	C. atlantica	130.28	0.29	14.44	0.0001[a]
	S. rosmarinus[a] *C. atlantica*	−329.37	1.12	−10.07	0.0005[a]

[a]Statistically significant at $p < 0.05$. EC_{50}: effective concentration for 50 percent of maximal effect. CP: *Coniophora puteana*, CV: *Coriolus versicolor*, GT; *Gloeophyllum trabeum*, and PP: *Poria placenta*.

been published on applying mixture design models to inhibit wood-decay fungi. This study is the first to do this. In this study, the binary mixture of 55% *S. rosmarinus* and 45% *C. atlantica* showed the highest inhibition effect against white and brown wood-decay fungi. The efficiency of this mixture could be due to the synergistic effect of the main components of *S. rosmarinus*, mainly 1,8-cineole (50.86 ± 3.5%), camphor (18.81 ± 2.1%), and α-pinene (9.36 ± 2.18%), on those of *C. atlantica* (β-himachalene (11.46 ± 2.3%), deodarnone (10.74 ± 3.3%), himachalol (10.52 ± 2.5%), (E)-α-atlantone (10.87 ± 2.3%), and α-himachalene (7.55 ± 1.1%)). The synergy produced in this mixture may be due to the interactions between 1,8-cineole and β-himachalene belonging to oxygenated monoterpene and sesquiterpene hydrocarbons (alcohols and ketones), respectively. The minor components may also have helped achieve synergy. This interaction between the different compounds is supported by the appearance of new compounds in binary mixtures, including myrtenal and α-cadinol. These results align with those indicated in the literature:[33] *S. rosmarinus* EO rich in camphor and 1,8-cineole presented a strong effect against *G. trabeum* and *P. placenta*. Another study found that *S. rosmarinus* essential oil containing 1,8-Cineole (19.60%), camphor (17.01%), and α -pinene (15.12%) presented an EC_{50} value of 517 μg/mL against *Hexagonia apiaria*.[61] Zhang et al.[62] found that monoterpenes significantly contribute to protecting wood against white wood-decay fungi, *Trametes hirsuta*, *Schizphylhls commune*, and *Pycnoporus sanguineus*. Camphor compound exhibited a low EC_{50} against *G. trabeum* and *C. versicolor*, with values of 0.123 and 1.5 mg/mL, respectively.[63]

https://doi.org/10.1021/acsomega.3c01970
ACS Omega 2023, 8, 27030−27043

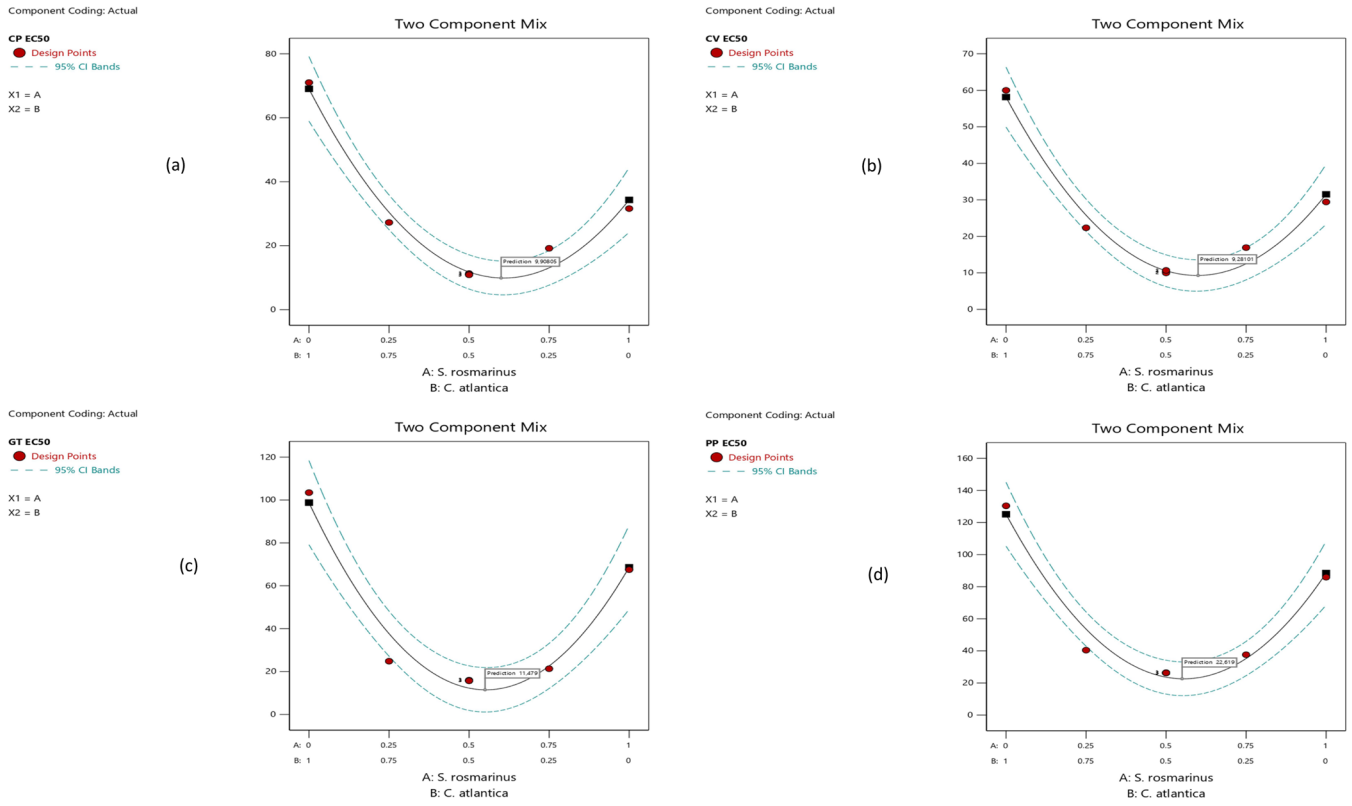


Figure 6. Binary mixture plot of (a) CP EC_{50}, (b) CV EC_{50}, (c) GT EC_{50}, and (d) PP EC_{50} according to the two plants *S. rosmarinus* and *C. atlantica*. CP: *Coniophora puteana*, CV: *Coriolus versicolor*, GT: *Gloeophyllum trabeum*, and PP: *Poria placenta*.

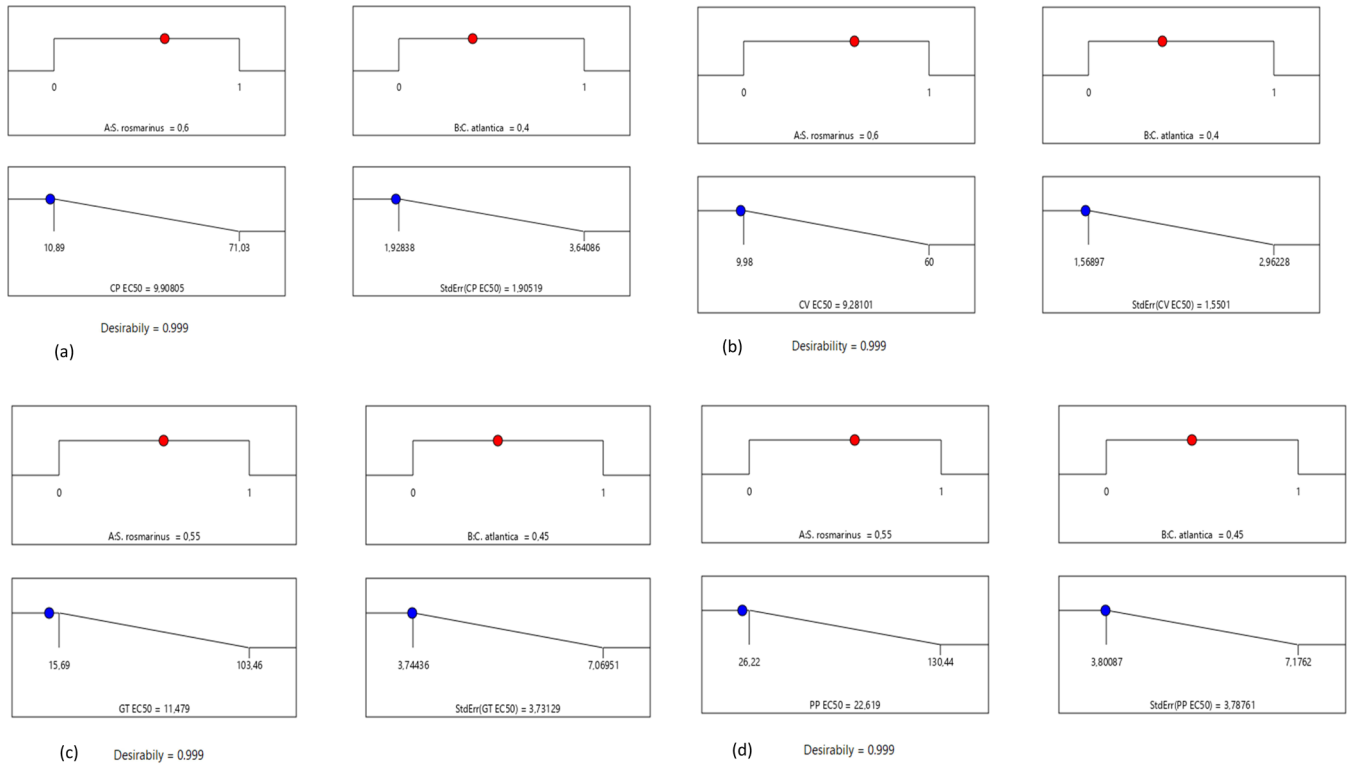


Figure 7. Desirabily plot of (a) CP EC_{50}, (b) CV EC_{50}, (c) GT EC_{50}, and (d) PP EC_{50}. CP: *Coniophora puteana*, CV: *Coriolus versicolor*, GT: *Gloeophyllum trabeum*, and PP: *Poria placent.*

Moreover, *C. atlantica* EO containing E-γ-Atlantone (19.73%), E-α-Atlantone (16.86%), 5-Isocedranol (11.68%), 9-iso-Thujopsanone (4.45%), Cedranone (4.13%), and Z α-Atlantone (4.02%) exhibited significant antifungal activity

https://doi.org/10.1021/acsomega.3c01970
ACS Omega 2023, 8, 27030−27043

against *T. versicolor*, *C. puteana*, *O. placenta*, and *G. trabeum* fungus, inhibiting their growth with 1/800, 1/400, 1/400, and 1/1000 v/v concentrations, respectively.[34] Rhafouri et al.[54] found that both wood-decay fungi, *G. trabeum* and *P. placenta*, stopped growth at a concentration of 1/250 v/v Atlas cedar seed essential oil. Another study indicated that the antifungal effect of sesquiterpenes against the brown-decay fungus *L. sulphureus* was high compared to that of the white-decay fungus *L. betulina*.[16] In addition, Ljunggren et al.[64] found that himachalene showed effective antifungal activity against *C. puteana*.

A test point was carried out on wood-decay fungi employing an optimal mixture to complete the testing of the proposed model's validity. Table 7 indicates no statistically significant difference between the experimental and predicted responses for the four wood-decay fungi. The binary mixture of 60% *S. rosmarinus*–40% *C. atlantica* demonstrated significant wood-decay fungal activity against *C. puteana* and *C. versicolor*, with EC_{50} values of 9.99 ± 1.52 and 9.33 ± 1.22 μg/mL, respectively. The binary mixture 55% *S. rosmarinus*–45% *C. atlantica* showed significant wood-decay fungal activity against *G. trabeum* and *P. placenta*, with EC_{50} values of 11.50 ± 1.98 and 22.53 ± 2.51 μg/mL, respectively. These findings support the theoretical results obtained by the desirability test, which revealed that the mixture of 60% *S. rosmarinus*–40% *C. atlantica* could inhibit *C. puteana* and *C. versicolor*, with EC_{50} values of 9.91 ± 1.91 and 9.28 ± 1.55 μg/mL, respectively. In contrast, the mixture of 55% *S. rosmarinus*–45% *C. atlantica* could inhibit *G. trabeum* and *P. placenta* with EC_{50} values of 11.48 ± 3.73 and 22.62 ± 3.79 μg/mL, respectively.

Table 7. Predicted and Observed Values for Test Points Achieved for the Optimal Mixture of Four Wood-Destroying Fungi

	mixture			
wood-decay fungi	*S. rosmarinus* (%)	*C. atlantica* (%)	predicted response values[a] a EC_{50} (μg/mL)	observed response values[b] EC_{50} (μg/mL)
Coniophora puteana	60	40	9.91 ± 1.91	9.99 ± 1.52
Coriolus versicolor	60	40	9.28 ± 1.55	9.33 ± 1.22
Gloeophyllum trabeum	55	45	11.48 ± 3.73	11.50 ± 1.98
Poria placenta	55	45	22.62 ± 3.79	22.53 ± 2.51

[a]The predicted value is provided with the response's standard deviation determined by the model. [b]The observed value of three replicates is given with standard deviation.

4. CONCLUSIONS

In this study, we investigated the anti-wood-decay fungal activity of the essential oils of *S. rosmarinus* and *C. atlantica*, alone and in binary combination, against four wood-decay fungi using a mixture design methodology. Most compounds of the studied mixture were the same as those of the EO alone, although new compounds appeared, namely, myrtenal. The percentage of these compounds decreased or increased in the mixture according to the proportions of *S. rosmarinus* and *C. atlantica*. The results indicated that the simultaneous extraction of the binary combination of *S. rosmarinus* and *C. atlantica* maximized anti-wood-decay fungal activity. The binary combination of 60% *S. rosmarinus* and 40% *C. atlantica* EOs resulted in the highest anti-wood-decay fungal activity against *G. trabeum* and *P. placenta*. The binary combination of 55% of *S. rosmarinus* and 45% of *C. atlantica* EOs exhibited the highest anti-wood-decay fungal activity against *C. puteana* and *C. versicolor*. These activities were explained by the synergistic effect between monoterpene and sesquiterpene, which consolidate to the appearance of myrtenal components. These results could allow for the use of the anti-wood-decay fungal properties of these mixtures in industrial wood preservation to prevent wood from deteriorating and protect the environment from chemical contamination. Future research should focus on developing natural products against wood rot fungi.

■ ASSOCIATED CONTENT

Data Availability Statement

All related data are within the manuscript.

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https://doi.org/10.1021/acsomega.3c01970
ACS Omega 2023, 8, 27030−27043

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Funding
This work was supported by the Researchers Supporting Project number (RSP2023R45) at King Saud University, Riyadh, Saudi Arabia.

Notes
The authors declare no competing financial interest.
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

■ ACKNOWLEDGMENTS

The authors extend their sincere appreciation to the Researchers Supporting Project number (RSP2023R45), King Saud University, Riyadh, Saudi Arabia, for financial support.

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