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Phytochemical Compounds of Branches from *P. halepensis* Oily Liquid Extract and *S. terebinthifolius* Essential Oil and Their Potential Antifungal Activity

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Abstract: In the present study, the antifungal activity of wood treated with *Pinus halepensis* branch *n*-hexane oily liquid extract (OLE) and *Schinus terebinthifolius* branch essential oil (EO) was evaluated against the growth of four phytopathogenic fungi—*Bipolaris oryzae*, *Fusarium oxysporum*, *Fusarium solani*, and *Rhizoctonia solani*. Air-dried wood samples of *Pinus roxburghii* were autoclaved, and each wood received 100 µL of the concentrated oils from *P. halepensis* and *S. terebinthifolius*. The main compounds identified in *S. terebinthifolius* branch EO were terpinen-4-ol (18.25%), *cis*- β -terpineol (15.60%), γ -terpinene (12.46%), sabinene (9.83%), α -terpinene (8.56%), and 4-thujanol (6.71%), while the main compounds in *P. halepensis* branch EO were 2-undecenal (22.25%), 4-hydroxy-10-methyl-3,4,7,8,9,10-hexahydro-2H-oxecin-2-one (8.43%), (Z)-2-decenal (6.88%), nonanal (5.85%), (2E)-2-decenal (4.65%), (E,E)-2,4-decadienal (4.41%), arachidonic acid methyl ester (4.36%), and 2-(7-heptadecynyloxy)tetrahydro-2H-pyran (4.22%). *P. halepensis* OLE at a concentration of 3% showed the highest inhibition percentage of fungal growth (IPFG) of *B. oryzae*, followed by *S. terebinthifolius* EO at 3% and 2%, with IPFG values of 80%, 74.44%, and 71.66%, respectively. At a concentration of 3%, branch oils from *S. terebinthifolius* and *P. halepensis* were found to have the highest IPFG values with 45.55% and 40.55%, respectively, against *F. oxysporum* growth. Moderate to weak activity was found against *F. solani* when *S. terebinthifolius* EO and *P. halepensis* OLE were applied to wood. EO and OLE-treated wood samples at 3% produced inhibitions of 54.44% and 41.11%, respectively, against *R. solani*.

Keywords: essential oil; oily liquid extract; *P. halepensis*; *S. terebinthifolius*; fungi; wood bio-fungicide

1. Introduction

Natural products extracted from forestry trees have a sustainable source of natural products from their different parts (wood, bark, roots, leaves, flowers, branches, seeds, and fruits). These natural products, such as essential oils (EOs), fixed oils, and polyphenolic and flavonoid compounds, have great effects in terms of their antimicrobial activities [1–9].

Bipolaris oryzae is the causal agent of rice brown spot disease and is responsible for significant economic losses, and in Egypt the disease is ranked second, coming next after blast disease [10]. Fusaria are common soil saprophytes; however, they are also known as phytopathogens—especially *Fusarium oxysporum*—that cause root or blight diseases in many host plants [11–13]. Two *Fusarium* species were recently included in the list of the top ten plant pathogenic fungi with both economic and scientific importance, with one of them being *F. solani*, which causes vascular wilt or root rot in more than 100 plant species [14,15]. *Rhizoctonia solani* is considered a distractive soil-borne disease of rice production worldwide, causing rice sheath blight disease. The initiation of an integrated disease management procedure is important to keep the disease under control [16].

Phytochemicals from all of the parts of Aleppo pine, *P. halepensis* Miller, have shown the presence of EOs, phenolic compounds, turpentine, and terpenes with valuable medicinal uses and antimicrobial agents [9–21]. Needle EOs have been shown to have many bioactive compounds, such as α -humulene, aromadendrin, and β -caryophyllene [22]; caryophyllene oxide, myrcene, α -pinene, and β -caryophyllene were also identified [23,24].

Schinus terebinthifolius Raddi produces resin that contains monoterpenes acting as a defense against attacking pathogenic agents [25]. Extracts and EOs, especially from fruits, have been studied extensively in dozens of works, e.g., oleic acid, δ -cadinene, α -phellandrene, 1b,5,5,6 α -tetramethyloctahydro-6H-indeno[1,2-b]oxiren-6-one, aromadendrene, hexadecanoic acid-2,3-dihydroxypropyl ester, α -caryophyllene, and germacrene D were the main compounds found in the ethyl ether extract of *S. terebinthifolius* fruits with good antifungal activity against *T. harzianum* and *A. niger* [2]. Acetone extract from ripened fruits with oleic acid, α -phellandrene, and δ -cadinene as main compounds had observed antibacterial activity [26]. Other compounds such as flavonoids, phenols, tannins, anthraquinones, quercetin, kaempferol, biphenyl esters, and steroids were isolated from the fruits of *S. terebinthifolius* [27–31], with potential antimicrobial activities. Methanol and Aqueous extracts of *S. terebinthifolius* showed potential activity against *C. albicans* [32,33]. Extracts of the stem of the plant showed the isolated compounds of schinol and biphenyl 4'-ethyl-4-methyl-2,2',6,6'-tetrahydroxy[1,1'-biphenyl]-4,4'-dicarboxylate with potential antifungal activity against *P. brasiliensis* [34].

Lignocellulosic materials such as wood can be affected or deteriorated by fungi in moist conditions, causing a loss in the overall quality or the surface quality of the wood, as well as some environmental problems as a result of their excretion of toxins [35–38]. Some works have been carried out to preserve or protect some commercial wood species from deterioration by fungi using natural extracts, and the results showed good antifungal activity, either inhibiting or preventing their growth [2,3,7,8]. No fungal growth of *A. tenuissima* and *F. culmorum* occurred on the *Acacia saligna* wood surface treated with the methanol extract of *Maclura pomifera* bark [39]. Recently, the treated wood samples of *Melia azedarach* with the water extract of *A. saligna* flowers, the ethanol extract of *Musa paradisiaca* peels, the *n*-hexane oily extracts from *Eucalyptus camaldulensis* and *Vitex agnus-castus*, or with *Withania somnifera* fruit acetone extract showed good antifungal activity against several molds, such as *F. culmorum*, *P. chrysogenum*, and *R. solani* [3,7,8,40]. Furthermore, new green pesticides that act as alternatives, such as natural product-based pesticides, are being developed to replace compounds lost due to the new regulations [41].

EOs are an interesting option for use as new pesticides reserving as anti; fungi, insects, and weeds. Moreover, many EOs can directly act as a natural insect repellent to provide protection against mosquitoes and other harmful arthropods, or to provide antifeedant activity against herbivores, such as eucalyptus oil [42,43]. MyggA® or Natural or Citriodiol® and Buzz away are other mosquito

repellent products based on p-Menthane-3,8-diol and citronellal monoterpene extracted from lemon eucalyptus E, which are marketed to use against public health pests and can help avoid ticks or biting flies [44–47]. Many researchers have reported that EO applications should be considered as resistance inducers and could affect pathogens through the production of phytoalexins and biochemical plant defense [48]. Indeed, even little amounts of EOs applied on plants in vivo have been shown to produce a greater reduction in disease severity [49]. There are many reasons that may contribute to the greater effect of EOs in vivo than in vitro, such as variation of the optimum temperature of fungal growth and the presence of microorganisms on the plant surface [50]. The present work aimed to evaluate the bioactivity of *P. halepensis* n-hexane oily liquid extract and *S. terebinthifolius* essential oil as wood-biofungicides.

2. Materials and Methods

2.1. Preparation of Essential Oil and n-Hexane Oily Liquid Extract

Green branches (wood and bark) of Brazilian Peppertree, *Schinus terebinthifolius* Raddi (Anacardiaceae), were rapidly washed by tap water to remove the dust, then smoothly dried with paper towel and cut into small pieces. About 100 g of the cut branches underwent extraction of their essential oil (EO) using the hydrodistillation method of the Clevenger apparatus for 3 h [51]. Meanwhile, the green branches (wood and bark) of Aleppo pine, *Pinus halepensis* Miller (Pinaceae), after being cut into small pieces, underwent extraction via the soaking method, where about 100 g of the branch sample was soaked in n-hexane solvent (200 mL) for 6 h under shaking. The n-hexane oily liquid extract (OLE) was separated by filtration with Whatman No. 1 filter paper under suction pressure then the OLE concentrated by evaporating the n-hexane solvent under vacuum using a rotary evaporator at 45 °C. EO and OLE were stored in brown tubes until use.

2.2. The Antifungal Activity of Wood Treated with Essential Oil and Oily Liquid Extract

Wood samples of *P. roxburghii* Sarg were prepared at the approximate dimensions of 2 × 1 × 0.5 cm, autoclaved at 121 °C for 20 min, and then left to cool. The four fungi of *Bipolaris oryzae*, *Fusarium oxysporum*, *Fusarium solani*, and *Rhizoctonia solani* were used for the bioassay. *S. terebinthifolius* (EO) and *P. halepensis* (OLE) were prepared at the concentrations of 1%, 2%, and 3%. For every wood sample, 100 µL of the concentrated EO or OLE was applied. The antifungal activity of the treated wood samples was compared with wood samples without oil treatments, which were used as a control. The fungal inhibition percentage was calculated with the formula of inhibition percentage of fungal growth (IPFG) (%) = $\left[\frac{DC-DT}{DC} \right] \times 100$, where DC is the diameter of fungal control and DT is the diameter of treatment. Four replicates were carried out for all of the treatments. The antifungal activities of the treated wood were measured following the method of our previous works [3,52–55] with minor modification, namely, ensuring that each wood sample examined for its antifungal activity had received the tested amount of EO or OLE.

2.3. Gas Chromatography-Mass Spectrometry (GC/MS) Analysis of the EO and OLE

The chemical compositions of the EO and OLE were analyzed using the Focus GC-DSQ Mass Spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m × 0.25 mm × 0.25 µm film thickness) apparatus at the Atomic and Molecular Physics Unit, Experimental Nuclear Physics Department, Nuclear Research Centre, Egyptian Atomic Energy Authority, Inshas, Cairo, Egypt. The column oven temperature was initially held at 45 °C, and then increased by 5 °C/min to 200 °C, held for 5 min, then increased again to 300 °C by 5 °C/min. Other conditions and identification of the compounds using the WILEY 09 and NIST 14 mass spectral databases can be found in previous works [1–5,56,57].

2.4. Statistical Analysis

Data of the antifungal activity were statistically analyzed with two factors [(type of oil (EO and OLE) and the concentration)] using the SAS system [58]. The differences among the means of the treatments were recorded using Fisher's least significant difference ($LSD_{0.05}$).

3. Results

3.1. Visual Observations of the Fungal Growth

Figure 1 shows the antifungal activity of the treated *Pinus roxburghii* wood with the *n*-hexane oily extract (OLE) from *Pinus halepensis* and the essential oil (EO) of *S. terebinthifolius* against the growth of *Bipolaris oryzae*. In the control treatment (wood without EO or OLE), the fungal growth almost reached full linear growth, but for the wood treated with both oils, the fungal mycelia were suspended and the inhibition was found at all of the concentrations used (1%, 2%, and 3%). Additionally, the fungal growth was found to grow on the side opposite to the treated wood with both EO and OLE.

Figure 1. Antifungal bioassay of wood treated with (B) *P. halepensis* oily liquid extract (OLE) and (F) *S. terebinthifolius* essential oil (EO) against the growth of *B. oryzae*. B1: 3%; B2: 2%; B3: 1%; F1: 3%; F2: 2%; F3: 1%.

Compared to the control condition, *Fusarium oxysporum* growth was inhibited and did not reach the treated wood with both EO and OLE, especially at the concentrations of 2% and 3% (Figure 2). On the other hand, the treated wood with both EO and OLE showed weak activity against *Fusarium solani* (Figure 3); however, *P. halepensis* OLE at 3% showed inhibition against its growth. Figure 4 shows that, in the control treatment, the fungal mycelia of *Rhizoctonia solani* covered and grew over the wood samples after the incubation period. Meanwhile, either the growth did not completely reach the direction of the treated wood, or no growth was found at all.

Figure 2. Antifungal bioassay of wood treated with (B) *P. halepensis* OLE and (F) *S. terebinthifolius* EO against the growth of *F. oxysporum*. B1: 3%; B2: 2%; B3: 1%; F1: 3%; F2: 2%; F3: 1%.

Figure 3. Antifungal bioassay of wood treated with (B) *P. halepensis* OLE and (F) *S. terebinthifolius* EO against the growth of *F. solani*. B1: 3%; B2: 2%; B3: 1%; F1: 3%; F2: 2%; F3: 1%.

Figure 4. Antifungal bioassay of wood treated with (B) *P. halepensis* OLE and (F) *S. terebinthifolius* EO against the growth of *R. solani*. B1: 3%; B2: 2%; B3: 1%; F1: 3%; F2: 2%; F3: 1%.

Furthermore, as shown in Figure 4, the fungal growth of *R. solani* was found in the opposite direction of the treated wood with the *P. halepensis* OLE and *Schinus terebinthifolius* EO.

3.2. Antifungal Activity of the Oils

Statistically, there was a significance effect of EO and OLE in terms of antifungal activity against *B. oryzae*, *F. oxysporum*, *F. solani*, and *R. solani* (Figure 5a), where *P. halepensis* OLE had the highest effect against the growth of *F. oxysporum* and *F. solani*. Meanwhile, *S. terebinthifolius* EO was found to have good activity against *B. oryzae* and *R. solani*. Furthermore, when increasing the EO or OLE concentration from 0% (control) to 3%, the inhibition percentage of fungal growth (IPFG) was increased (Figure 5b).

Figure 5. Overall bioactivity of wood treated with *P. halepensis* OLE and *S. terebinthifolius* EO; (a) EO and OLE source and (b) EO and OLE concentration against the fungal linear growth of *B. oryzae*, *F. oxysporum*, *F. solani*, and *R. solani*.

For the effect of EO/OLE-treated wood as affected by the interaction between the EO/OLE type and their concentrations (Table 1), the highest activity against *B. oryzae* growth was found for *P. halepensis* OLE applied at a concentration of 3%, followed by *S. terebinthifolius* EO at 3% and 2%, with IPFG values of 80%, 74.44%, and 71.66%, respectively. *S. terebinthifolius* EO and *P. halepensis* OLE at a

concentration of 3% observed the highest IPFG, with values of 45.55% and 40.55%, respectively, against *F. oxysporum* growth.

Wood treated with *P. halepensis* OLE at the 3% and 2% concentration levels showed significant activity against the growth of *F. solani*, with IPFG values of 30.55% and 29.44%, respectively. Also, *S. terebinthifolius* EO (2%) and *P. halepensis* OLE (1%) showed IPFG values of 21.11% and 20%, respectively. The highest activity against the growth of *R. solani* was observed for wood treated with *S. terebinthifolius* EO and *P. halepensis* OLE at 3%, with IPFG values of 54.44% and 41.11%, respectively. However, *S. terebinthifolius* EO and *P. halepensis* OLE at 2% showed IPFG values of 35% and 30.55%, respectively, against *R. solani*.

Table 1. Inhibition percentages of fungal growth (%) as affected by wood treated with *P. halepensis* OLE and *S. terebinthifolius* EO.

Oil/Extract Type	Concentration (%)	Fungal Inhibition (%)			
		<i>B. oryzae</i>	<i>F. oxysporum</i>	<i>F. solani</i>	<i>R. solani</i>
<i>P. halepensis</i> OLE	0 (control)	0.00	0.00	0.00	0.00
	1	50.55 ± 4.92	31.11 ± 4.05	20 ± 9.07	24.44 ± 4.05
	2	70 ± 5.28	35.55 ± 6.60	29.44 ± 4.20	30.55 ± 7.34
	3	80 ± 1.81	40.55 ± 4.58	30.55 ± 2.12	41.11 ± 2.86
<i>S. terebinthifolius</i> EO	0 (control)	0.00	0.00	0.00	0.00
	1	61.11 ± 2.86	23.44 ± 2.93	13.88 ± 2.12	28.33 ± 3.79
	2	71.66 ± 3.79	33.33 ± 1.81	21.11 ± 2.86	35 ± 2.12
	3	74.44 ± 2.86	45.55 ± 2.86	23.88 ± 2.12	54.44 ± 6.91
<i>p</i> -value		**	**	**	**

** : High significance difference.

3.3. Chemical Composition of Branches from *S. terebinthifolius* Essential Oil and *P. halepensis* Oily Liquid Extract

The chemical composition of the EO identified in *S. terebinthifolius* branch is shown in Table 2. The main compounds were terpinen-4-ol (18.25%), *cis*- β -terpineol (15.60%), γ -terpinene (12.46%), sabinene (9.83%), α -terpinene (8.56%), 4-thujanol (6.71%), (-)- α -terpineol (4.34%), *o*-cymene (4.05%), D-limonene (2.79%), α -phellandrene (2.46%), and β -caryophyllene (2.34%). The chemical compounds of *P. halepensis* branch OLE are presented in Table 3. The main compounds were 2-undecenal (22.25%), 4-hydroxy-10-methyl-3,4,7,8,9,10-hexahydro-2H-oxecin-2-one (8.43%), (Z)-2-decenal (6.88%), nonanal (5.85%), (2E)-2-decenal (4.65%), (E,E)-2,4-decadienal (4.41%), arachidonic acid methyl ester (4.36%), 2-(7-heptadecyloxy)tetrahydro-2H-pyran (4.22%), 1-nonadecene (3.50%), 12,15-octadecadiynoic acid, methyl ester (3.1%), Z-(13,14-epoxy)tetradec-11-en-1-ol acetate (3.04%), 7-methyl-Z-tetradecen-1-ol acetate (3.01%), (Z)-2-tridecenal (2.9%), O-benzylserine (2.22%), methyl octadeca-13,16-diynoate (2.21%), myristyl chloride (1.97%), (Z)-oleic acid (1.48%), *n*-caprylaldehyde (1.31%), and 1-heptatriacotanol (1.15%).

Table 2. Phytochemicals of essential oil from *S. terebinthifolius* branches by gas chromatography-mass spectrometry (GC-MS).

Compound	Chemical Classification	Percentage in the Oil (%)	SI ¹	RSI ²
α -Thujene	Monoterpene	0.94	935	954
α -Pinene	Monoterpene	1.21	960	962
Sabinene	Monoterpene	9.83	961	968
β -Pinene	Monoterpene	0.32	942	952
Myrcene	Monoterpene	1.88	943	945
α -Terpinene	Monoterpene	8.56	939	944
D-Limonene	Monoterpene	2.79	926	929
α -Phellandrene	Monoterpene	2.46	918	921
<i>o</i> -Cymene	Monoterpene	4.05	926	937
γ -Terpinene	Monoterpene	12.46	936	941
Artemisia ketone	Monoterpene	0.33	774	974
4-Thujanol	Monoterpene	6.71	888	890
Linalool	Monoterpene	0.74	922	934
<i>cis</i> - β -Terpineol	Monoterpene	15.60	928	937
<i>para</i> -Menth-3-en-1-ol	Monoterpene	1.85	888	893
<i>cis-para</i> -2-menthen-1-ol	Monoterpene	0.64	903	918
Camphor	Monoterpene	0.23	765	820
Terpinen-4-ol	Monoterpene	18.25	931	937
(<i>Z</i>)-Piperitol	Monoterpene	0.28	873	927
(-)- α -Terpineol	Monoterpene	4.34	941	945
<i>trans</i> -Piperitol	Monoterpene	0.14	866	925
Linalyl acetate	Monoterpene	1.63	899	921
2,2-Dimethyl-3-vinyl-bicyclo[2.2.1]heptane	Hydrocarbons	0.26	748	807
Thymol	Monoterpene	1.19	866	903
β -Caryophyllene	Sesquiterpene	2.34	922	941
γ -Elemene	Sesquiterpene	0.95	849	885

¹ SI: Standard index; ² RSI: Reverse standard index.**Table 3.** Phytochemicals of oily liquid extract from *P. halepensis* branches by GC/MS.

Compound	Chemical Classification	Percentage in the Extract (%)	SI ¹	RSI ²
2,7-dimethyl-1-Octanol	Terpene hydrocarbon	0.70	706	783
2,6-Dimethyloctane-1,8-diol	Terpene hydrocarbon	0.25	675	675
(<i>E</i>)-2-Decen-1-ol	Unsaturated aldehydes	0.57	705	775
(2-Hexylcyclopropyl)acetic acid	Hydrocarbons	0.29	718	758
1-Dodecene	Unsaturated aldehydes	0.47	742	742
<i>n</i> -Caprylaldehyde	Alkyl aldehyde	1.31	803	934
Isopinocarveol	Pinane monoterpene	0.97	708	748
(<i>E</i>)-9-Tetradecen-1-ol acetate	Unsaturated aldehydes	0.45	751	783
<i>trans</i> -2-Decenal	Unsaturated aldehydes	0.40	884	921
(<i>Z</i>)-2-Decenal	Unsaturated aldehydes	6.88	901	932
(2 <i>E</i>)-2-Decenal	Unsaturated aldehydes	4.65	884	919
Nonanal	Fatty aldehydes	5.85	870	887
Oxacyclotetradecan-2-one	Ketone	0.32	751	752
Methyl octadeca-13,16-diynoate	Fatty acid methyl ester	2.21	742	750
1-Tetradecanol	Saturated fatty alcohol	0.32	767	768
(<i>Z</i>)-2-Tridecenal	Unsaturated aldehydes	2.9	771	814
Undecanoic acid, hydroxy-, lactone	Fatty acid	0.19	740	757
1,2-15,16-Diepoxyhexadecane	Alkane hydrocarbon	0.74	780	782
5-heptyldihydro-2(3H)-furanone	gamma butyrolactones	0.32	734	745
(<i>E,E</i>)-2,4-Decadienal	Unsaturated aldehydes	4.41	838	870

Table 3. Cont.

Compound	Chemical Classification	Percentage in the Extract (%)	SI ¹	RSI ²
(Z)-Oleic acid	Fatty acid	1.48	770	770
2-Undecenal	Unsaturated aldehydes	22.25	890	903
Olealdehyde	Aldehydes	0.69	727	792
Arachidonic acid methyl ester	Fatty acid methyl ester	4.36	753	760
Myristyl chloride	Fatty acid	1.97	735	755
Z,Z,Z-1,4,6,9-Nonadecatetraene	Unsaturated aldehydes	0.68	741	741
4-Hydroxy-10-methyl-3,4,7,8,9,10-hexahydro-2H-oxecin-2-one	Ketone	8.43	844	890
2-methylene-(3 β ,5 α)-Cholestan-3-ol	Steroids	0.87	772	782
(Z)-7-Hexadecenal	Unsaturated aldehydes	0.46	747	890
Vitamin A aldehyde (Retinal)	Retinoids	0.21	701	771
9-Hexadecenoic acid	Fatty acid	1.08	788	789
1-Heptatriacotanol	Fatty alcohol	1.15	728	730
O-Benzylserine	Amino acid	2.22	766	776
12,15-Octadecadiynoic acid, methyl ester	Fatty acid	3.1	743	753
2-(7-heptadecynyloxy)tetrahydro-2H-Pyran	Hydrocarbons	4.22	697	697
1-Nonadecene	Hydrocarbons	3.50	724	757
Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	Hydrocarbons	3.04	785	794
7-Methyl-Z-tetradecen-1-ol acetate	Hydrocarbons	3.01	747	762

¹ SI: Standard index; ² RSI: Reverse standard index.

4. Discussion

Pinus halepensis OLE and *Schinus terebinthifolius* EO showed the presence of several bioactive compounds, as identified by Gas Chromatography-Mass Spectrometry (GC/MS). 2-undecenal, as well as other compounds such as 4-hydroxy-10-methyl-3,4,7,8,9,10-hexahydro-2H-oxecin-2-one, (Z)-2-decenal, nonanal, (2E)-2-decenal, (E,E)-2,4-decadienal, arachidonic acid methyl ester, and 2-(7-heptadecynyloxy)tetrahydro-2H-pyran, were found in the OLE of *P. halepensis*.

Several cited chemical compositions of *P. halepensis* have been published regarding terpenes, fatty acids, aldehydes, and cyclic phenolic compounds [59–61]. The lipophilic extractives (resin acids, terpenes, fatty acids, glycerides, steryl esters, sterols) were extracted using n-hexane then the hydrophilic ones (stilbenes, flavanols, monosaccharides, sugar alcohols) with acetone/water from sapwood and heartwood of *P. halepensis* stem [62]. Additionally, lipophilic and hydrophilic extractives were analyzed with GC-MS that were more abundant in heartwood than in sapwood of *P. halepensis* [63]. Seed oil of *P. halepensis* extracted with hexane was rich in fatty acid composition with linoleic and oleic acids accounting for more than 76% [64].

Terpinen-4-ol, *cis*- β -terpineol, γ -terpinene, sabinene, α -terpinene, and 4-thujanol were identified as the main compounds in *S. terebinthifolius* branch EO. The EO from *S. terebinthifolius* showed the presence of several compounds related to monoterpenes, sesquiterpene, and some other organic compounds [65–71]. Another study showed that the most abundant component of *S. terebinthifolius* EO was limonene [6]; also, α -phellandrene, β -phellandrene, α -pinene, and limonene were reported in the EO of *S. terebinthifolius* [69,72].

S. terebinthifolius EO compounds such as α - β -pinene, α -funebrene, Z-salvene, sabinene, and limonene [73] were isolated from fruits, while the compounds β -Longipinene, bicyclogermacrene, germacrene D, and β -pinene were found in leaves [74]. Although, mixed monoterpenes and sesquiterpenes were reported in unripe fruits; namely, α -cadinol, δ -cadinene, elemole, Δ^3 -carene, germacrene D-4-ol, β -phellandrene, epi- α -cadinol, and germacrene D [67]. On the other hand, the terpenes limonene, sabinene, Δ^3 -carene, and *p*-cymene were detected as main components of *S. terebinthifolius* ripe fruit EO [27].

In the present study, both oils were observed to have potential antimicrobial activity in terms of microbial inhibition when treating wood. The monoterpene or sesquiterpene hydrocarbons and their oxygenated derivatives are considered as potential antimicrobial agents by many studies. Thus, these results agree with other previous works [9,18,21,26,75,76].

Recently, schinol and a new biphenyl compound (4'-ethyl-4-methyl-2,2',6,6'-tetrahydroxyl(1,1'-biphenyl)-4,4'-dicarboxylate), isolated from hexane and dichloromethane fractions obtained from leaves and stems, showed marked antifungal activity against *P. brasiliensis* [34]. Santos et al. [77] found that EOs from *S. terebinthifolius* and *S. molle* could be a possible alternative to control diseases caused by phytopathogenic fungi that affect agricultural production. The EOs from *S. terebinthifolius* showed a pronounced fungicide effect against *Botrytis* ssp isolated from "gerberas" and roses [73]. The antifungal activity may be attributed to the presence of the said chemical components in the EO; for instance, *O*-cymene and limonene have been shown to have strong antifungal properties [78]. Nevertheless, the mechanism of antifungal activity of this EO is still unknown. However, a recent study on the induced damage to the cell membrane structure of yeast and isolated mitochondria suggests that phytoconstituents from the EO of *S. terebinthifolius* are likely to disrupt the permeability barrier of the cell membranes and thereby inhibit respiration [79,80]. Furthermore, it has been suggested that the differences in susceptibility of tested organisms to monoterpenes and the differences in the efficacy of different monoterpenes may possibly be explained by the variation in the rate of monoterpene penetration and characteristic membrane structure [80].

Several authors who have studied the antimicrobial activity of Pinaceae family [81–83] report that monoterpenes possess a high antifungal activity. The absence of monoterpenes in our EO can explain the absence of a very high activity of Aleppo pine EO, but aldehydes are often found as constituents of plant products [84] and may play an important role in the observed antimicrobial activity of plant aldehyde-containing material [85]. Their action, very likely due to an alteration in the function of membrane-associated proteins, seems to be exerted mainly at the cell surface. However, the capability to penetrate the outer layer of cells can help to explain the antimicrobial activity of some aldehydes, especially against Gram-negative bacteria [86,87]. On the other hand, the antifungal activity of our EO may be due to the presence of *n*-caprylaldehyde (hexanal) which is commercially used to produce fruity flavors and to prevent fruit spoilage. Meanwhile, another study confirmed that the saturated aldehydes hexanal, nonanal, and (*E*)-2-octenal showed almost no antimicrobial activity against some clinical bacteria [88], while the same study proved that the complex of long chain aldehydes—which highly presented in our EO—has very good antimicrobial activity, as they stated that α,β -unsaturated aldehydes ((*E*)-2-eptenal, (*E*)-2-nonenal, (*E*)-2-decenal, and (*E,E*)-2,4-decadienal) were tested together (ratio = 1:1:1:1) against ATCC bacterial isolates and clinically isolated microbial strains, and a strong synergic effect was observed [88]. Moreover, the effectiveness of the aldehydes seems to depend not only on the presence of the α,β -double bond, but also on the chain length from the enal group and on the microorganism tested. Finally these oil α,β -unsaturated aldehydes might be good alternatives to other highly toxic disinfectants, such as glutaraldehyde [89]. In another study [18], the *P. halepensis* EO was shown to possess antifungal activity against *A. flavus*, *A. niger*, *F. oxysporum*, and *R. stolonifera*, and thus can be used as a natural treatment for fungal infections, as well as a natural preservative in food.

The treated wood of *Acacia saligna* at the concentration of 5% with the methanolic extracts of *Morus alba* heartwood, *Cupressus sempervirens* wood, and *Maclura pomifera* bark showed no fungal growth of *T. harzianum* on the surface of the wood [52]. An inhibition zone was found against *T. harzianum* for *A. saligna* wood treated with the methanolic extract of *C. sempervirens* wood at concentrations of 5%, 10%, and 20% [52]. Wood samples of *P. sylvestris*, *F. sylvatica*, and *P. rigida* treated with the EOs or extracts from *E. camaldulensis* leaves or *P. rigida* wood had a potential effect against the growth of some phytopathogenic fungi [54,56].

Acer saccharum bark acetone extract, in combination with citric acid, applied to *Leucaena leucocephala* wood showed some activities against *T. viride*, *F. subglutinans*, and *A. niger* [4]. No growth of *A. flavus* and *C. gloeosporioides* was observed for sheets of Papyrus (*Cyperus papyrus* L.) strips pre-treated with

P. rigida and *E. camaldulensis* or *Salix babylonica* extracts [90]. *Melia azedarach* wood samples treated with *A. saligna* flowers water extract showed activity and growth inhibition against *F. culmorum*, *R. solani*, and *P. chrysogenum* [8], while the same treated with the methanol extract of *Musa paradisiaca* peels showed potential antifungal activity against *F. culmorum* and *R. solani* [7]. Additionally, the same wood treated with oils extracted with *n*-hexane from *E. camaldulensis* and *Vitex agnus-castus* showed promising antifungal activities against *F. culmorum*, *R. solani*, and *P. chrysogenum* [3].

Among the chemical components of the oils, there are synergistic or antagonistic interaction effects. The mode of action of EOs has been investigated by many authors, who suggested that antimicrobial activity is produced by interactions provoked by terpenes in the enzymatic systems related with energy production and in the synthesis of structural components of the microbial cells [91]. Other reports have suggested that the components of the EOs cross the cell membrane, interacting with the enzymes and proteins of the membrane such as the H⁺-ATPase pumping membrane, thus producing a flux of protons toward the cell exterior, which induces changes in the cells and, ultimately, their death [92]. Furthermore, several works [93–96] have explained how the antimicrobial ability of terpenes stop the functions of cell membranes, not only the permeability, how they might penetrate the membrane to the cell interior organs and interact critically with intracellular sites. Moreover, Daferera et al. [97] reported that the antifungal activity of EOs may be due to formation of hydrogen bonds between the hydroxyl group of oil phenols and the active sites of the target enzymes.

5. Conclusions

The antifungal activity of the wood of *Pinus roxburghii* treated with *P. halepensis* branch *n*-hexane oily liquid extract and *S. terebinthifolius* branch essential oil was tested against *B. oryzae*, *F. oxysporum*, *F. solani*, and *R. solani*. Both the extracted essential oil and oily liquid extract inhibited *F. oxysporum* isolate growth with a percentage of ca. 40% at a concentration of 3%, and showed good bioactivity against the growth of *B. oryzae*, *F. oxysporum*, and *R. solani*. These activities could be related to monoterpenoids, the most abundant compounds identified in the extracts. Therefore, both natural extracted materials could be considered as a wood-biofungicide agent and for their possible use on a larger scale.

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