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Evaluation of usage three natural extracts applied to three commercial wood species against five common molds

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ABSTRACT

Natural extracts have become of high interest in the past ten years for their inhibiting the growth of molds over wood and wood products surfaces in service or during the storage of building materials. In the present study, the antifungal effects of three natural extracts applied to three woods against five common molds were assessed. The growth of fungal hyphae of *Alternaria alternata*, *Fusarium subglutinans*, *Chaetomium globosum*, *Aspergillus niger*, and *Trichoderma viride* on the surfaces of *Pinus sylvestris*, *Pinus rigida* and *Fagus sylvatica* woods treated with extracts of *Pinus rigida* (heartwood), *Eucalyptus camaldulensis* (leaves) and *Costus speciosus* (rhizomes) was visually estimated. GC/MS and FTIR analyses were used to identify the chemical constituents and the functional groups of extracts. α-terpineol (24.91%), borneol (10.95%), terpin hydrate (9.60%), D-fenchyl alcohol (5.99%), and limonene glycol (5.05%), which are the main constituents of *P. rigida* heartwood methanol extract. The main chemical compounds of methanol extract from *Eucalyptus camaldulensis* leaves were spathulenol (18.89%), cryptone (5.79%), 4,6,6-trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo[5.1.0.0(2,4)]octane (5.79%), (3,3-dimethylcyclohexylidene)-(E)-acetaldehyde (5.57%), and ascaridole (4.32%). The main constituents identified in the distilled water extract from *Costus speciosus* rhizomes were *meso*-erythritol (12.21%), methyl-2-methyl-1,3-oxothiolan-2-yl-ketone (11.61%), (all-Z)-5,8,11,14,17-eicosapentaenoic acid-methyl ester (9.74%), diosgenin (5.07%), 2-ethyl-3-hydroxy-4H-pyran-4-one (4.43%), 3′,4′,7-trimethylquercetin (3.17%), and digitoxin (2.77%). Wood specimens treated at the level of 2% concentration of *P. rigida* heartwood extract observed good inhibition to the mold growth under laboratory conditions. These findings support the potential use of natural extracts for natural wood protection against mold infestation for surface treatment of wood. The results indicate that wood extracts may be useful for reducing the incidence of mold on wood products, but none of the materials evaluated completely inhibited the test fungi. These extracts may provide a useful value-added application for by-products of lumber production from these species.

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1. Introduction

Molds can grow on surfaces of wood and other organic materials and utilize the wood carbohydrates as a nutrient source and have colorless or pale colored mycelium and colored spores over the wood and wood products surfaces causing discoloration by their pigmented spores and mycelia, also soft rotting could be appeared (Zabel and Morrell, 1992; Mansour and Salem, 2015; Xu et al., 2015). Molds usually do not damage the wood structure and they can consume the compounds present in ray cells of sapwood and axial cell lumens like simple sugars and starch (Kerner-Gang and Schneider, 1969; Blanchette et al., 1992; Fabbri et al., 1997; Breuil, 1998; Mesquita et al., 2009; Mansour and Salem, 2015; Salem et al., 2016; Salem, 2016).

Molds can be washed or planed off from wood surfaces but, in some cases, they are able to decompose cellulose and lignin causing more damage (Bridžiuvienė and Raudonienė, 2013). Ascomycetes and deuteromycetess (soft rot fungi), can affect and modify the

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lignin by degrading wood polysaccharides more readily than lignin (Istek et al., 2005; da Silva et al., 2007; Pournou and Bogomolova, 2009) and considered to be the first decay type of wood colonization (Eaton and Hale, 1993).

The decay mechanisms caused by soft rot fungi have been reported by producing reactive oxygen species (ROS) (Hammel et al., 2002), where its pattern could be appeared as a thinning and, erosion of the cell walls, chain of cavities following the microfibril angle in the secondary cell wall, and/or erosion from the cell lumen towards the middle lamella (Daniel, 2003; Popescu et al., 2011).

Molds like *Alternaria*, *Trichoderma*, *Aspergillus*, *Chaetomium* and *Fusarium* species and other fungi are capable to biodegrade wood and other lignocellulosic materials by producing cellulolytic enzymes (Ueno, 1985; Filho et al., 1996; Parenicová et al., 2000; Payne and Bruce, 2001; Kaushal et al., 2012; Ljaljević-Grbić et al., 2013; Bridžiuvienė and Raudonienė, 2013). Moreover, the surface elemental compositions of *Pinus rigida*, *Juglans nigra*, and *Fagus sylvatica* woods (Salem, 2016); *Citharexylum spinosum* and *Morus alba* (Mansour et al., 2015a) were superficially changed as inoculated with the molds *Penicillium selerotigenum*, *Paecilomyces variotii*, and *Aspergillus niger*.

Natural extracts have been widely used in several studies and become potentially useful for protecting wood against the growth of molds and destroying fungi (Jeloková and Šindler, 1997; Philp et al., 1995; Qi and Jellison, 2004; Wang et al., 2005; Yang and Clausen, 2007; Bakar et al., 2013; Salem et al., 2016; Mansour and Salem, 2015; Zhang et al., 2016). Natural extracts could be a possible approach for developing new wood preservatives (Sen et al., 2002).

In the present study, three natural extracts from *Pinus rigida* (heartwood), *Eucalyptus camaldulensis* Dehnh. (leaves) and *Costus speciosus* (rhizomes) were evaluated against the growth of five common molds namely, *Alternaria alternata*, *Fusarium subglutinans*, *Chaetomium globosum*, *Aspergillus niger*, and *Trichoderma viride*.

Previously, different extracts from *E. camaldulensis* had antifungal activity *Microsporium gypseum*, *Trichophyton mentagrophytes* and *Candida albicans* (Babayi et al., 2004; Essien and Akpan, 2004). Saponin and *P*-coumaric acid methyl ester from *C. speciosus* showed good antifungal activity against *Botrytis cineria*, *Alternaria* sp, *Cladosporium cladosporioides*, *Colletotrichum gleosporioides*, *Curvularia* sp. and *Penicillium* sp. (Bandara et al., 1988; Singh and Srivastava, 1992). On the other hand, for our knowledge, there is little information about the antifungal activity of extracts from heartwood of *P. rigida*, where in our previously work (Salem et al., 2016), the essential oils from heartwood observed good antifungal activity when applied to the three commercial woods against the same molds.

The chemical compositions of the extracts could be analyzed and screened by means of gas chromatography–mass spectrometry (GC/MS) technique (Salem et al., 2016). Additionally, the functional groups of different components can be identified in solid wood and different extracts of wood by means of FTIR (Chow, 1971; Ajuong and Breese, 1998; Nuopponen et al., 2003; Aluong and Redington, 2004; Nuopponen, 2005).

The aim of the present work was to evaluate the antifungal effect of three natural extracts from against the growth of five common molds. The evaluation was done by application of the three natural extracts for preservation of three commercial wood species (*Pinus sylvestris*, *Pinus rigida* and *Fagus sylvatica*) against the biodeterioration caused by the studied molds. Additionally, the suggested chemical compositions of the extracts were identified by means of GC/MS. In addition, the FITR spectrophotometer technique was used to identify the functional groups of extracts.

2. Materials and methods

2.1. Preparation of wood blocks

In the present study, sapwood blocks with dimensions of 10 × 10 × 5 mm from Scots pine (*Pinus sylvestris* L., Pinaceae), Pitch pine (*Pinus rigida* Mill., Pinaceae), and European beech (*Fagus sylvatica* L., Fagaceae) were air-dried to constant weights.

2.2. Preparation of extracts

P. rigida heartwood, *Costus speciosus* rhizomes and *Eucalyptus camaldulensis* leaves were air-dried at room temperature and ground to 40–60 mesh. Methanol (80%) with *P. rigida* wood and *E. camaldulensis* leaves and distilled water (DW) with *C. speciosus* rhizomes were used for the extraction. About 50 g from each material were macerated in 150 ml of the respective solvent for one week in a dark place in normal room temperature (Salem et al., 2013). It should be noted here, a few drops of ethanol were added to DW in the maceration procedure for *C. speciosus* rhizomes to prevent any contamination that could be appeared by bacterial or fungal growth. The solvents were evaporated using a rotary vacuum evaporator at 45 °C and then the extracts dried and stored in sealed vials at 4 °C until further use. The extracts of *P. rigida*, *E. camaldulensis* and *C. speciosus* were prepared at different concentrations of 2%, 1%, 0.5%, 0.25%, 0.13%, and 0.063% by diluting the respective amount of extract in 10% dimethyl sulfoxide (DMSO, Sigma-Aldrich).

2.3. Wood treatment by extracts

Wood blocks of 10 × 10 × 5 mm from each wood type were autoclaved at 121 °C for 20 min and left to cool. 15 samples from each wood type were immersed in a solution of each concentration of extracts (2%, 1%, 0.5%, 0.25%, 0.13%, and 0.06%) for 8 h (Fig. 1) and then the samples were air-dried for 24 h (Mansour and Salem, 2015).

2.4. Biodeterioration of wood by molds in vitro

The treated and untreated commercial woods with extracts were studied for their biodeterioration by five common molds namely; *Alternaria alternata*, *Fusarium subglutinans*, *Chaetomium globosum*, *Aspergillus niger*, and *Trichoderma viride* (Provided from stock cultures of Microbiology Laboratory, Conservation Department, Faculty of Archaeology, Cairo University). 15-day-old PDA culture of each fungus was prepared. After treating wood samples with extracts, they were inoculated with a disc (5 mm diameter) of each fungus in a Petri dish that contained 15 ml of PDA-culture and were incubated for three days for *T. viride* and one week for the other fungi at 25 ± 1 °C. Three replicates were used for each particular concentration. DMSO (10%) was used as an alternative in the control sample. The inhibition zones (IZs, mm) of extracts around the treated wood samples against each fungus were measured and recorded using recommendations of previously published work (Satish et al., 2007; Essa and Khallaf, 2014; Mansour and Salem, 2015; Mansour et al., 2015b).

2.5. Visual observation

The extent of fungal growth was visually evaluated after two week, and 4 months of inoculation by the naked eye (Salem et al., 2016) and the fungicidal activity was estimated by fungal growth retardation, using the following visually determined marks recommended by Humar and Pohleven (2005) as shown in Table 1.

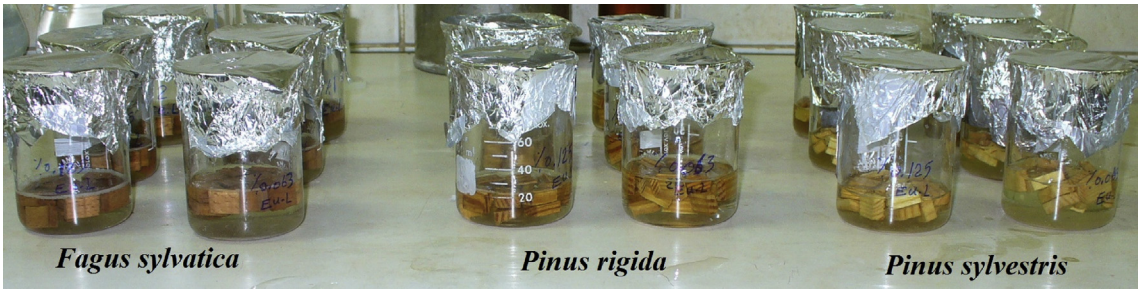


Fig. 1. Immersion method used for treating wood samples with natural extracts.

Table 1
Visual of fungal growth retardation marks.[a]

Mark	Mycelial growth
0	mycelium growth more intense than control
1	normal growth, insignificant retardation (area of colony ≥90% of area of controls)
2	visible signs of retardation (colony <90% and ≥60% of controls)
3	pronounced retardation (colony <60% and ≥25% of controls)
4	very marked retardation (colony <25% of controls)
5	no growth

[a] Measured according to Humar and Pohleven (2005).

2.6. GC/MS analysis of the extracts

Analysis of chemical compositions of the studied extracts was carried out using GC/MS apparatus at Atomic and Molecular Physics Unit, Experimental Nuclear Physics Department, Nuclear Research Center, Egyptian Atomic Energy Authority, Inshas, Cairo, Egypt.

The chemical composition of the extracts were performed using Trace GC Ultra-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m × 0.25 mm × 0.25 μm film thickness). The column oven temperature was initially held at 120 °C and then increased by 5 °C min^{-1} to 200 °C with holding 2 min then increased to 280 °C (10 °C min^{-1}). The injector and detector (MS transfer line) temperatures were kept at 250 °C. Helium was used as a carrier gas at a constant flow rate of 1 ml min^{-1}. The solvent delay was 2 min and diluted samples of 1 μl were injected automatically using Autosampler AS3000 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of *m*/*z* 40–550 in full scan mode. The ion source and transfer line temperatures were set at 200 and 250 °C, respectively. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 (Oberacher, 2011) and NIST 11 Mass Spectral (2011) databases.

2.7. FTIR spectroscopic analysis

Wood samples were analyzed with a Perkin Elmer Spectrum One spectrometer equipped. Extractives were analyzed on a (Perkin Elmer-FT-IR system-spectrum BX) spectrometer, at the Micro Analytical Center, Cairo University, using the KBr pellet method at a resolution of 4 cm^{-1} ranging from 400 to 4000 cm^{-1}. Standard Ø13 mm diameter pellets were prepared by mixing and pressing 10 mg of the dried wood extractive sample in 300 mg of KBr for 5 min under 200 bar pressure. Three parallel measurements were performed. The obtained FTIR spectra were further processed using the computer software, Spectrum One (ver. 5.0.1) (Miklečić et al., 2012).

2.8. Statistical analysis

Results of application of extracts from *P. rigida* (wood), *E. camaldulensis* (leaves) and *C. speciosus* (rhizomes) on the three studied wood types (*P. sylvestris*, *P. rigida* and *F. sylvatica*) against the growth of each fungus were statistically analyzed using Analysis of variance (ANOVA) procedure in SAS version 8.2 (2001). Fisher's Least Significant Difference (LSD) at 5% level of probability was used to measure the differences among the means.

3. Results and discussion

3.1. Visual observation after 14 days

Antifungal effects of extracts applied to the three wood types against five common molds were assessed and compared with the control sample and the visual observations of the growth are presented in Figs. 2–4 with growing marks shown in Table 2.

Application of *Pinus rigida* wood extracts (Fig. 2) to the three studied wood types showed remarkable effects against the studied fungi. Complete inhibition against the growth of *Alternaria alternata*, *Fusarium subglutinans*, *Chaetomium globosum*, *Aspergillus niger* and *Trichoderma viride* was found (Fig. 2). The highly fungal growth inhibition was observed by applying of *P. rigida* wood extracts at high (2%) and low (0.063%) concentrations of the extract, even against *T. viride* at low concentration there was no fungal growth around the treated woods with the extract. The application of *E. camaldulensis* leaves extract for the three wood types did not show activity against the growth of the studied fungi at high or low concentration in comparison with the control sample (Fig. 3). The extracts from *C. speciosus* (rhizomes) had only activity against *C. globosum* and some activity was observed against *F. subglutinans* at the high concentration (2%). Other fungi showed resistance to the extracts of *C. speciosus* rhizomes (Fig. 4).

Table 2 summarizes the visual fungicidal activity by fungal growth retardation which ranged from 1 to 5 marks (Humar and Pohleven, 2005). Methanol extract from *P. rigida* wood applied at all the concentrations showed good retardation of the studied fungi where the marks ranged from 3 to 5 against all the studied fungi.

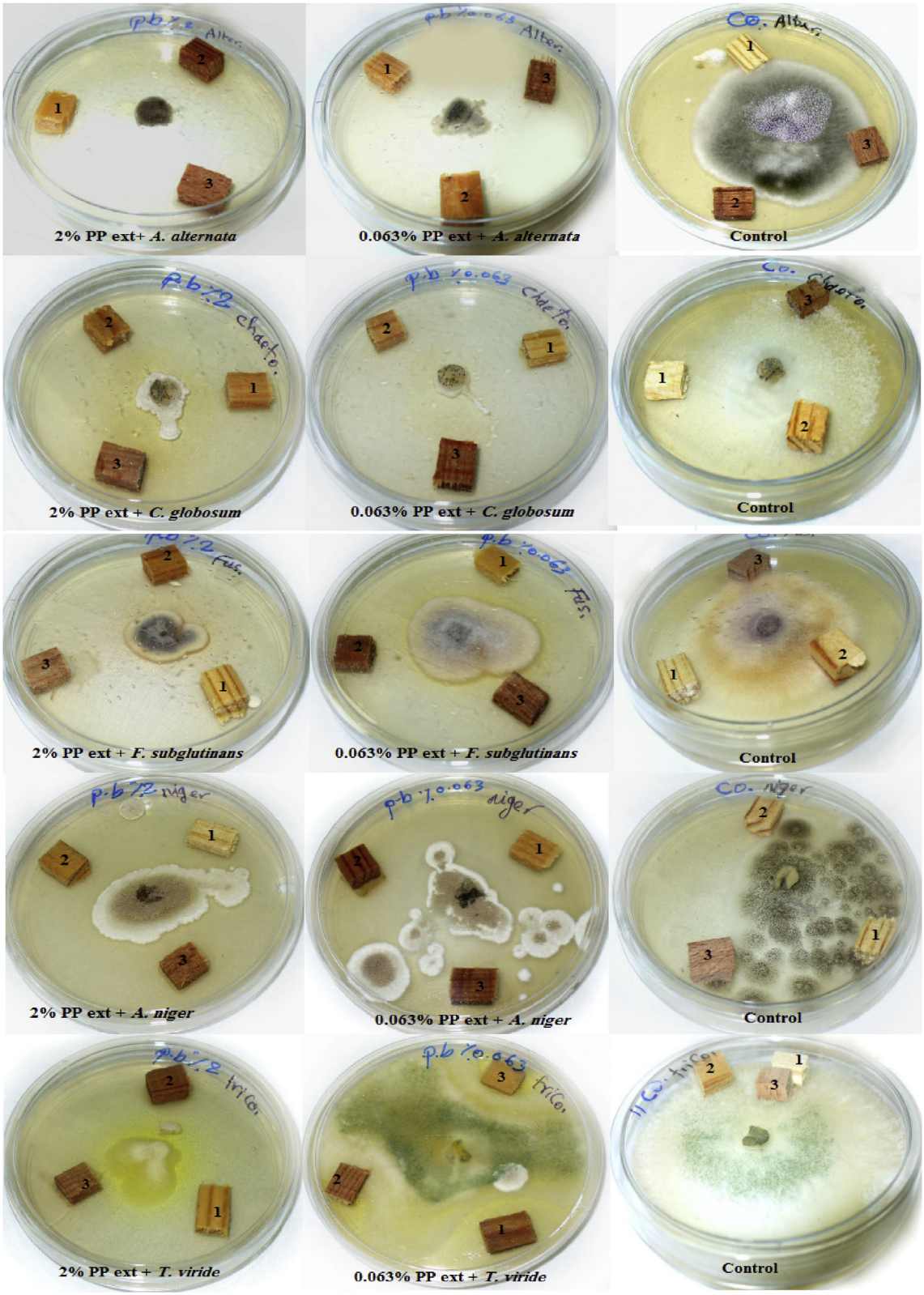


Fig. 2. Effect of *P. rigida* heartwood methanol extract on the growing of five molds over surfaces of three commercial woods (1- *P. sylvestris*, 2- *P. rigida* and 3- *F. sylvatica*).

The growth of *A. alternata* and *C. globosum* was suspended at the medium concentration (0.5%). Extract of *E. camaldulensis* leaves showed only good retardation against the growth of *A. niger* with mark 3 at 2% and the extract showed weak activity against other tested fungi. DW extract of *C. speciosus* rhizomes showed good activity against the growth of *A. alternata*, *A. niger*, *C. globosum*, and *F. subglutinans* where the marks ranged between 3 and 4.

The inhibitory effect wood specimens soaked in different

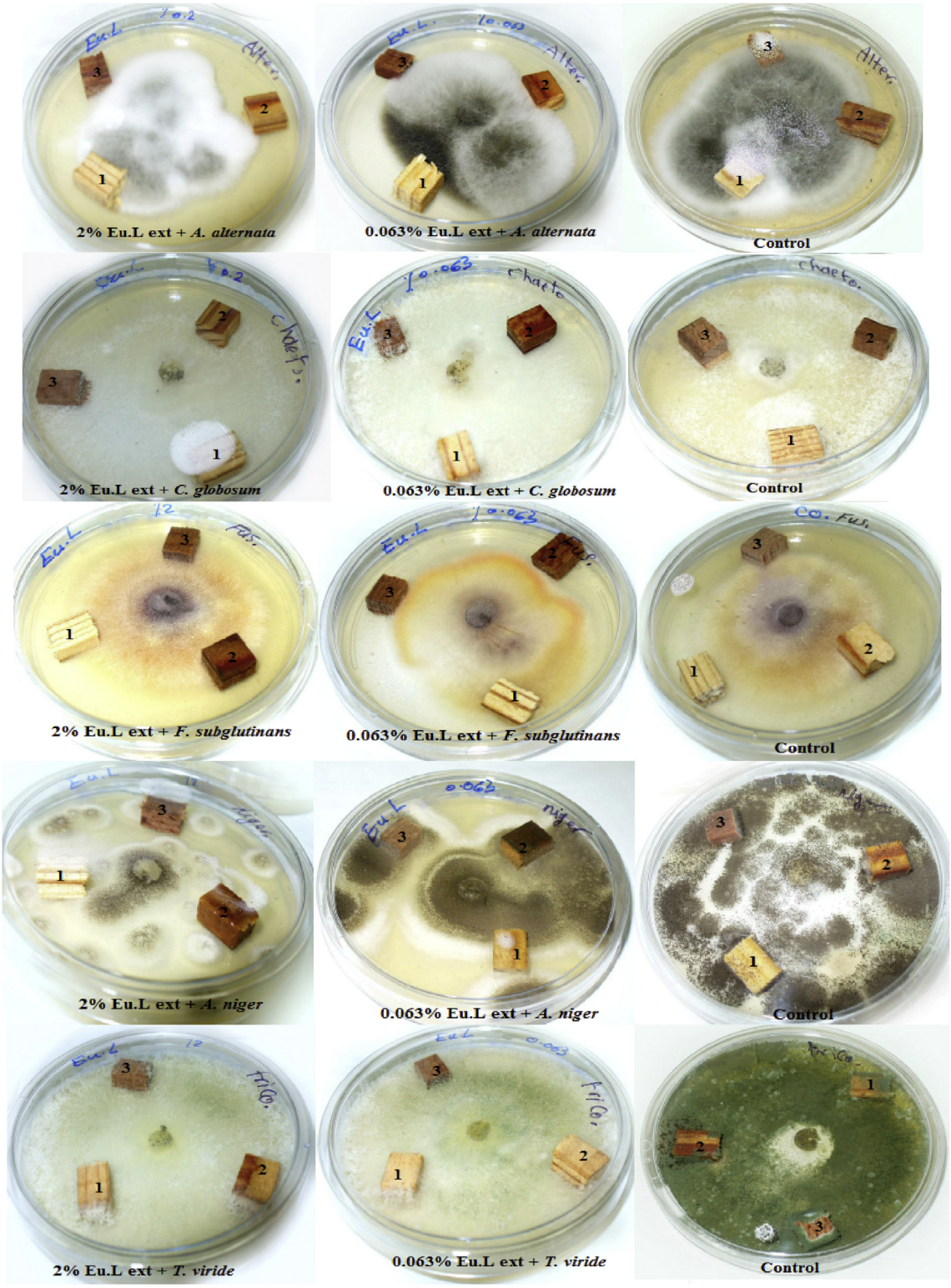


Fig. 3. Effect of *E. camaldulensis* leaves methanol extract on the growth of five molds over surfaces of three commercial woods (1- *P. sylvestris*, 2- *P. rigida* and 3- *F. sylvatica*).

extracts was low for the extracts from *E. camaldulensis* and very weak with *C. speciosus* (rhizomes). *P. rigida* wood extracts completely inhibited all test fungi. These results are in consistence with our previous work with the same wood species, where the essential oil from wood of *P. rigida* showed the highest activity against the same studied mold fungi (Salem et al., 2016).

Molds isolated from painted or preservative-treated woods are recognized as soft-rot fungi (Kim et al., 2007; Pournou and Bogomolova, 2009; Råberg et al., 2009). On wood joints, *Alternaria* fungi caused distinctly seen dark grey discoloration (Domsch et al., 2007). *A. alternata* was the most active tyrosinase producer as well as phenoloxidase activity that degrades lignin (Bridžiuvienė

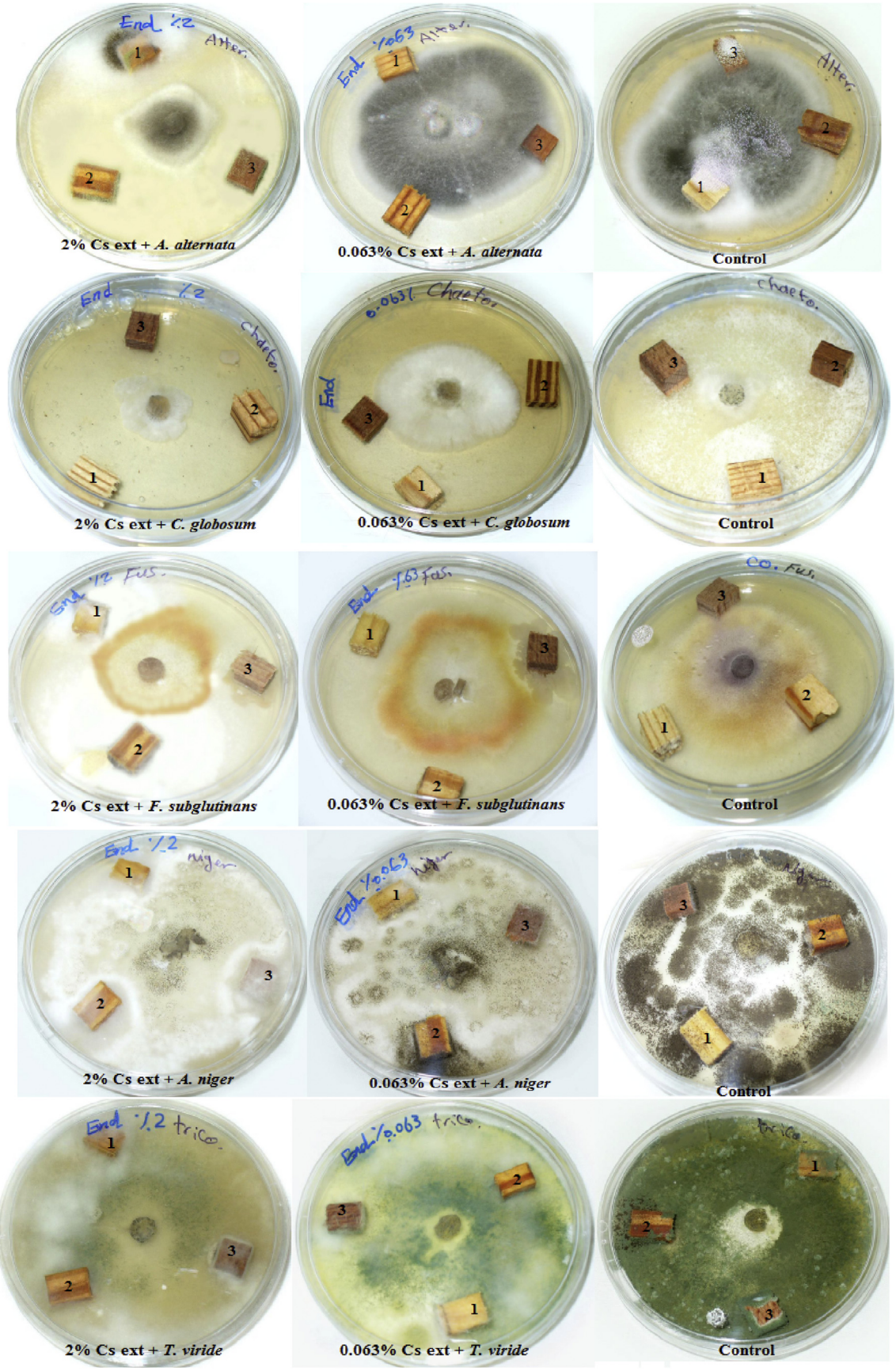


Fig. 4. Effect of *C. speciosus* rhizomes DW extract on the growth of five molds over surfaces of three commercial woods (1- *P. sylvestris*, 2- *P. rigida* and 3- *F. sylvatica*).

Table 2
Results of fungal growth retardation marks.[a]

Treatments		Fungal growth retardation marks				
Extract	Concentration (%)	*A. alternata*	*F. subglutinans*	*C. globosum*	*A. niger*	*T. viride*
P. rigida wood methanol extract	2%	5	4	5	4	5
	1%	5	4	5	4	4
	0.50%	5	3	5	3	4
	0.25%	4	3	4	3	4
	0.13%	5	4	4	3	3
	0.06%	4	3	4	3	4
E. camaldulensis leaves methanol extract	2%	2	2	2	3	1
	1%	1	1	2	3	1
	0.50%	1	1	1	1	1
	0.25%	1	1	1	1	0
	0.13%	0	1	1	1	0
	0.06%	0	0	0	1	0
C. speciosus rhizomes DW extract	2%	3	3	4	4	2
	1%	2	3	3	3	2
	0.50%	2	3	3	3	1
	0.25%	1	3	3	2	1
	0.13%	0	2	2	1	1
	0.06%	0	2	3	1	1

[a] Measured according to Humar and Pohleven (2005).

and Raudonienė, 2013).

Trichoderma species produces cellulase and hemicellulases enzymes (Gautam, 2010). *T. viride* hyphae are shown in ray parenchyma cells of common radiata pine (Xiao et al., 1999), and green mold contamination was observed in wood, wooden pallets and concrete (Abosriwil and Clancy, 2002). Among four species of *Trichoderma*, the *T. viride* enzyme achieved the highest glucose conversion (90.09%) of cellulose from steam pretreated *Lespedeza* stalk (*Lespedeza crytobotrya*) (Feng et al., 2011). *Trichoderma* species have been shown to degrade pit membranes and increase penetration of preservatives (Green et al., 1996).

The degradation action of *A. niger* was reported by the produced pectinases and hemicellulose enzymes like xylanases and arabinases (Delgado et al., 1992; Van Peij et al., 1997; Parenicová et al.,

Table 3
Means of inhibition zones (mm) of natural extracts at different concentrations applied to *F. sylvatica* wood against the growth of *A. alternata*, *F. subglutinans*, *C. globosum*, *A. niger*, and *T. viride*.

Treatments		Inhibition zone (IZ, mm)														
		A. alternata[a]			*F. subglutinans*[a]			*C. globosum*[a]			*A. niger*[a]			*T. viride*		
Extract (A)	Concentration (%) (B)	Mean	−95%	+95%	Mean	−95%	+95%	Mean	−95%	+95%	Mean	−95%	+95%	Mean	−95%	+95%
P. rigida wood methanol extract	Control (0%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2%	19.0	16.5	21.4	10.3	6.5	14.1	17.0	10.4	23.5	7.0	2.0	11.9	10.3	5.1	15.5
	1%	15.0	12.5	17.4	8.6	7.2	10.1	13.0	8.0	17.9	7.3	4.4	10.2	9.0	6.5	11.4
	0.50%	14.0	7.4	20.5	6.0	3.5	8.4	13.0	8.7	17.3	4.0	1.5	6.4	7.6	1.4	13.9
	0.25%	13.6	−0.4	27.7	8.3	4.5	12.1	15.0	10.0	19.9	4.3	−0.8	9.5	5.6	0.5	10.8
	0.13%	13.6	−0.4	27.7	8.0	3.0	12.9	14.0	9.0	18.9	3.6	2.2	5.1	8.0	3.7	12.3
	0.06%	13.3	3.9	22.7	5.3	−2.6	13.3	11.6	6.5	16.8	3.0	0.5	5.4	3.0	−1.3	7.3
E. camaldulensis leaves methanol extract	Control (0%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2%	1.3	−0.1	2.7	0.3	−1.1	1.7	1.6	−2.1	5.4	1.3	−0.1	2.7	0.0	0.0	0.0
	1%	0.3	−1.1	1.7	0.3	−1.1	1.7	1.6	−1.2	4.5	0.6	−0.7	2.1	0.0	0.0	0.0
	0.50%	0.0	0.0	0.0	0.0	0.0	0.0	1.0	−1.4	3.4	1.0	−1.4	3.4	0.0	0.0	0.0
	0.25%	0.0	0.0	0.0	0.0	0.0	0.0	1.0	−1.4	3.4	0.3	−1.1	1.7	0.0	0.0	0.0
	0.13%	0.0	0.0	0.0	0.0	0.0	0.0	0.6	−0.7	2.1	0.0	0.0	0.0	0.0	0.0	0.0
	0.06%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. speciosus rhizomes DW extract	Control (0%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2%	3.0	0.5	5.4	4.0	1.5	6.4	10.0	5.0	14.9	7.6	6.2	9.1	0.6	−0.7	2.1
	1%	3.3	1.9	4.7	3.6	−1.5	8.8	9.0	6.5	11.4	5.3	2.4	8.2	0.0	0.0	0.0
	0.50%	2.3	0.9	3.7	2.3	−3.4	8.1	9.3	7.9	10.7	3.6	−0.1	7.4	0.0	0.0	0.0
	0.25%	1.6	0.2	3.1	2.6	−1.1	6.4	7.0	4.5	9.4	3.0	0.5	5.4	0.0	0.0	0.0
	0.13%	0.3	−1.1	1.7	2.3	0.9	3.7	5.6	4.2	7.1	2.0	−0.4	4.4	0.0	0.0	0.0
	0.06%	0.0	0.0	0.0	0.3	−1.1	1.7	6.3	4.9	7.7	0.0	0.0	0.0	0.0	0.0	0.0
Mean (B)	Control (0%)	0.0			0.0			0.0			0.0			0.0		
	2%	7.7			4.8			9.5			5.3			3.6		
	1%	6.2			4.2			7.8			4.4			3.0		
	0.50%	5.4			2.7			7.7			2.8			2.5		
	0.25%	5.1			3.6			7.6			2.5			1.8		
	0.13%	4.6			3.4			6.7			1.8			2.6		
	0.06%	4.4			1.8			6.0			1.0			1.0		
L.S.D. at 0.05	**A**	**1.29**			**0.80**			**0.85**			**0.61**			**0.64**		
	B	**1.98**			**1.22**			**1.30**			**0.93**			**0.98**		
	A x B	**3.24**			**2.22**			**2.49**			**1.99**			**1.73**		

[a] Values of ±95% are confidence limits (mm).

2000). *A. niger* produced soft-rot decay type I (cavity formation) and soft-rot decay type II (erosion), as sapwoods of *Pinus halepensis* and *Ficus sycomorus* degraded in soil (Hamed, 2013).

C. globosum was found in building materials, which produce the highly cytotoxic chaetomins and chaetoglobosins that inhibit cell division and glucose transport (Ueno, 1985; Andersen and Nissen, 2000). The hyphae of *C. globosum* grow almost exclusively in the S2 layer of the wood cell wall resulted as distinct decay cavities revealed in longitudinal sections of lime wood (Popescu et al., 2011).

F. subglutinans produce extracellular enzymes with strong activity like β-glucosidase and amylase, where amylase is the enzyme, which degrades amylose in starch polymers and β-glucosidase degrades D-cellobiose to glucose (Kwon et al., 2007).

3.2. Antifungal activity of F. sylvatica, P. rigida and P. sylvestris wood blocks treated with extracts

Results observed in Tables 3–5 show that there were highly significant effects of the studied extracts applied to the three woods against the growth of *A. alternata*, *F. subglutinans*, *C. globosum*, *A. niger*, and *T. viride*.

Antifungal activities values of the applied extracts to *F. sylvatica* wood showed that the highest inhibition zones (IZs) against the studied molds around the treated woods were recorded by the methanol extract of *P. rigida* heartwood with IZs values of 19.0 mm, 10.3 mm, 17.0 mm, 7.0 mm and 10.3 mm against the growth of *A. alternata*, *F. subglutinans*, *C. globosum*, *A. niger*, and *T. viride*, respectively (Table 3). Methanol extract from *E. camaldulensis* leaves and *C. speciosus* rhizomes DW extract showed weak activity against the five mold fungi, except against *C. globosum*, where the IZ values reached 10.0 mm at the concentration of 2% with DW extract of *C. speciosus* rhizomes.

Wood of *P. rigida* as a control did not observe any IZ against the growth of the studied molds, but at 2% concentration of its methanol extract, good antifungal activities were found with IZs values of 20.6 mm, 10.0 mm, 19.0 mm, 9.6 mm, and 12.0 mm, against the growth of *A. alternata*, *F. subglutinans*, *C. globosum*, *A. niger*, and *T. viride*, respectively (Table 4). Furthermore, *A. alternata* showed good response to all the concentrations used of methanol extract of *P. rigida* heartwood and the IZs values ranged from 20.6 mm (2%) to 15.3 mm (0.063%).

The methanol extract from *E. camaldulensis* leaves and *C. speciosus* rhizomes DW extract showed weak activity against the tested molds, except against *C. globosum*, where the IZs values ranged from 11.0 mm (2%) to 7.3 mm (0.063%) and moderate activity at 2% against *F. subglutinans* (IZ, 6.0 mm) and *A. niger* (IZ, 5.0 mm) with *C. speciosus* rhizomes DW extract.

Treated wood samples of *P. sylvestris* with *P. rigida* methanol extract showed good IZs against the growth of *A. alternata*, *F. subglutinans*, *C. globosum*, *A. niger*, and *T. viride* with values of 16.6 mm, 10.0 mm, 13.0 mm, 12.6 mm, and 11.3 mm, respectively, at 2% concentration (Table 5). Additionally, all the concentrations from *P. rigida* methanol extract observed good antifungal activity against the studied mold fungi. The methanol extracts from *E. camaldulensis* leaves did not show measurable IZs against the five mold fungi, while the DW extract of *C. speciosus* rhizomes showed good activity against *C. globosum* and *A. niger* with IZs values of 10.0 mm,

Table 4
Means of inhibition zones (mm) of natural extracts at different concentrations applied to *P. rigida* wood against the growth of *A. alternata*, *F. subglutinans*, *C. globosum*, *A. niger*, and *T. viride*.

Treatments		Inhibition zone (IZ, mm)														
		A. alternata[a]			*F. subglutinans*[a]			*C. globosum*[a]			*A. niger*[a]			*T. viride*		
Extract (A)	Concentration (B)	Mean	−95%	+95%	Mean	−95%	+95%	Mean	−95%	+95%	Mean	−95%	+95%	Mean	−95%	+95%
P. rigida wood methanol extract	Control (0%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2%	20.6	15.5	25.84	10.0	3.4	16.5	19.0	16.5	21.4	9.6	5.8	13.4	12.0	4.5	19.4
	1%	17.6	13.8	21.46	10.0	5.0	14.9	13.6	9.8	17.4	9.3	7.9	10.7	9.6	5.8	13.4
	0.50%	16.3	8.3	24.32	5.6	1.8	9.4	14.6	10.8	18.4	9.0	2.4	15.5	7.3	−0.6	15.3
	0.25%	14.3	10.5	18.13	8.6	4.8	12.4	12.3	6.0	18.5	5.0	2.5	7.4	8.6	7.2	10.1
	0.13%	17.6	9.6	25.65	9.0	4.0	13.9	12.3	7.1	17.5	4.3	0.5	8.1	8.6	7.2	10.1
	0.06%	15.3	0.1	30.51	6.6	5.2	8.1	9.3	7.9	10.7	3.0	−1.3	7.3	6.0	−0.5	12.5
E. camaldulensis leaves methanol extract	Control (0%)	0.0	0.0	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2%	1.6	0.2	3.10	1.3	−0.1	2.7	2.3	−1.4	6.1	1.6	−2.1	5.4	0.0	0.0	0.0
	1%	0.6	−0.7	2.10	0.3	−1.1	1.7	1.6	−1.2	4.5	1.3	−1.5	4.2	0.0	0.0	0.0
	0.50%	0.0	0.0	0.00	0.0	0.0	0.0	1.0	−1.4	3.4	0.6	−0.7	2.1	0.0	0.0	0.0
	0.25%	0.0	0.0	0.00	0.0	0.0	0.0	1.0	−1.4	3.4	0.3	−1.1	1.7	0.0	0.0	0.0
	0.13%	0.0	0.0	0.00	0.0	0.0	0.0	1.0	−1.4	3.4	0.3	−1.1	1.7	0.0	0.0	0.0
	0.06%	0.0	0.0	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. speciosus rhizomes DW extract	Control (0%)	0.0	0.0	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2%	4.0	1.5	6.48	6.0	3.5	8.4	11.0	2.0	19.9	5.0	5.0	5.0	1.3	−0.1	2.7
	1%	3.3	1.9	4.77	4.3	−0.8	9.50	10.0	3.4	16.5	4.3	2.9	5.7	0.0	0.0	0.0
	0.50%	2.3	−1.4	6.13	2.6	−2.5	7.8	10.3	6.5	14.1	4.3	2.9	5.7	0.0	0.0	0.0
	0.25%	2.0	−0.4	4.48	3.3	−1.8	8.5	8.0	3.7	12.3	2.6	1.2	4.1	0.0	0.0	0.0
	0.13%	0.6	−0.7	2.10	2.6	1.2	4.1	7.6	6.2	9.1	1.0	−1.4	3.4	0.0	0.0	0.0
	0.06%	0.0	0.0	0.00	1.0	−1.4	3.4	7.3	2.1	12.5	0.3	−1.1	1.7	0.0	0.0	0.0
Mean (B)	Control (0%)	0.0			0.0			0.0			0.0			0.0		
	2%	8.7			5.7			10.7			5.4			4.4		
	1%	7.2			4.8			8.4			5.0			3.2		
	0.50%	6.2			2.7			8.6			4.6			2.4		
	0.25%	5.4			4.0			7.1			2.6			2.8		
	0.13%	6.1			3.8			7.0			1.8			2.8		
	0.06%	5.1			2.5			5.5			1.1			2.0		
L.S.D. at 0.05	**A**	**1.16**			**0.81**			**0.99**			**0.65**			**0.74**		
	B	**1.769**			**1.245**			**1.51**			**0.99**			**1.13**		
	Ax B	**3.15**			**2.02**			**2.75**			**1.64**			**1.90**		

[a] Values of ±95% are confidence limits (mm).

Table 5
Means of inhibition zones (mm) of natural extracts at different concentrations applied to *P. sylvestris* wood against the growth of *A. alternata*, *F. subglutinans*, *C. globosum*, *A. niger*, and *T. viride*.

Treatments		Inhibition zone (IZ, mm)														
		A. alternata[a]			*F. subglutinans*[a]			*C. globosum*[a]			*A. niger*[a]			*T. viride*		
Extract (A)	Concentration (%) (B)	Mean	−95%	+95%	Mean	−95%	+95%	Mean	−95%	+95%	Mean	−95%	+95%	Mean	−95%	+95%
P. rigida wood methanol extract	Control (0%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2%	16.6	11.5	21.8	10.0	7.5	12.4	13.0	10.5	15.4	12.6	7.5	17.8	11.3	6.1	16.5
	1%	14.3	10.5	18.1	9.0	9.0	9.0	12.3	6.0	18.5	11.6	7.8	15.4	9.3	5.5	13.1
	0.50%	16.3	12.5	20.1	7.6	6.2	9.1	11.3	7.5	15.1	10.6	5.5	15.8	7.3	1.0	13.5
	0.25%	13.6	9.8	17.4	8.6	2.4	14.9	12.0	4.5	19.4	11.6	2.9	20.3	8.6	−0.0	17.3
	0.13%	15.0	4.1	25.8	8.0	3.0	12.9	9.6	−9.6	28.9	13.0	4.0	21.9	7.3	−3.0	17.6
	0.06%	14.6	−7.8	37.2	5.3	1.5	9.1	13.6	10.8	16.5	6.3	2.5	10.1	4.3	−1.4	10.1
E. camaldulensis leaves methanol extract	Control (0%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2%	1.3	−0.1	2.7	0.6	−0.7	2.1	2.0	−0.4	4.4	1.3	−2.4	5.1	0.0	0.0	0.0
	1%	0.6	−0.7	2.1	0.3	−1.1	1.7	2.0	2.0	2.0	1.0	−1.4	3.4	0.0	0.0	0.0
	0.50%	0.6	−0.7	2.1	0.0	0.0	0.0	1.6	0.2	3.1	1.0	−1.4	3.4	0.0	0.0	0.0
	0.25%	0.0	0.0	0.0	0.3	−1.1	1.7	1.0	−1.4	3.4	0.3	−1.1	1.7	0.0	0.0	0.0
	0.13%	0.0	0.0	0.0	0.0	0.0	0.0	1.3	−0.1	2.7	0.3	−1.1	1.7	0.0	0.0	0.0
	0.06%	0.3	−1.1	1.7	0.0	0.0	0.0	1.0	−1.4	3.4	0.0	0.0	0.0	0.0	0.0	0.0
C. speciosus rhizomes DW extract	Control (0%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2%	2.3	0.9	3.7	5.3	3.9	6.7	10.0	2.5	17.4	10.3	6.5	14.1	0.0	0.0	0.0
	1%	2.0	2.0	2.0	5.0	−1.5	11.5	9.0	6.5	11.4	10.3	5.1	15.5	0.0	0.0	0.0
	0.50%	1.0	−1.4	3.4	4.3	−0.8	9.5	10.0	7.5	12.4	9.3	7.9	10.7	0.0	0.0	0.0
	0.25%	0.6	−0.7	2.1	4.0	−0.9	8.9	9.3	7.9	10.7	7.6	3.8	11.4	0.0	0.0	0.0
	0.13%	0.0	0.0	0.0	2.6	−1.1	6.4	8.3	4.5	12.1	8.3	6.9	9.7	0.0	0.0	0.0
	0.06%	0.0	0.0	0.0	1.0	−1.4	3.4	6.6	1.5	11.8	0.6	−0.7	2.1	0.0	0.0	0.0
Mean (B)	Control (0%)	0.0			0.0			0.0			0.0			0.0		
	2%	6.7			5.3			8.3			8.1			3.7		
	1%	5.6			4.7			7.7			7.6			3.1		
	0.50%	6.0			4.0			7.6			7.0			2.4		
	0.25%	4.7			4.3			7.4			6.5			2.8		
	0.13%	5.0			3.5			6.4			7.2			2.4		
	0.06%	5.0			2.1			7.1			2.3			1.4		
L.S.D. at 0.05	**A**	**1.46**			**0.79**			**1.37**			**0.99**			**0.94**		
	B	**2.23**			**1.21**			**2.09**			**1.52**			**1.44**		
	Ax B	**3.65**			**2.05**			**3.90**			**2.80**			**2.92**		

[a] Values of ±95% are confidence limits (mm).

and 10.3 mm, respectively, at 2% concentration. In addition, the extracts from *E. camaldulensis* leaves and *C. speciosus* rhizomes were not active against *A. niger*, and *T. viride*. *P. sylvestris* wood treated with the extracts from *Muscari neglectum* and *Gynandriris sisyrinschium* were shown good antifungal activity against the decayed fungi *Postia placenta* and *Trametes versicolor* (Goktas et al., 2010).

3.3. Chemical composition of the extracts

3.3.1. GC/MS analysis of methanol extract from P. rigida heartwood

Suggested chemical compositions by GC/MS of the methanol extract from *P. rigida* heartwood are presented in Table 6. The main constituents were α-terpineol (24.91%), borneol (10.95%), terpin hydrate (9.60%), D-fenchyl alcohol (5.99%), limonene glycol (5.05%), 8-hydroxycarvotanacetone (2.62%), exo-2-hydroxycineole (2.45%), epoxylinalol (2.35%), (Z)-9-octadecenoic acid (2.29%), D-verbenone (2.16%), carvone hydrate (2.09%), verbenol (2.02%), caryophyllene (1.68%), *cis*-pinonic acid (1.48%), and sandaracopimaradiene (1.20%). Some carotenoids like lycoxanthin and dihydroxanthin were found in small amounts with 0.28% and 0.063%, respectively.

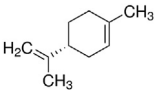
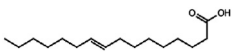
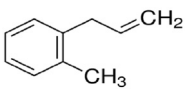
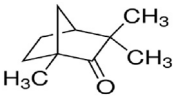
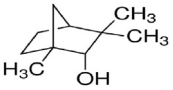
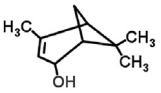
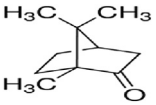
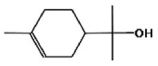
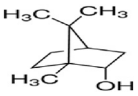
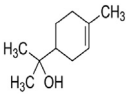
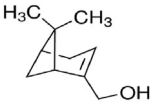
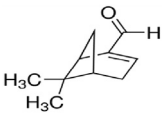
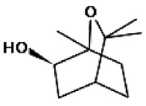
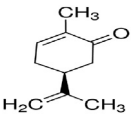
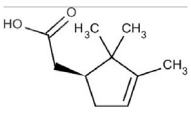
3.3.2. GC/MS analysis of methanol extracts from E. camaldulensis leaves

Suggested chemical compositions analyzed by GC/MS of *E. camaldulensis* leaves methanol extract are presented in Table 7. The main compounds were spathulenol (18.89%), cryptone (5.79%), 4,6,6-trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo [5.1.0.0(2,4)] octane (5.79%), (3,3-dimethylcyclohexylidene)-(E)-acetaldehyde (5.57%), ascaridole (4.32%), 4,6,6-trimethylbicyclo-[3.1.1]-heptane-3,4-diol (3.11%), 3-ethoxy-3-ethyl-1-pentyne (3.07%), *O*-cymene (2.90%), octahydro-8a-hydroxy-1(2H)-naphthalenone (2.67%), α-phellandrene epoxide (2.29%), nona-2,3-dienoic acid, ethyl ester (2.14%), and terpinen-4-ol (2.14%). FTIR spectra (Fig. 5) of the methanol extract from *E. camaldulensis* leaves are summarized in Table 9, which present the intensity of the functional groups found in the extract.

The phytochemical analysis of the crude extracts of *Eucalyptus* species revealed the presence of saponin, saponin glycosides, steroid, cardiac glycosides, tannins, volatile oils, phenols and balsam gum (Babayi et al., 2004). Polyphenols, flavonols, flavonones, proanthocyanidins and ellagitannis (tannins) and essential oils were isolated from *E. camaldulensis* (Siddiqui et al., 1997; Brophy and Southwell, 2002).

Extracts from *E. camaldulensis* have shown to have antifungal effects (Babayi et al., 2004; Essien and Akpan, 2004). The ethanol extract of *E. camaldulensis* leaf had marked fungicidal effect against *Microsporium gypseum* and *Trichophyton mentagrophytes* (Essien and Akpan, 2004). The methanol extract of *E. Camaldulensis* has been reported to inhibit the growth of *Candida albicans* (Babayi et al., 2004) and showed antifungal activity against *Microsporum canis*, *Microsporum gypseum*, *Tricophyton rubrum*, *Tricophyton schoenleinii*, *Tricophyton mentagrophytes* and *Epedermophyton floccosum* (Falahati et al., 2005). At 10% concentration, the methanol extract gave 66% of *Fusarium solani* biomass reduction (Bashir and Tahira, 2012).

Table 6
Chemical compositions of the methanol extract from *P. rigida* heartwood.

RT	Compound name	Chemical structure	Area %
3.35	R)-(+)- Limonene		0.26
3.43	9-Hexadecenoic acid		0.12
4.10	1-Allyl-2-methylbenzene		0.35
4.19	L-Fenchone		0.17
4.55	D-Fenchyl alcohol		5.99
5.00	2-Pinen-4-ol		4.18
5.11	Camphor		0.22
5.19	cis-α-Terpineol		0.57
5.42	Borneol		10.95
5.75	α-Terpineol		24.91
5.90	Myrtenol		0.22
5.96	Myrtenal		0.23
6.34	Exo-2-hydroxycineole		2.45
6.75	D-Carvone		0.40
7.37	α-Campholenic acid		0.13

(*continued on next page*)

Table 6 (*continued*)

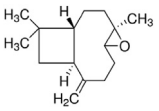
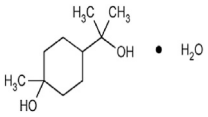
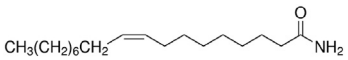
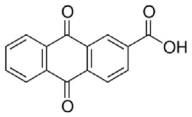
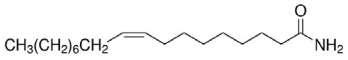
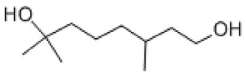
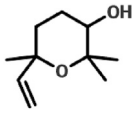
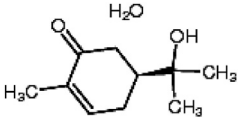
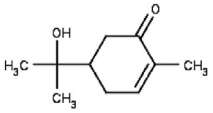
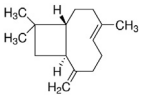
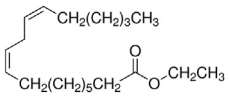
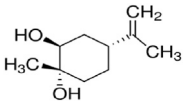
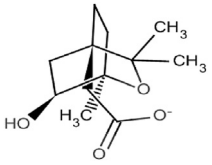
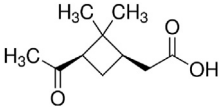
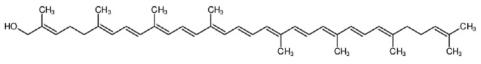
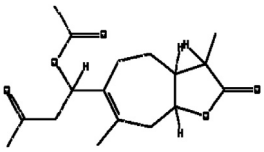
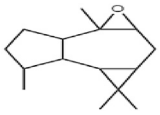
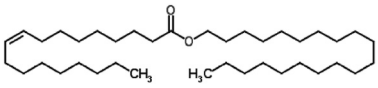
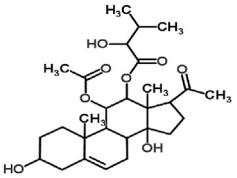
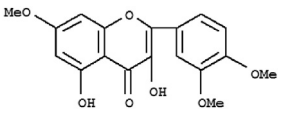
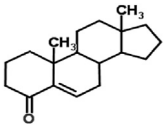
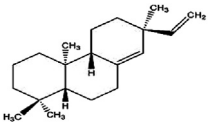
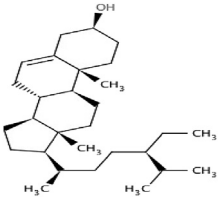
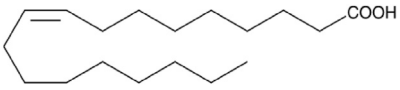
RT	Compound name	Chemical structure	Area %
7.59	Caryophyllene oxide		0.34
7.95	Terpin hydrate		9.60
8.63	Oleic acid		0.77
8.90	2-Anthraquinonecarboxylic acid		0.22
9.15	Oleamide		0.26
9.43	hydroxycitronellol		0.28
9.52	Epoxylinalol		2.35
9.70	Carvone hydrate		2.09
10.59	8-Hydroxycarvotanacetone		2.62
10.74	Caryophyllene		1.68
11.82	Linoleic acid ethyl ester		0.22
12.15	limonene glycol		5.05
12.64	2-Acetoxy-1,8-cineole		0.69
14.64	*cis*-Pinonic acid		1.48
15.49	Lycoxanthin		0.06

Table 6 (*continued*)

RT	Compound name	Chemical structure	Area %
15.59	Dihydroxanthin		0.28
16.27	Isoaromadendrene epoxide		0.43
17.81	Oleic acid, eicosyl ester		0.13
19.55	Drebyssogenin f		0.10
21.46	Quercetin-7,3′,4'-trimethoxy		0.65
21.85	Androst-5-en-4-one		0.47
22.53	Sandaracopimaradiene		1.20
23.60	β-Sitosterol		0.76
23.79	Oleic acid		2.29

3.3.3. GC/MS analysis of DW extracts from C. speciosus rhizomes

The suggested chemical composition of the DW extract from *C. speciosus* rhizomes used in the present study is presented in Table 8. The main constituents identified in the DW extract were *meso*-erythritol (12.21%), methyl-2-methyl-1,3-oxothiolan-2-yl-ketone, (11.61%), (all-Z)-5,8,11,14,17-eicosapentaenoic acid, methyl ester (9.74%), 6-oxo-heptanoic acid (8.01%), 1,3-dihydroxypropanone (7.44%), diosgenin (5.07%), 2-ethyl-3-hydroxy-4H-pyran-4-one (4.43%), 3′,4′,7-trimethylquercetin (3.17%), and digitoxin (2.77%).

Different extracts of *C. speciosus* rhizomes showed the presence of polyphenolic compounds that are responsible for good antifungal activity (Khare, 2007; Abirami et al., 2014). Several studies reported the chemical compositions of *C. speciosus* rhizomes; for example, 24-hydroxytriacontan-26-one, 24-hydroxytriacontan-27-one, methyl triacontanoate, diosgenin, and sitosterol (Gupta et al., 1981), 5α-stigmast-9(11)-en-3β-ol (Rastogi and Mehrotra, 2004), β-sitosterol-β-D-glucoside, prosapogenins A and B of dioscin, dioscin, and gracillin (Gupta et al., 1983), diosgenin (Sulakshana and Rani, 2014) were characterized. Steroid saponins and sapogenins from *C. speciosus* exhibited antifungal activity (Singh and Srivastava, 1992), whereas saponin B was found to be highly

Table 7
Chemical compositions of the methanol extract from *E. camaldulensis* leaves.

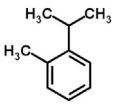
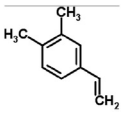
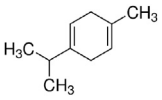
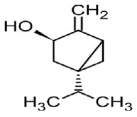
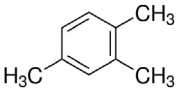
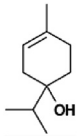
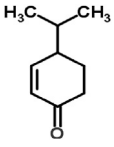
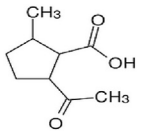
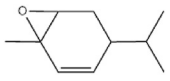
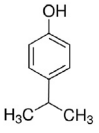
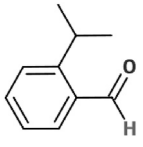
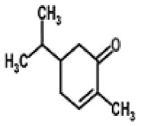
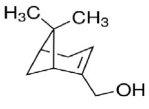
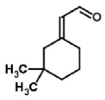
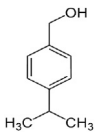
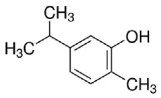
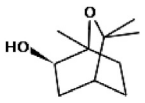
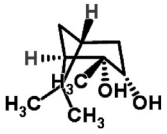
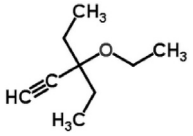
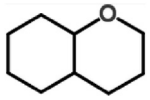
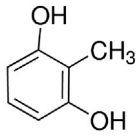
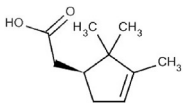
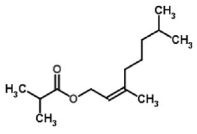
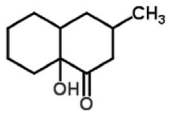
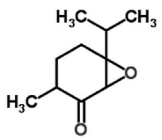
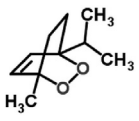
RT	Compound name	Chemical structure	Area %
3.29	*O*-Cymene		2.90
3.41	4-vinyl-1,2-dimethyl-benzene		1.23
3.67	γ-Terpinene		0.15
4.56	Sabinol		0.16
5.41	1,2,4-Trimethyl-benzene		1.02
5.56	Terpinen-4-ol		2.14
5.75	Cryptone		5.79
5.85	Nepetonic acid		0.23
5.99	α-Phellandrene epoxide		2.29
6.15	4-Isopropylphenol		1.65
6.72	2-Isopropylbenzaldehyde		1.83
6.85	Carvotan acetone		0.95
7.25	Myrtenol		0.93

Table 7 (*continued*)

RT	Compound name	Chemical structure	Area %
7.47	Acetaldehyde, (3,3-dimethylcyclohexylidene)-, (E)-		5.57
7.60	Cuminyl alcohol		4.01
7.67	Carvacrol		1.74
8.03	*exo*-2-Hydroxycineole		0.43
8.20	(1R,2R,3S,5R)-(-)-Pinanediol		3.11
8.26	3-Ethoxy-3-ethyl-1-pentyne		3.07
8.42	*cis*-2-Oxabicyclo[4.4.0]decane		1.18
8.61	2-Methylresorcinol		1.24
9.17	α-Campholonic acid		1.25
9.81	Isobutyrate, (Z)-2-octen-1-ol,3,7-dimethyl		1.90
10.31	8a-Hydroxy-3-methyloctahydro-1(2H)-naphthalenone		2.67
10.67	3-Methyl-6-(1-methylethyl)-7-oxabicyclo[4.1.0]heptan-2-one		0.25
10.91	Ascaridole		4.32

(*continued on next page*)

Table 7 (*continued*)

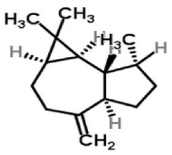
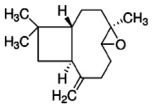
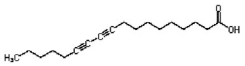
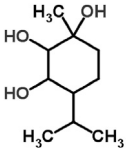
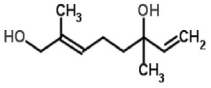
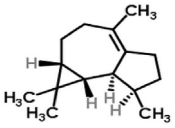
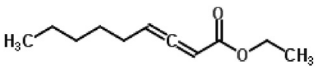
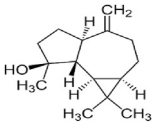
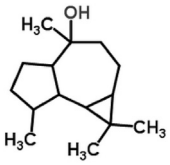
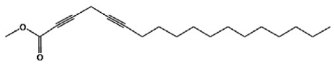
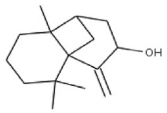
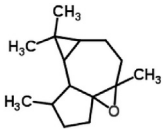
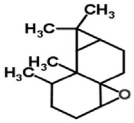
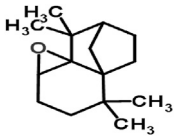
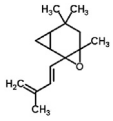
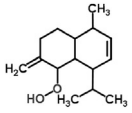
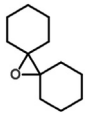
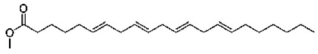
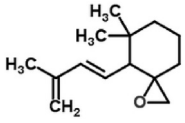
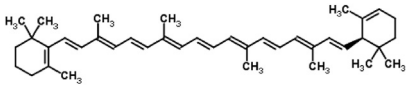
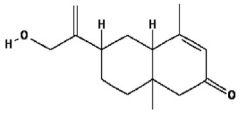
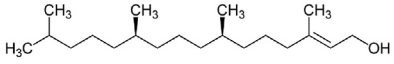
RT	Compound name	Chemical structure	Area %
11.17	Aromadendrene		0.66
11.41	Caryophyllene oxide		0.36
11.67	10,12-Octadecadiynoic acid		0.25
11.82	*p*-Menthane-1,2,3-triol		1.27
11.99	8-Hydroxylinalool		0.60
12.37	Ledene		0.52
13.32	Nona-2,3-dienoic acid, ethyl ester		2.14
14.28	Spathulenol		18.89
14.57	Epiglobulol		0.26
14.75	2,5-Octadecadiynoic acid methyl ester		0.33
15.56	*trans*-Longipinocarveol		1.82
15.98	Ledene oxide-(II)		0.48
16.32	Calarene epoxide		0.29

Table 7 (*continued*)

RT	Compound name	Chemical structure	Area %
17.25	Isolongifolene epoxide		0.76
17.40	4,6,6-Trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo[5.1.0.0(2,4)]octane		5.79
19.20	Murolan-3,9(11)-diene-10-peroxy		0.77
19.90	13-Oxadispiro[5.0.5.1]tridecane		0.95
20.84	6,9,12,15-Docosatetraenoic acid methyl ester		0.11
21.04	5,5-dimethyl-4-(3-methyl-1,3-butadienyl)-1-oxaspiro[2.5]octane		0.74
21.49	(+)-α-Carotene		0.31
22.05	4a,5,6,7,8,8a-hexahydro-6-[1-(hydroxymethyl)ethenyl]-4,8a-dimethyl-,2(1H)-Naphthalenone		0.42
23.55	Phytol		0.82

effective against conidial germination of *Botrytis cineria* and *Alternaria* sp. *P*-coumaric acid methyl ester was found as a principle antifungal activity against plant pathogenic fungi *Cladosporium cladosporioides*, *Colletotrichum gleosporioides*, *Curvularia* sp. and *Penicillium* sp. (Bandara et al., 1988). On the other hand, Saraf (2010) reported that methanol and aqueous extracts of *C. specious* rhizome did not exhibit any antimicrobial activity.

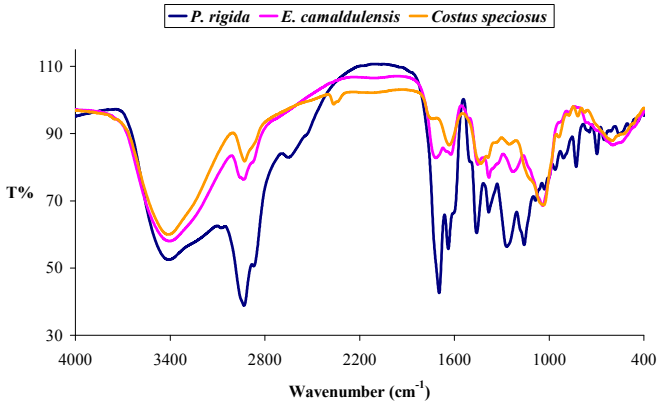


Fig. 5. FTIR spectra of the studied extracts.

3.4. FTIR spectra of the extracts

FTIR spectra (Fig. 5) showed the functional groups of the studied extracts. The obtained FTIR spectra of extractives are different according to the species, the type of solvent used for extraction, which could be expected due to the different content, and the type of extractives in studied wood species. A decrease or an increase of absorption bands of carbonyl, aromatic, alcohol groups, and amine groups indicates possible chemical reactions of extractives (Table 9). The chemical composition of wood extractives is very complicated since several hundreds have been identified in many different wood species (Miklečić et al., 2012). Many groups of extractives often have functional groups that can be quite reactive, for example terpenoids, phenolic compounds, quinones, de-esterified waxes and fats can contain hydroxyls (−OH), carbonyls (−C═O) and/or carboxylic groups (−COOH). Moreover, the most important substances extractable by DW are condensed tannins and water-soluble low-molecular weight carbohydrates.

Table 8
Chemical compositions of the DW extract from *C. speciosus* rhizomes.

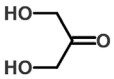
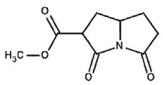
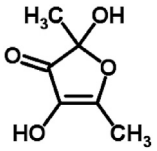
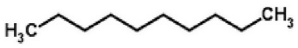
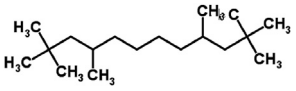
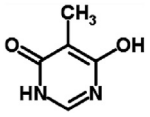
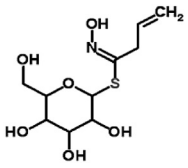
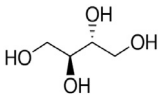
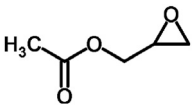
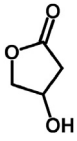
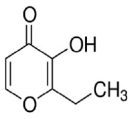
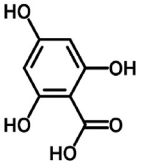
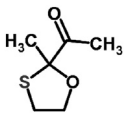
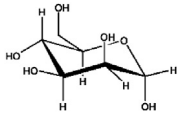
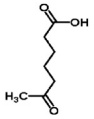
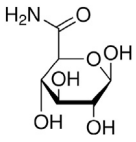
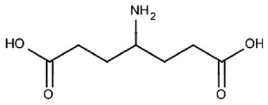
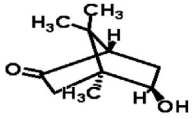
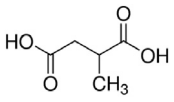
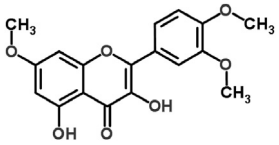
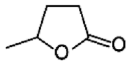
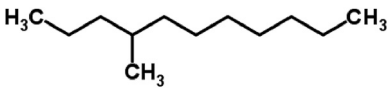
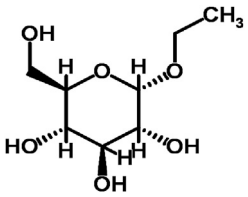
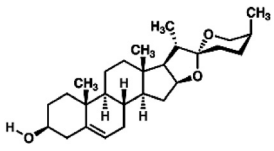
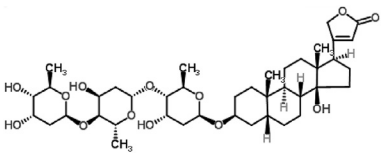
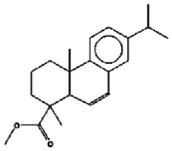
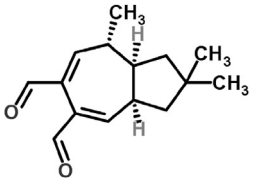
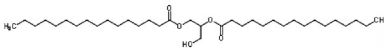
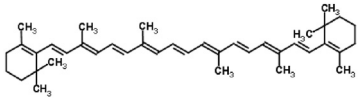
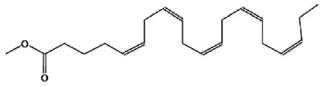
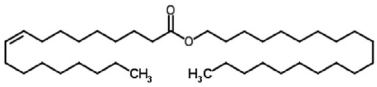
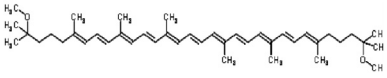
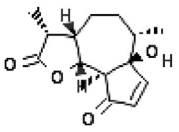
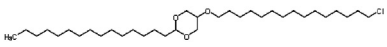
RT	Compound name	Chemical structure	Area %
2.07	1,3-Dihydroxypropanone		7.44
2.37	1H-pyrrolizine-2-carboxylic acid-hexahydro-3,5-dioxo-, methyl ester		0.29
2.80	2,4-Dihydroxy-2,5-dimethyl-3(2 h)-furan-3-one		0.55
2.88	Decane		0.58
3.30	2,2,4,9,11,11-Hexamethyl-dodecane		0.69
3.53	1-Decanamine		1.01
3.76	Pyrimidine-4,6-diol, 5-methyl-		2.08
4.33	Desulphosinigrin		0.17
4.56	*meso*-Erythritol		12.21
4.72	1,2-Epoxy-3-propyl acetate		0.43
4.82	Dihydro-4-hydroxy-2(3H)-furanone		0.27
4.94	2-Ethyl-3-hydroxy-4H-pyran-4-one		4.43
6.20	2,4,6-Trihydroxybenzoic acid		0.15

Table 8 (*continued*)

RT	Compound name	Chemical structure	Area %
6.46	-Methyl-2-methyl-1,3-oxothiolan-2-yl-ketone		11.61
6.72	d-Mannose		0.37
7.09	6-Oxo-heptanoic acid		8.01
7.32	D-Glucuronic acid amide		0.61
7.99	4-Amino-heptanedioic acid		0.74
8.24	endo-Ketoborneol		0.11
8.38	2-Hydroxy-3-methylsuccinic acid		1.59
9.20	3′,4′,7-Trimethylquercetin		3.17
9.84	γ-Lactone		1.65
10.31	4-Methylundecane		1.54
11.59	Ethyl-α-D-glucopyranoside		0.18
13.39	Diosgenin		5.07

(*continued on next page*)

Table 8 (*continued*)

RT	Compound name	Chemical structure	Area %
13.69	Digitoxin		2.77
16.44	Abieta-6,8,11,13-tetren-18-oic acid methyl ester		0.16
20.04	Velleral		0.25
20.74	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester		0.34
21.69	β-Carotene		1.29
22.49	(all-Z)-5,8,11,14,17-Eicosapentaenoic acid-methyl ester		9.74
23.57	Oleic acid, eicosyl ester		0.27
24.29	3,4,3′,4'-tetrahydrospirilloxanthin		0.34
24.74	Decahydro-7,9- dihydroxy-6,9a-dimethyl-3-methylene-(3aS,6S,6aS,7S,9R,9aS,9bR)-Azuleno[4,5-b]furan-2(3H)-one,		2.23
26.23	5-(Hexadecyloxy)-2-pentadecyl-trans-1,3-Dioxane		0.15

Guzmán et al. (2014) reported that the spectra of *E. Camaldulensis* were indicated the presence of long-chain linear aliphatic compounds which associated with the intense and thin bands at 2916 and 2917 cm^{-1} and at 2849 cm^{-1} [asymmetric and symmetric vibrations of the methylene groups, CH_2], as well as smaller bands at 1462 and 1463 cm^{-1} and at 719 cm^{-1}. In addition, they found that the absorptions at 1713 and 1719 cm^{-1} and their shoulders at 1686 and 1687 cm^{-1} [C=O stretching vibration of the carbonyl bonds; (C=O)] could be correspond to soluble compounds with ketone or aldehyde functional groups. These vibrations together with the vibrations at 1168 cm^{-1} and at 1105 and 1104 cm^{-1} [asymmetric and symmetric C–O–C stretching vibrations of the ester bonds; (C–O–C) and (C–O–C), respectively] may also be associated with esterified soluble lipid compounds. Moreover, bands at 3363 cm^{-1} was observed to stretching of the hydroxyl group of terpineol 1674 cm^{-1} and 1373 cm^{-1} assigned to C=C bond of the alkene system and $-CH_3$ group, and the bands came from stretching and bending of C–O bond (C–O at the tertiary alcohol) was observed at 1157 and 1126 cm^{-1} (Wijayati et al., 2011).

On the other hand, from the FTIR data of extractives, it is very difficult to draw any firm structural conclusions, other than to deduce the presence or absence of certain functional groups, because it is highly unlikely that the extract materials represent a single pure substance (Chow, 1971; Ajuong and Breese, 1998; Nuopponen et al., 2003; Aluong and Redington, 2004; Nuopponen, 2005).

3.5. Visual observation of fungal growth after 120 days

Methanol extract from *P. rigida* heartwood completely inhibited the growth of all the test fungi for at least 20 weeks (marked 5). *E. camaldulensis* leaves and *C. speciosus* rhizomes extracts showed

Table 9
FTIR data of the studied extracts.

Referenced data*				*P. rigida* (wood)	*E. Camaldulensis* (leaves)	*C. specious* (rhizomes)
Functional groups	Compound Type	Frequency range (cm^{-1})	Appearance	Wavenumber (cm^{-1})	Wavenumber (cm^{-1})	Wavenumber (cm^{-1})
C–H	Alkanes	2960–2850	strong- stretch	2932.2	2933.2	2928.4
	Alkyl	2870–1380	medium- weak	1382.7	1380.8	1386.6
	Alkenes	675–1000	–	–	874.6	875.5
	Vinyl	800–840	medium	829.2	–	818.6
	Aromatics	690–710	strong-medium	697.1	–	776.2
O–H	Alcohols, Phenols	3160–3640	stretch, broad	3408.6	3403.7	3412.4
	Carboxylic acids	2500–3000	broad, stretch	2652.6	–	938.2
C–O	Alcohols	1150–1200	medium	1158.0	–	–
	Carboxylic acids	1250–1300	strong, stretch	1268.9	–	–
	alcohols, esters, carboxylic acids	1260–1000	strong, stretch		1042.3	1034.6
	ethers, aromatic	1220–1260	medium, stretch		1228.4	1250.6
C=O	aldehydes, esters, carboxylic acids, ketones	1760–1670	–	–	1716.3	1737.6
	α, β -unsaturated aldehydes, ketones	1665–1710	strong, stretch	1697.1	–	–
C–C	Dienes, with benzene ring	1600–1625	strong	–	1621.8	–
	aromatics	1400–1500	stretch (in –ring)	1458.9	1451.2	1431.9
N–H	amines	1580–1650	medium, bend	1638.2	–	1632.5

* Data according to Chow (1971), Ajuong and Breese (1998), Nuopponen et al. (2003), Aluong and Redington (2004), Sundqvist (2004), Nuopponen (2005), Miklečić et al. (2012), and Sulakshana and Rani (2014).

huge growth of the studied fungi at the fourth week with all the measured concentrations as well as some other contaminates.

During the process of papermaking from *E. camaldulensis*, large amounts of waste such as leaves are disposed of, also during the woodworking of pitch wood, larger amount of sawdust and wood shavings are available. The possibility of utilizing these wastes (leaves and wood sawdust and shavings) as well as the medicinal plants from the herbarium stores is worthy of investigation and can be used in the production of natural extracts. The application of natural extracts for preserving wood also gave good protection against the growth of mold. Environmentally friendly wood preservatives should encourage the use of natural extracts as wood preservatives, since the extracts have substantial advantages for wood protection and provide good decay resistance against fungi.

4. Conclusions

We herein report the application of three natural extracts on three commercial wood types to control the biodeterioration caused by growth of five common mold fungi. Extract from *P. rigida* wood is efficacious against the growth of *A. alternata*, *F. subglutinans*, *C. globosum*, *A. niger* and *T. viride*. The application of *E. camaldulensis* leaves extract for the three wood types did not show activity against the growth of the studied fungi at high or low concentrations in comparison to the control sample. The extracts from *C. speciosus* (rhizomes) showed activity only against *C. globosum* and some activity observed against *F. subglutinans* at the high concentration (2%). Other fungi showed resistance to the extracts of *C. speciosus* rhizomes.

α-terpineol, borneol, terpin hydrate, D-fenchyl alcohol, limonene glycol, *cis*-pinonic acid, (Z)-9-octadecenoic acid and sandaracopimaradiene were of the main constituents of *P. rigida* wood extract. The main compounds of the methanol extract from *E. camaldulensis* leaves were spathulenol, cryptone, (3,3-dimethylcyclohexylidene)-(E)-acetaldehyde, and ascaridole. The main constituents identified in the water extract from *C. speciosus* rhizomes were *meso*-erythritol, methyl-2-methyl-1,3-oxothiolan-2-yl-ketone, (all-Z)-5,8,11,14,17-eicosapentaenoic acid-methyl ester, 6-oxo-heptanoic acid, 1,3-dihydroxypropanone, 2-ethyl-3-hydroxy-4H-pyran-4-one, diosgenin, 3′,4′,7-trimethylquercetin, and digitoxin.

The present study proves that the application of natural extracts, such as methanol extract from *P. rigida*, can be used as a wood preservative against molds, thereby improving product durability.

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