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SOME BIOACTIVE PROPERTIES OF *ACACIA DEALBATA* EXTRACTS AND THEIR POTENTIAL UTILIZATION IN WOOD PROTECTION

A study was made of the potential use of Acacia dealbata wood extracts as bio-protective agents. Initially, extracts were obtained from Acacia dealbata sapwood, heartwood and bark, and their antioxidant, antimicrobial and anti-quorum sensing activities were determined. Next, the decay resistance of Scots pine wood samples impregnated with these extracts was examined against the brown rot fungus, Coniophora puteana. The impregnation procedure was performed according to the ASTM D (1413) standard test method at two different concentrations, 3% and 5% by weight, using hot water and methanol as extraction solvents. The strongest antioxidant, antimicrobial and anti-quorum sensing activities were those of the bark extract. Hot water extraction led to lower performance than methanol extraction. According to EN 113 testing methods, the highest level of preservative effect against wood-decaying fungi was observed in the case of 5% methanol extract from the bark.

Keywords: *Acacia dealbata*, antioxidant, antimicrobial, anti-quorum sensing, impregnation

Introduction

As chemical toxicity levels have increased over the years, research into the bioactive properties of natural products has become widespread due to the important role of these products in pharmacy and medicine. Among these natural products, trees offer a number of distinctive properties. Trees can live for years or even centuries because of their secondary metabolites, which play an effective role in providing protection from microorganisms and in adaptation and pollination [Luis et al. 2012]. *Acacia* species are among the trees that contain

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a high level of secondary metabolites [Seigler 2003]. Different parts of *Acacia* species, including flowers, leaves, barks and gum, have been reported as being used for the treatment of diseases [Sowndhararajan et al. 2013]. The bark of *A. dealbata* produces a gum, resembling gum arabic, which is used especially in the treatment of bronchial disorders [Sowndhararajan et al. 2015].

Natural plant extracts and tannins are well-known alternatives in the wood preservation industry. Extracts obtained from naturally durable species can be used to treat non-durable wood species [Harju et al. 2003; Turner and Conradie 1995]. *Acacia* trees (family Mimosaceae) are known as a source of bioactive components. Most species of this genus contain protective compounds such as phenolics, flavonoids, terpenes, tannins, amines and alkaloids [Seigler 2003]. The use of extracts of durable tree species such as *Acacia dealbata* Link to protect non-durable woods represents a highly environmentally friendly approach.

It is reported that secondary metabolites such as phenolics and flavonoids exhibit antioxidant, antimicrobial, antitumor and antifungal activity [Ryu et al. 1994; Aziz et al. 1997; Ribeiro et al. 2007] and have the ability to inhibit the bacterial communication mechanism called 'quorum sensing' [Taganna et al. 2011]. In addition, these components may exhibit anti-insect and feeding deterrent activity against subterranean termites. Even though the bioactive properties of *Acacia dealbata* have been studied extensively [Sowndhararajan et al. 2013; Tascioglu et al. 2013], evidence for its anti-quorum sensing activity has yet to be provided. In this study, the antioxidant, antimicrobial and anti-quorum sensing activities of extracts from the sapwood, heartwood and bark of *Acacia dealbata* were investigated. These parts were prepared as treatment solutions at different levels (3% and 5% by weight) using two different extraction solvents (hot water and methanol). Scots pine wood samples were impregnated with each solution, and efficacy against *Coniophora puteana* was determined.

Materials and methods

Material

Acacia dealbata Link was obtained from Trabzon province in northeastern Turkey. The location of the study area was 40°59'56.0"N and 39°40'59.3"E. The samples were obtained at heights of 1-1.20 m from a 10-year-old tree. The wood was divided into sapwood, heartwood and bark, and these parts were stored separately.

Preparation of the extracts

Heartwood, sapwood and bark parts were dried in a dryer (Profilo PFD1350W, Turkey) for 24 h at 60°C, then ground in a basic micro-fine grinder and passed through a 1-millimeter sieve (IKA Werke MF10, Germany). Approximately 5 g

powdered samples were dissolved in 50 mL methanol (99%). The mixture was continuously stirred using a shaker (Heidolph Promax 2020, Schwabach, Germany) at room temperature for 24 h. Particles were removed using Whatman No. 4 filter paper (pore size 20-25 μm). The solutions were then filter-sterilized using 0.45 μm hydrophilic polyvinylidene fluoride (PVDF) filters.

Antioxidant activity

Determination of polyphenolic content

The polyphenolic contents of the methanol extracts were evaluated on three different bases: total phenolic content (TPC), total flavonoid (TF) and condensed tannin (CT) content. For determination of the total phenolic content, the Folin-Ciocalteau procedure was employed and gallic acid was used as standard [Slinkard and Singleton 1977]. The results were expressed as mg gallic acid equivalent (GAE) per g of methanol extract.

Determination of flavonoid content

The concentration of total flavonoid in the methanol extracts was measured using a spectrometric assay. The total flavonoid concentration was expressed as mg quercetin equivalent (QE) per g of sample [Fukumoto and Mazza 2000].

Determination of condensed tannin content

The concentration of condensed tannin was determined by the method previously used by Julkunen-Titto [Julkunen-Titto 1985]. The results were expressed as mg catechin equivalent (CE) per g of sample.

Determination of antioxidant capacity

The antioxidant capacity was determined on the basis of ferric reducing antioxidant power (FRAP). This method is based on the reduction of tripyridyltriazine complex (Fe $(TPTZ)^{3+}$) to blue-colored $Fe(TPTZ)^{2+}$ by antioxidants in an acidic medium [Benzie and Strain 1996]. FRAP values were expressed for the wet weight of the samples as μmol of Iron(II) Sulfate Heptahydrate ($FeSO_4 \cdot 7H_2O$) equivalent per g of sample.

Bioactivity against bacteria and yeast

The tree extracts were tested for antimicrobial activity by the agar well diffusion method according to the Clinical & Laboratory Standards Institute (CLSI) guidelines [Wayne 2012] against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 7002, *Listeria*

monocytogenes ATCC 43251, *Candida parapsilosis* ATCC 22019 and *Candida albicans* ATCC 10231. The microorganisms were obtained from the Department of Medical Microbiology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey. Bacteria and yeast were cultured in Luria Bertani (LB) and Sabouroud Dextrose agar (LABM, UK) respectively. Fresh cultures (18 h) of bacteria and yeast were used to make suspensions in 5 mL of sterile isotonic sodium chloride, and the turbidity was adjusted to 0.5 McFarland. Agar plates were seeded with each suspension, and 0.6 cm agar wells were cut out using a sterile pipette tip. Fifty microliters of *Acacia dealbata* extracts were transferred into each agar well and the cultures were incubated at 37°C for 24 hours. Ampicillin, gentamicin, cefotaxime, tetracycline and amphotericin B solutions and DMSO were used as positive and negative controls respectively. The antimicrobial activity was determined by visual inspection and by measurement of the diameter of clear inhibition zones around the agar wells. The minimal inhibitory concentration (MIC) of the extracts showing positive antimicrobial activity in the agar diffusion method was determined using the liquid microdilution test method. The well with the lowest concentration that did not show any microbial growth was considered to represent the MIC of the tested extract.

Anti-quorum sensing activity

Anti-quorum sensing activity was determined using the microdilution method as described for the antimicrobial activity test above [Damte et al. 2013]. The anti--QS activity of the extracts was tested against *Chromobacterium violaceum* ATCC 12472*,* a violacein-producing bioindicator strain. Briefly, the MIC of each extract was determined as described above, and sub-MIC concentrations were used for the inhibition of pigment production by *C. violaceum*. For the anti-QS assay, each extract was added to a fresh culture of the bioindicator strains in LB broth and incubated for 24 h. After incubation, 1 mL of culture was centrifuged, and a pellet was resuspended in 1 ml of DMSO and vortexed at the highest speed for pigment extraction. Supernatant was removed and absorbance values of the pigments were determined at OD 585 nm using a microplate reader [Damte et al. 2013; Norizan et al. 2013]. Because vanilla has been proved to exhibit anti-QS activity against *C. violaceum*, vanilla extract was used as a positive control [Choo et al. 2006]. Vanilla was purchased from a commercial supplier.

Efficacy against wood-decaying basidiomycete fungi

Extract solutions were prepared with the fine powders obtained from different parts of the tree dissolved in distilled hot water and methanol. Briefly, hot water extraction was carried out on a hot plate at 80°C for a period of 2 hours under continuous stirring with a magnetic stirrer. All extract solutions were filtered

after cooling using filter paper (Whatman, No. 4). Methanol extraction at 3-5% (by weight) was carried out at room temperature for 2 hours.

Treatment method

Scots pine sapwood was cut parallel to the grain directions and sawn into specimens measuring 1.5 × 0.5 × 2.5 cm (tangential × radial × longitudinal). The specimens were treated according to the ASTM D 1413 standard test method. The samples were impregnated in a medium scale impregnation container under vacuum-pressure of 635 mm Hg for 40 min followed by 15 min at atmospheric pressure. The retention for each concentration was calculated (in kg/m^3) using the following formula:

$$R = (G \times C/V) \times 10\ kg/m^3 \quad (1)$$

where R is the retention in kg per cubic meter, G is the weight in grams of treatment solution absorbed by the wood specimen (initial weight of specimen subtracted from the weight of the treated specimen), C is the percentage concentration or solution strength of the treatment (%), and V is the volume of the samples in cubic centimeters.

After impregnation, the samples were allowed to stand for one week for conditioning. The treated wood blocks were stored in a conditioning room at 20 ±2°C and 65 ±3% relative humidity until they attained a stable weight prior to decay resistance testing.

Decay resistance test

The fungal decay test was performed according to the EN 113 standard test method using a brown rot fungus, *Coniophora puteana* BAM Ebw.15, for both treated (test) and untreated (control) samples. The tests were repeated ten times. Because of the smaller sample sizes, the incubation time was approximately 7 weeks at 22°C and 70% humidity. After incubation, the samples were dried at 103 ±2°C and weighed. The weight loss caused by the fungal attack was calculated as follows:

$$\text{Weight loss (\%)} = [(m_o - m_d)/m_o] \times 100 \quad (2)$$

where m_o is the oven-dried weight prior to the test, and m_d is the oven-dried weight after the test.

Statistical calculations

The data were analyzed using the SPSS 21.0 statistical software package and based on a 95% confidence level. Statistically significant differences between the results of assays were identified by simple variance analysis. The mean values were compared with Duncan homogeneity groups if the effect was significant.

Results and discussion

Antioxidant activity

Total phenolic content, total flavonoids, condensed tannin content and FRAP (μmol$FeSO_4 \cdot 7H_2O$/g) values obtained for *A. dealbata* wood parts are presented in table 1.

Table 1. Total phenolics, total flavonoids, condensed tannins and FRAP values of *A. dealbata* parts

Part of *Acacia dealbata*	TP [mg GAE/g]*	TF [mg QE/g]*	Condensed Tannins [mg CE/g]*	FRAP [μmol $FeSO_4 7 \cdot 7H_2O$/g]*
Sapwood	2.076 ±0.267[a]	0.081 ±0.005[a]	0.620 ±0.081[a]	47.110 ±5.346[a]
Heartwood	31.893 ±1.041[b]	2.468 ±0.074[b]	4.473 ±0.120[b]	322.35 ±10.235[b]
Bark	321.499 ±1.035[c]	6.600 ±0.230[c]	4.789 ±0.025[c]	3525.0 ±15.636[c]

[a]Means having the same superscript letter(s) in the same column are not significantly different ($P > 0.05$) according to Duncan's multiple range test.
*Results given as mean ± standard deviation

In recent years, researchers have shown increasing interest in phenolic compounds obtained from natural sources, due to their potential beneficial effects on human health [Manach et al. 2004]. In this study, the highest total phenolic content was found in the bark of *A. dealbata*; the value was 321.499 ±1.035 mg GAE/g, 160 times greater than the total phenolic content of the sapwood (2.076 ±0.267 mg GAE/g). The phenolic content obtained for the bark was greater than the phenolic content previously determined in various extractions of *A. dealbata* (ethanolic, hydroalcoholic, methanolic, acetone extracts: 243.80, 290.65, 241.81, 203.10 mg GAE/g of dry mass, respectively) [Luis et al. 2012]. In another study, it was reported that the highest content of total phenolics was detected in ethyl acetate extract (74.18 mg GAE/g), followed by methanol (36.51 mg GAE/g), hydroalcoholic (33.48 mg GAE/g) and dichloromethane (28.14 mg GAE/g) extracts. The lowest total phenolics were obtained in n-hexane (18.06 mg GAE/g) [Amoussa et al. 2015]. The ordering of the total phenolic contents of the *A. dealbata* parts was as expected (bark > heartwood > sapwood), and these contents were found to differ significantly from each other ($P < 0.05$) by Duncan's multiple range test. These results indicate that the bark of *A. dealbata* is a natural source of phenolic compounds.

Total flavonoid content

Tannins, flavonoids, lignans, stilbenes, terpenes and terpenoids can be listed as major extracted chemicals, and are well known for their protective properties against biological degradation of woods [Windeisen et al. 2002]. Reyes-Chilpa et al. [1995] isolated two flavonoid compounds, castillen D and castillen E, from the bark of the tropical tree *Lonchocarpus castilloi*, and determined the feeding deterrent activity of those compounds against dry wood termites *Cryptotermes brevis*. In this study, the highest flavonoid content was found in the bark of *A. dealbata*; this value was 6.600 ±0.230 mg QE/g, six times higher than the total flavonoid content of the sapwood (0.081 ±0.005 mg QE/g). Thus, the quantity of flavonoids in the bark may be one of the factors in the tree's natural durability. The results for total flavonoid content differed significantly from each other ($P < 0.05$) according to Duncan's multiple range test.

Condensed tannin content

The *Acacia* genus is known to contain high quantities of secondary metabolites such as tannins, flavonoids and gum. Tannins have been reported to exhibit antimicrobial, antimutagenic, anticarcinogenic and antioxidant activity [Chung et al. 1998; Cowan 1999]. The high tannin content of *Acacia* trees has been shown to be effective in deterring herbivores [Owen-Smith 1993]. In our study, compared with the other assays (total phenolic content, total flavonoid content), a smaller difference was observed in the quantity of condensed tannin between heartwood and bark (4.473 ±0.120 and 4.789 ±0.025 mg CE/g respectively). The results were lower than those reported for some other *Acacia* species (*A. raddiana* 82.27 ±4.20, *A. tortilis* 48.92 ±2.71 mg CEQ/g) [Rohner and Ward 1997]. It can be concluded that the heartwood of *A. dealbata* is a natural source of condensed tannin, like the bark of *Acacia dealbata* tree.

FRAP

Acacia barks have been reported as natural sources of antioxidants. Acetone and methanol extracts of *A. leucophloea*, *A. ferruginea*, *A. dealbata* and *A. pennata* barks have previously been shown to possess very strong antioxidant properties, using various *in vitro* chemical assays [Sowndhararajan et al. 2013]. In this study, the highest ferric reducing antioxidant power was found in the bark (3525.0 ±15.636 μmol $FeSO_4 \cdot 7H_2O$/g); this is similar to the results of the other assays. In a previous study, the ferric reducing antioxidant power of *A. pennata* bark was determined using two different extraction methods, maceration and Soxhlet extraction, as 1850 and 1770 μmol $FeSO_4$/g respectively [Feregrino-Pérez et al. 2011]. It can be concluded that the antioxidant activity of such natural materials is dependent on the extraction method and solvent, as well as on the species of tree.

Bioactivity against bacteria and yeast

The antimicrobial activity of the *A. dealbata* extracts was measured by agar well diffusion and minimal inhibitory concentration (MIC) tests. The results are presented in table 2 and table 3 respectively.

Table 2. Antimicrobial activity of *Acacia dealbata* extracts

Materials	Agar well diffusion [mm zone diameter]									
	S. aureus	*E. coli*	*P. aeruginosa*	*E. faecalis*	*C. albicans*	*C. parapsilosis***	*S. typhimurium*	*P. mirabilis*	*K. pneumoniae*	*L. monocytogenes*
Sapwood	2	0	0	0	0	0	0	…	2	1
Heartwood	2	0	0	0	0	0	0	…	2	1
Bark	4	0	0.2	0	0.1	0	0	4	3	1
Ampicillin	> 30	16-17	…	> 30	…	…	27	…	…	…
Gentamicin	…*	…	21-22	…	…	…	…	…	21	…
Amphotericin B	…	…	…	…	30	…	…	…	…	…
Tetracycline	…	…	…	…	…	…	…	…	…	25
Cefotaxime	…	…	…	…	…	…	…	37	…	…

*Not tested.
**May have acquired bacterial resistance to the control used. The tests were not repeated, as no extract had any positive effect.

As shown in table 2, extracts from the sapwood and heartwood of *A. dealbata* inhibited the growth of *S. aureus, K. pneumoniae* and *L. monocytogenes*. The bark extract inhibited the same microorganisms as well as *P. aeruginosa, C. albicans* and *P. mirabilis.* Tree barks contain various substances serving to protect the wood against many factors. In a study of the antimicrobial activity of wood and bark extracts from 14 hardwood species in eastern North America, it was reported that 36% of the extracts were active against at least one fungal strain, and the bark extracts were more active than the wood extracts [Omar et al. 2000]. In another study, structurally novel diterpenoid constituents from the stem bark of *Azadirachta indica* (Meliaceae) showed antibacterial activity against various Gram-positive and Gram-negative bacteria [Ara et al. 1989]. In a study of the antimicrobial properties of chloroform, methanol, aqueous and ethanol extracts of the stem bark of *Saraca indica* (Caesalpiniaceae) the methanol and aqueous extracts exhibited antimicrobial activity with MIC 0.5-2% and 1-3% respectively. Furthermore, the methanol extracts exhibited the strongest activity against both bacteria and fungi

[Sainath et al. 2009]. It may be concluded that the solvent used and the extraction method can affect the antimicrobial activity of materials of this type.

Table 3. Minimal inhibitory concentration test results for extracts

Sample	Minimal inhibitory concentration (MIC) test results						
	S. aureus	*E. coli*	*P. aeruginosa*	*C. albicans*	*P. mirabilis*	*K. pneumoniae*	*L. monocytogenes*
Sapwood	78.125	…*	…	…	…	625	625
Heartwood	39.1	…	…	…	…	625	625
Bark	156.25	…	625	2.5	156.25	312.5	312.5
Ampicillin	78.125	2.5	…	…	…	…	…
Gentamicin	…	…	1.25	…	…	156.25	…
Amphotericin B	…	…	…	78.125	…	…	…
Tetracycline	…	…	…	…	9.76	…	…
Cefotaxime	…	…	…	…	…	…	78.125

* Not tested (1.25 and 2.5 mg/mL; other values expressed as µg/mL).

Minimal inhibitory concentration is the lowest concentration that inhibits the growth of microorganisms. As shown in table 3, the MIC value against *S. aureus* was lower for heartwood (39.1 µg) than for sapwood (78.125 µg). It may be concluded that the heartwood extract is more effective than the sapwood extract. Owolabi et al. [2007] studied the antifungal and antibacterial activity of ethanol and aqueous extracts of *Kigelia africana* (Bignoniaceae) stem bark, reporting that the aqueous extract exhibited no antibacterial or antifungal activity, and that the MIC values for the ethanol extract were 6.25 ±1.07 mg/ml against *S. aureus* and 7.92 ±1.52 mg/ml against *C. albicans.* The MIC values obtained in the study were generally lower than those obtained in the above-mentioned study. It may be concluded that MIC values may be dependent on tree type, part of tree, extraction methods and concentrations, and the microorganisms tested.

Anti-quorum sensing activity

Graphs showing the anti-QS activities of bark, heartwood and sapwood of *A. dealbata* appear in figures 3-5. Vanilla and DMSO were used as positive and negative controls, respectively (figs. 1-2). The anti-quorum sensing activity test is considered positive when an extract inhibits the quorum sensing activity – in this case, pigment production decrease or ends by *C. violaceum* – without affecting bacterial growth. In this study, only the extract of the bark of *Acacia*

dealbata showed anti-QS activity (fig. 3), and the efficiency of anti-QS activity was lower than that of vanilla, the positive control.

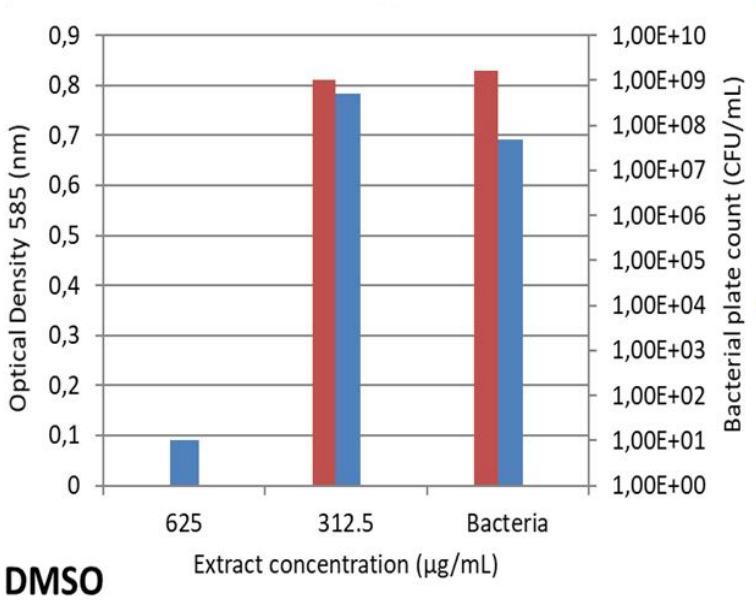

Fig 1. DMSO anti-QS activity

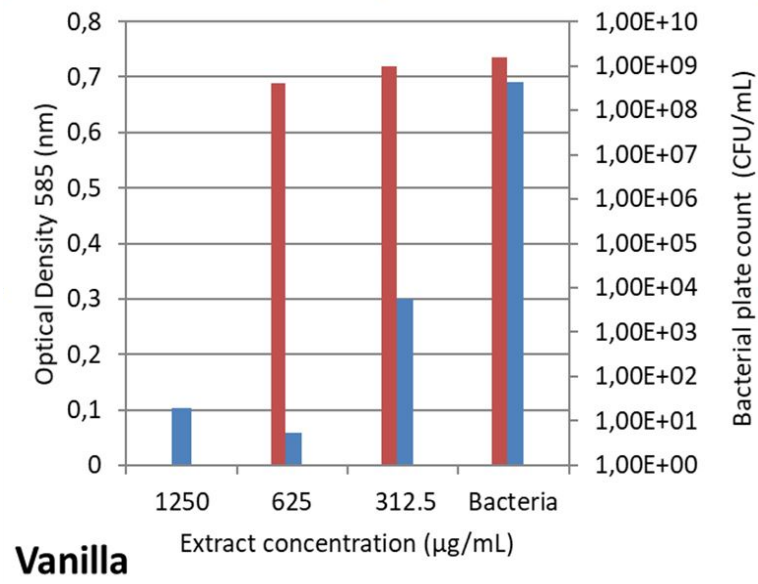

Fig. 2. Vanilla anti-QS activity

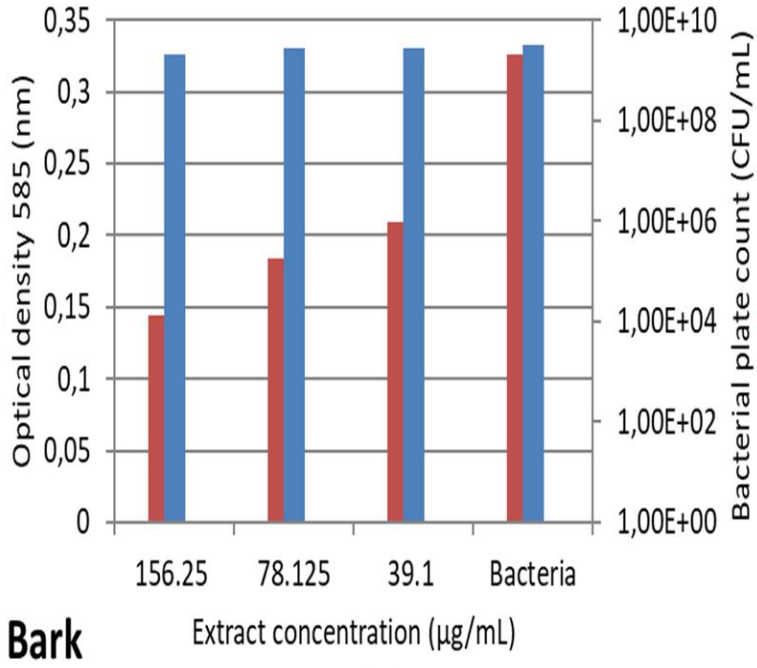

Fig. 3. Bark anti-QS activity

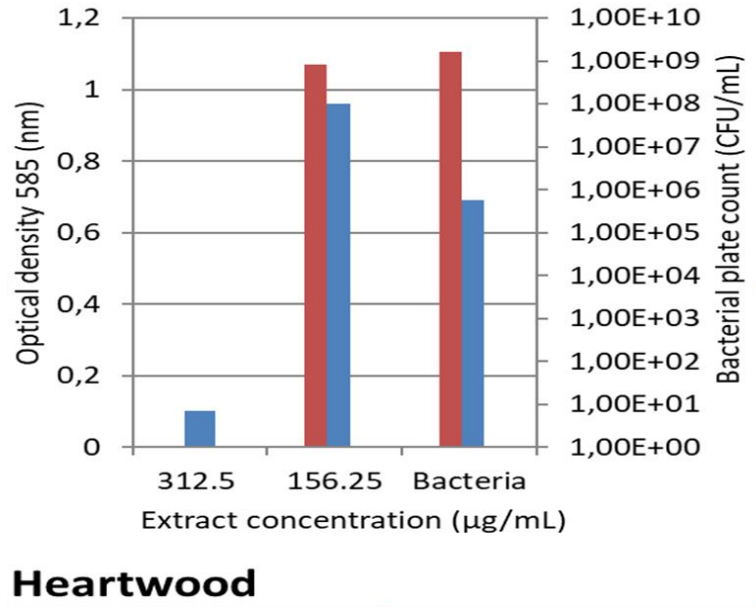

Fig 4. Heartwood anti-QS activity

As shown in figures 4 and 5, the sapwood and heartwood of *A. dealbata* did not present any anti-QS activity. Both of them exhibited antimicrobial activity in a concentration of 312.5 µg/ml. In a previous study, the molecular composition of *Quercus cortex* (oak bark) extract and its antibacterial and anti-quorum sensing effects were investigated [Deryabin and Tolmacheva 2015]. It was reported that the *Q. cortex* extract showed weak antibacterial and significant anti-QS activity, and that this activity was retained and completely restored when the samples were dried and rehydrated. Luís et al. [2016] studied the antioxidant, antibacterial and anti-quorum sensing activities of *Eucalyptus globulus* and *Eucalyptus radiata* essential oils, and reported both essential oils as quorum sensing inhibitors [Luís et al. 2016]. Thus, these results indicate that trees, especially their barks, may contain potential quorum sensing inhibitors.

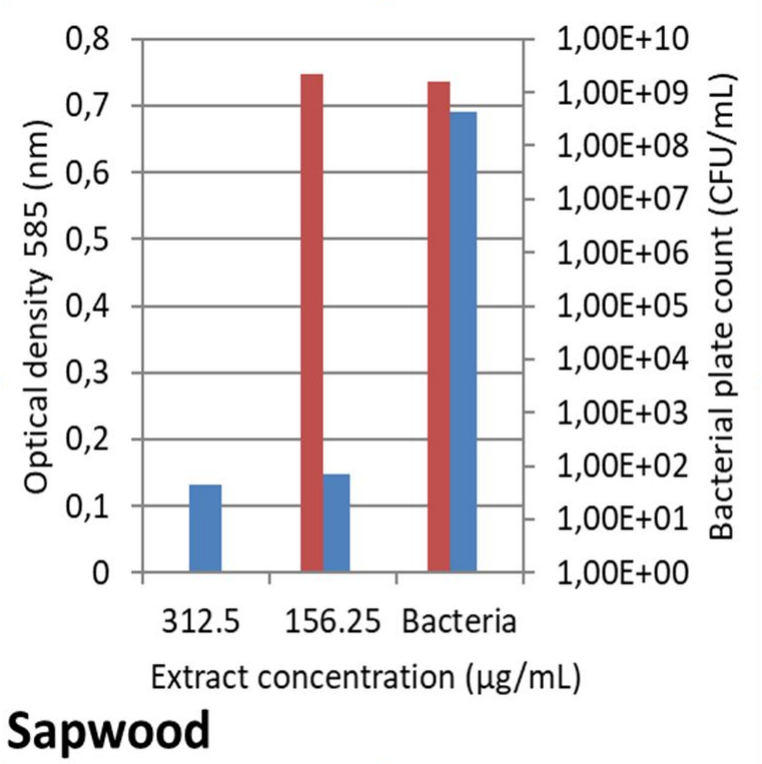

Fig 5. Sapwood anti-QS activity

Efficacy against wood-decaying basidiomycete fungi

Retentions (kg/m^3) of treated Scots pine are shown in table 4.

As may be seen in table 4, the retention of solutions prepared with methanol was lower than that of solutions prepared with hot water extraction. An increase

Table 4. Retention values [kg/m^3]

Solution and concentration	Water	Methanol
Sapwood (3%)	22.8 ±2.9	19.8 ±1.6
Sapwood (5%)	37.9 ±6.6	35.3 ±6.5
Heartwood (3%)	21.9 ±2.1	18.8 ±1.3
Heartwood (5%)	38.2 ±6.6	35.6 ±6.5
Bark (3%)	22.5 ±2.6	19.0 ±1.4
Bark (5%)	32.1 ±6.3	29.0 ±4.2

in concentration had a positive effect on retention. In a similar study using natural extracts, it was also shown that an increase in the solution concentration resulted in increased retention [Sen et al. 2009]. The retention values of sapwood solution prepared with methanol (19.8 ±1.5 kg/m^3) and of heartwood solution prepared with water (38.2 ±6.6 kg/m^3) were the lowest and highest values respectively. These retention levels were found to be higher than those reported in a previous study in which four different concentrations of mimosa (*Acacia mollissima*), quebracho (*Schinopsis lorentzii*) and pine (*Pinus brutia*) bark extracts were investigated [Tascioglu et al. 2013].

Weight loss (%) for the control and impregnated wood samples is shown in figure 6. Among the impregnated wood samples, the highest weight loss (23.5%) was determined in case of the sapwood samples impregnated at 3% concentration and extracted with hot water. Weight loss decreased as the solution concentration increased. The lowest weight loss (7.45%) was observed in the wood samples treated with 5% methanol extracts from bark. The hot water extract performed less well than the methanol extract. As shown in figure 6, the performance ranking of the protective solutions was bark > heartwood > sapwood. This can be explained by the fact that different parts of the tree show different inhibitory effects.

According to the EN 113 test method, for a new impregnation solution to be approved for use, the weight loss of impregnated examples should be less than 3%. In this study, the lowest weight loss (7.45%) was determined in wood samples treated with 5% bark solution in methanol. Even though this value is not sufficient to meet the requirements of the European standards, the findings appear promising; it can be investigated using higher concentrations and more efficient extraction methods.

Conclusions

In this study, a determination was made of the antioxidant, antimicrobial and anti-quorum sensing activities of extracts of *Acacia dealbata* wood parts

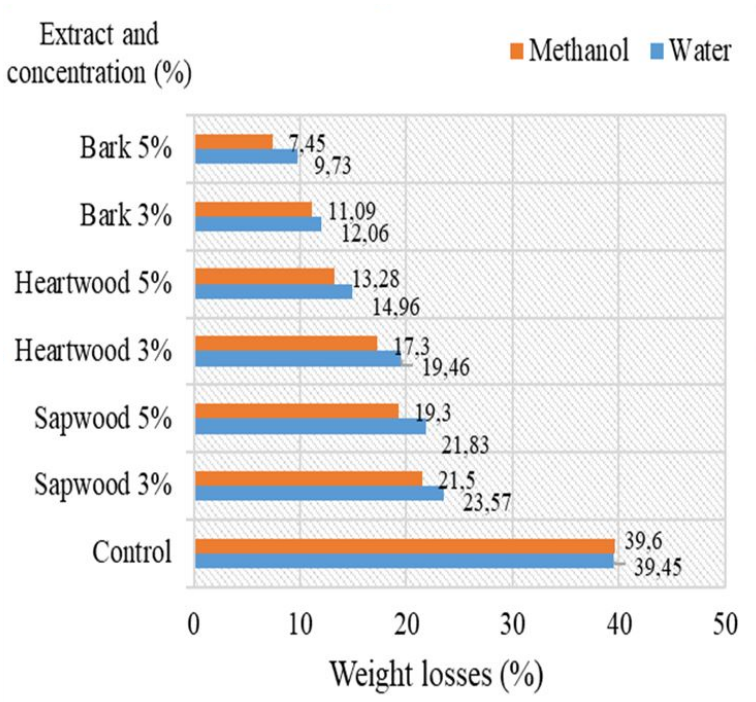

Fig. 6. Weight loss of control and impregnated wood samples (%)

(sapwood, heartwood and bark) in methanol. Additionally, possibilities for the use of *A. dealbata* sapwood, heartwood and bark extracts (obtained using hot water and methanol) as bio-protective agents were investigated using a fungal decay test. The highest antioxidant, antimicrobial and anti-quorum sensing activities were found in the case of bark extract. Antifungal activity was higher in the case of methanol extracts than with the hot water extracts. The greatest protective effect was observed in case of a 5% methanol extract prepared from bark. These treatments showed promising results, although they do not yet provide adequate protection to meet the requirements of European standards. In further investigations of fungal toxicity against brown rot fungus (*Coniophora puteana*), the extracts may be mixed with different antifungal materials such as herbal or non-toxic chemicals. Additionally, higher concentration levels with more efficient extraction methods may be tested. Consequences of outdoor exposure such as leaching, weathering and evaporation should be discussed and considered in further investigations.

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