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Investigation of Some Tree Bark Extracts and Essential Oils for Antioxidant, Antimicrobial and Anti-Quorum Sensing Activities

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Abstract

Aim of study: The objective of this research is to uncover the bioactive characteristics of ethanol extracts and essential oils from 15 different tree barks (*Thuja plicata, Sequoia sempervirens, Eucalyptus globulus, Pinus nigra, Platanus orientalis, Fagus orientalis, Populus tremula, Castanea sativa, Pinus sylvestris, Pinus pinaster, Picea orientalis, Populus nigra, Cryptomeria japonica, Abies nordmanniana, Quercus robur*).

Material and method: In this study, the bioactive properties of 15 different tree barks were investigated. Ethanol extracts were analyzed for their polyphenolic content, antioxidant activity, and antimicrobial properties. Additionally, the essential oils were evaluated for their anti-quorum sensing capabilities.

Main results: Several barks demonstrated high potential as sources of polyphenolic compounds, tannins, and antioxidant activity, indicating their potential as bioactive resources for the forestry and pharmaceutical industries.

Research highlights: The ethanol extracts of *Eucalyptus globulus*, *Castanea sativa*, and *Quercus robur* barks showed remarkable results regarding total polyphenolic content, antioxidant activity, and antimicrobial properties. Essential oils from the barks of *Populus nigra*, *Populus tremula*, and *Platanus orientalis* exhibited positive anti-quorum sensing activity. Moreover, both *Quercus robur* and *Eucalyptus globulus* displayed high bioactive potential.

Keywords: Wood Bark, Ethanol Extract, Essential Oil, Bioactive Properties

Bazı Ağaç Kabuğu Ekstraktlarının ve Uçucu Yağlarının Antioksidan, Antimikrobiyal ve Anti Quorum Sensing Aktivitelerinin Araştırılması

Öz

Çalışmanın amacı: On beş farklı ağaç kabuğunun (*Thuja plicata, Sequoia sempervirens, Eucalyptus globulus, Pinus nigra, Platanus orientalis, Fagus orientalis, Populus tremula, Castanea sativa, Pinus sylvestris, Pinus pinaster, Picea orientalis, Populus nigra, Cryptomeria japonica, Abies nordmanniana, Quercus robur*) etanol ekstreleri ve uçucu yağlarının biyoaktif özelliklerinin ortaya çıkartılmasıdır.

Materyal ve yöntem: Bu çalışmada, 15 farklı ağaç kabuğunun biyoaktif özellikleri araştırıldı. Etanol ekstreleri, polifenol içerikleri, antioksidan ve antimikrobiyal aktiviteler açısından test edildi; esansiyel yağlar ise anti-quorum sensing aktivitesi için test edildi.

Temel sonuçlar: Ana sonuçlar: Birçok kabuk, polifenolik bileşenlerin, tanen içeriğinin ve antioksidan aktivitenin ortalaması açısından yüksek potansiyel sergiledi, bu da onları orman ve farmasötik endüstri için potansiyel biyoaktif kaynaklar haline getirmektedir.

Araştırma vurguları: E. globulus, C. sativa ve *Q. robur* kabuklarının etanol ekstreleri, toplam polifenol içeriği, antioksidan ve antimikrobiyal özellikler açısından dikkate değer sonuçlar gösterdi. *Populus nigra, Populus tremula* ve *Platanus orientalis* kabuklarının esansiyel yağları ise olumlu anti-QS aktivitesi sergiledi. Bu bilgilere ek olarak *Q. robur* ve *E. globulus* yüksek biyoaktif potansiyele sahip olduğunu gösterdi.

Anahtar Kelimeler: Ağaç Kabuğu, Etanol Ekstresi, Uçucu Yağ, Biyoaktif Özellikler

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Introduction

In recent decades, with the advent of and with the progressive development of the variable multi-drug resistant superbugs, scientists are focusing their efforts on every way to find solutions for this problem. This situation increased the interest toward natural resources as a main origin for bioactive compounds, particularly with antimicrobial activity because of the extraordinary number of microorganisms inhabiting the environment that are in consistent situation of attacks and self-defense.

Plants produce many bioactive molecules like phenolic compounds, flavonoids, terpenes, stilbene, pinosylvin, alkaloids and others. These compounds participate to the defense mechanism of plants against bacteria, fungi, and insects (Cowan, 1999; Compean and Ynalvez, 2014). Although plants have been profoundly investigated for antimicrobial active compounds, most of the studies have only considered lower plants like grass, or the green parts of the trees, such as leafs, fruits, seeds or flowers (Sharmeen et al., 2012; Javid et al., 2015; Marasini et al., 2015; Egamberdieva et al., 2017; Bereksi et al., 2018). Nevertheless, the heartwood, knot wood, phloem and the bark of the trees may also be precious in term of bioactive molecules and may incorporate phenolic compounds, flavonoids, essential oils and others (Miranda et al., 2012; Metsämuuronen and Siren, 2014; Özgenç et al., 2017). In addition, alternatives to the formal antimicrobial molecules have been under investigation, referring to different methodologies and techniques like nanotechnology (Santos et al., 2013; Wang et al., 2017), molecular genetics tools (Westwater et al., 2003), phage therapy (Parisien et al., 2008), chemicals synthesis of antibiotic derivatives (Rahman and Gray, 2005; Al Bari et al., 2006) and using probiotics (Bidarkar et al., 2014; Fijan, 2016). One of the alternative methods that is still under research, is the alteration of the quorum sensing (QS) mechanism in the infective bacterium. QS is a mechanism of cell to cell signalization that permits for each bacterial cell to be in coherence with its population (Jayaraman and Wood, 2008). Communication takes place through diffusible molecules identified by their corresponding receptors on the bacterial cell surface (Hmelo, 2017). The general design of the mechanism is similar between the different bacterial cells whereas the molecules incriminated in the system are variables between the different species and genera (Jayaraman and Wood, 2008). QS system is a kind of sensor that transmits the information about the environmental conditions to the bacterial cell, which will perceive the information and accommodate its own growth to keep the harmony with the other cells inside the same population (Goryachev, 2009). Inside the bacterial cell, the QS effector molecules are correlated to genetic precursors and regulate the expression of some bacterial phenotypes such as the cellular replication and growth, pigmentation, biofilm formation, symbiotic relationships and the production of some molecules (Goryachev, 2009; Li and Tian, 2012; Kendall and Sperandio, 2016). Particularly QS mechanism is incriminated in the regulation of the pathogenic parameters of bacteria such as the production of virulence and pathogenicity Key elements that significantly contribute to the mechanisms of bacterial infection and proliferation (Yarwood and Schlievert, 2003; Hughes and Sperandio, 2008; Rutherford and Bassler, 2012). Quorum sensing mechanism may also control the relations between different bacterial species or genera and may even occur between different microorganisms to regulate their symbiotic relationships (Lowery et al., 2008; Ramanan et al., 2016).

The QS mechanism is highly proposed as an alternative solution for antimicrobial resistance mainly by using anti-QS blockers (quorum quenching mechanism) or by regulating the system to target some essential bacterial genes, particularly those affecting the bacterial virulence and pathogenicity (Hentzer and Givskov, 2003; Bjarnsholt et al., 2005; LaSarre and Federle, 2013). One of the first known and well-studied bacterial QS models is the *Chromobacterium violaceum (C. violaceum)* which synthesized a violet pigment called violacein that is essentially regulated by its QS system (Stauff and Bassler, 2011). This system is extensively employed in vitro, particularly for researching QS or anti-QS effectors (Norizan et al., 2013;

Tan et al., 2013; Vasavi et al., 2014; Abudoleh and Mahasneh, 2017). Tree barks constitute the main protective layer against external stress and are usually very rich in bioactive compounds such as polyphenols that are found to have the ideal chemical structure to remove free radicals (Sokół-Łętowska et al., 2007; Miranda et al., 2012).

This work aimed to test the bioactive potential of some wood extracts from different tree bark samples taken from the provinces of Trabzon in Turkey. The study focused on evaluating the antioxidant and antimicrobial properties of ethanol extracts derived from tree barks, with a parallel investigation into the anti-quorum sensing activity of the essential oils.

Materials and Methods

Material and Sample Preparation

Tree samples are listed in Table 1.

Table 1. Sample species and their numbers

Sample	Latin name
S1	*Thuja plicata*
S2	*Sequoia sempervirens*
S3	*Eucalyptus globulus*
S4	*Pinus nigra*
S5	*Platanus orientalis*
S6	*Fagus orientalis*
S7	*Populus tremuloides*
S8	*Castanea sativa*
S9	*Pinus sylvestris*
S10	*Pinus pinaster*
S11	*Picea orientalis*
S12	*Populus nigra*
S13	*Cryptomeria japonica*
S14	*Abies nordmanniana*
S15	*Quercus robur*

The 15 different samples were obtained from the provinces of Trabzon in northeastern Turkey. The study areas are located at 40°59'56.0"N and 39°40'59.3"E. Tree samples are listed in Table 1 with their species name, family and common name. Bark samples were air-dried, and they were ground and sieved as 60 mesh (250 um) particle size using Thomas-Wiley Laboratory Mill Model 4 and shaker sieve.

Ethanol Extracts Preparation

The organic extraction was applied by mixing 5 g of bark meal in 40 mL of ethanol (purity ≥ 99%). The blends were incubated for 24 hours at room temperature, subjected to agitation at 210 rpm using a Heidolph Promax 2020 shaker from Schwabach, Germany. The mixture was subsequently clarified by removing wood particles through Whatman No. 4 filter paper with a pore size of 20 - 25 µm. The resulting solutions underwent sterile filtration using 0.45 µm hydrophilic polyvinylidene fluoride (PVDF) filters. The different extracts were dried at 40°C for 24 - 48 h. Finally, each extract was dissolved in dimethyl sulfoxide (DMSO) (10%) in order to constitute working solutions of 10 mg/ml.

Essential Oil Extraction

The bark samples were air-dried at room temperature and subsequently underwent individual hydro-distillation sessions lasting 3 hours, employing a Clevenger-type apparatus (Yılmaz & Deniz, 2017). Following the distillation process, the essential oils (EO) were gathered and preserved in amber vials at -20°C until analysis. Approximately 30 g of bark meal from each sample was utilized for the isolation of essential oils.

Phenolic Contents and Antioxidant Activity

Polyphenolic compounds of the ethanolic extracts were investigated by testing the total phenolic, flavonoid and the condensed tannin content using Folin-Ciocalteu procedure (Slinkard and Singleton, 1977), spectrometric assay (Fukumoto and Mazza, 2000) and the Julkunen-Tiitto method (Julkunen-Tiitto, 1985) respectively. The outcomes of the total phenolic content were quantified in milligrams (mg) of gallic acid (GA) per gram (g) of bark extract. Total flavonoid contents were measured in milligrams of Quercetin Equivalents (QE) per gram of sample extract, while condensed tannin content results were determined in grams of Catechin equivalent (CE) per gram of wood powder extract. The assessment of antioxidant activity involved the examination of Ferric Reducing Antioxidant Power (FRAP) activity. (Benzie and Strain, 1996). Every test was repeated 3 times and the results were calculated as the means of the measurements.

Anti-microbial Activity

The antimicrobial activity of ethanolic extracts was assessed using the agar well

diffusion method, following the guidelines outlined by the Clinical & Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2016) The tests were conducted on Mueller Hinton agar plates against various microorganisms, including *Staphylococcus aureus (S. aureus)* ATCC 25923, *Escherichia coli (E. coli)* ATCC 25922, *Enterococcus faecalis (E. faecalis)* ATCC 29212, *Pseudomonas aeruginosa (P. aeruginosa)* ATCC 27853, *Klebsiella pneumoniae (K. pneumoniae)* ATCC 13883, *Acinetobacter haemolyticus (A. haemolyticus)* ATCC 19002, *Bacillus cereus (B. cereus)* ATCC 6633, and *Candida albicans (C. albicans)* ATCC 10231. Luria Bertani agar (LB) (LABM, UK) was used to cultivate the microorganisms and fresh cultures of 18 h in LB broth were used to prepare the suspensions of 0.5 McFarland in 2 mL of sterile isotonic sodium chloride. Mueller–Hinton (MH) agar lates were cultivated with each suspension using sterile cotton swab, then 0.6 cm wells were opened into the agar. Extracts were transferred as 50 µl per well for each sample. DMSO (10%) was used as negative control, and antibiotics were used as positive control: ampicillin (10 µg) was applied for *S. aureus*, *B. subtilis* and *E. faecalis*; gentamycin (10 µg) was used for *E. coli*, *K. pneumoniae*, and *A. haemolyticus* whereas amphotericin B (20 µg) was used with *C. albicans*. Petri dishes were placed in an incubator at a temperature of 37°C for a duration of 24 hours.

Anti-quorum Sensing Activity

The effectiveness of essential oils in inhibiting quorum sensing (anti-QS) was assessed using the microdilution method on a 96-micro-well plate, focusing on the bacterium *C. violaceum* ATCC 12472. Quorum sensing activity was examined by assessing the pigment production in the bacterial culture both with and without the application of essential oils. The results were then compared to the bacterial growth to evaluate the impact of the essential oils on QS. By hypothesis, an anti-QS bioactive compound will significantly decrease the production of bacterial pigmentation without affecting the bacterial cells growth, otherwise its activity is considered as antibacterial growth inhibitor instead of anti-QS.

In summary, each well of the micro-well plate was initially filled with 100 µl of LB broth. Subsequently, 100 µl of each essential oil was introduced into the first well of every row, and a sequential 1/2 dilution was performed by transferring 100 µl from the initial well to the next in the same row. This process continued until the last well, which served as the positive culture control for *C. violaceum*. For the preparation of the bacterial suspension, overnight cultures were adjusted to 0.5 McFarland (equivalent to 108 CFU/ml), taking into account a previous optimization of bacterial plate count based on the OD600 parameter in LB broth culture. The 0.5 McFarland bacterial suspension was further diluted to a 1/100 factor, and 5 µl of this dilution was added to each well. The 11th well in each row remained without bacteria, serving as the negative control. Each extract underwent testing twice, and two concentrations of vanilla (3.12 µg/ml and 1.56 µg/ml) were employed as positive controls for anti-QS sensing activity (Choo et al., 2006).

Following a 24-hour incubation period, the Minimum Inhibitory Concentration (MIC) was determined for each sample. In the case of volatile oils, the original solution was considered 100% concentration, and their relative percentages were assessed based on the serial dilution that occurred during the microdilution process. SubMIC concentration (½ MIC) and the subsequent smaller ratio (½ SubMIC) were analyzed for anti-QS activity for each sample. Bacterial count and pigment production were evaluated in these wells. For bacterial count determination, 10 µl was taken from each well, mixed with 90 µl of 0.9% NaCl to create a 1/10 dilution, and a series of 1/10 dilutions was continued until reaching a 10-8 dilution factor. The diluted solutions were then dropped on MH agar, incubated at 37°C for 24 hours, and bacterial colonies were counted for each dilution. The original bacterial count was calculated by multiplying the count by the dilution factor and by 10 to obtain the final count as colony-forming units (CFU) in the 100 µl culture. For pigment production assessment, the 96-well plates were subjected to dry at 50°C. Subsequently, 100 µl of dimethyl sulfoxide was introduced into each well and allowed to incubate on a microplate shaker for 2 hours in darkness.

After complete pigment extraction, DMSO solutions were transferred to a new plate, and their absorbance was measured at 595 nm. Each test was repeated twice for every concentration and at least three times for the bacterial positive control. The bacterial count and pigment absorbance of the volatile oils were statistically compared to the results of the positive control (bacterial growth without extract) using SPSS® Statistics software v.20 (IBM® Corporation, USA). The analysis utilized One-Way ANOVA, followed by the Tukey HSD method for data with homogenous variance or the Tamhane test for data with non-homogeneous variances. The tests were conducted in duplicate, and the the statistical analysis was conducted with a significance level set at 95%.

Results and Discussion

Antioxidant Activity

Total polyphenolic contents and antioxidant activity of bark's ethanol extract are presented in Table 2.

Table 2. Total polyphenol contents and antioxidant activity of bark's ethanol extract

Sample	Total phenolic (mg GAE/g)	Total flavonoid (mg QE/g)	Condensed Tannin (mg CE/g)	FRAP (µmol $FeSO_4.7H_2O$/g)
Thuja plicata	8.856±0.025^{c}	N.D.*	9.429±0.122^{g}	294.433±5.890^{bc}
Sequoia sempervirens	5.548±0.043^{b}	0.009±0.000^{i}	1.544±0.019^{a}	449.564±3.900^{ef}
Eucalyptus globulus	27.427±0.146^{m}	0.0008±0.0001^{f}	21.445±0.035^{m}	444.050±0.601^{bcd}
Pinus nigra	13.414±0.814^{e}	0.0003±0.000^{a}	7.973±0.153^{e}	277.187±4.763^{b}
Fagus orientalis	11.942±0.069^{d}	0.0004±0.000^{b}	8.362±0.082^{f}	301.427±4.445^{bcd}
Populus tremula	22.629±0.201^{k}	0.0014±0.000^{h}	13.386±0.042^{i}	396.358±3.445^{cde}
Castanea sativa	25.833±0.058^{l}	0.0004±0.000^{b}	9.399±0.017^{g}	528.728±5.098^{f}
Pinus sylvestris	19.477±0.623^{i}	0.0007±0.000^{e}	19.178±0.028^{k}	411.047±2.370^{de}
Pinus pinaster	21.657±0.024^{j}	0.0011±0.000^{g}	17.464±0.061^{j}	203.210±1.373^{b}
Picea orientalis	18.365±0.204^{h}	0.0006±0.000^{d}	10.777±0.033^{h}	316.754±3.456^{bcd}
Populus nigra	15.403±0.060^{g}	0.0005±0.000^{c}	5.237±0.088^{c}	204.681±3.567^{b}
Cryptomeria japonica	3.519±0.094^{a}	N.D.	2.222±0.003^{b}	63.040±0.006^{a}
Abies nordmandiana	14.206±0.153^{f}	0.0004±0.000^{b}	7.854±0.014^{d}	288.644±4.700^{bc}
Quercus robur	25.963±0.554^{l}	0.0005±0.000^{c}	21.042±0.012^{l}	505.063±0.090^{ef}

ND*: not detected, GAE: gallic acid equivalent, QE:quercetin equivalent, CE: condensed tannin equivalent, $FeSO_4.7H_2O$: iron(ii) sulfate 296eptahydrate

The highest content of phenolic compouds was obtained by *E. globulus* (27.427±0.146 mg GAE/g), *Populus tremula* (22.629±0.201 mg GAE/g), *Castanea sativa* (25.833±0.058 mg GAE/g), *Pinus pinaster* (21.657±0.024 mg GAE/g) and *Quercus robur* (25.963±0.554 mg GAE/g). Samples from all species showed high antioxidant activity with FRAP results between (396.358±3.445 µmol $FeSO_4.7H_2O$/g and 528.728±5.098 µmol $FeSO_4.7H_2O$/g). The highest antioxidant activity was registered for *Castanea sativa*, which registered low values of flavonoid and condensed tannin content. This may evoke that total phenolic content are the reason for the obtained antioxidant activity. *Pinus sylvestris* exhibited elevated FRAP results despite having a low quantity of total phenolic and total flavonoid content. This suggests that either condensed tannins or other molecules are implicated in the observed antioxidant activity. On the other hand, *Quercus robur* demonstrated high values in total phenolic content, condensed tannin content, and FRAP value, indicating that this species is a potent candidate as a source of polyphenolic molecules and antioxidant bioactive compounds. Considerably low content of total flavonoids were found with no detection notated in *Thuja plicata* and *Cryptomeria japonica* extracts. Other study working on Quercus species found high ratios of total phenolic and total flavonoids contents with polar extraction using hot water (Valencia-Avilés et al., 2018). Their results were around 700 mg GAE/g and 860 mg GAE/g for phenolic contents, and between 20 mg QE/g and 45 mg QE/g for flavonoid contents. As for condensed tannin content, ethanol extracts of *Quercus robur* and *Eucalyptus globulus* had the highest values among the studied samples. The lowest condensed tannin content was obtained from *Sequoia sempervirens* extract. Other study working on *Quercus robur* sp.

investigated water and ethanol-water extract for phenolic and flavonoid content. They showed results around 70 mg of gallic acid/g, and 72 mg of catechin/g for phenolic content and flavonoid contents respectively. The same study showed results of condensed tannin content (Proanthocyanidins) in hot water extract similar to ours with values ranging from 9.4 to 25.7 mg CE/g) (Valencia-Avilés et al., 2018). Tree barks are known to be rich in tannin contents. Researchers reported that spruce bark is a natural condensed tannin source for industry and that tannin industry is in continuous progress while looking for new cheap raw materials to increase the production with the desired properties (Kemppainen et al., 2014). In a previous study, a strong positive correlation (0.906) was found between total phenolic content and FRAP activity (Dudonné et al., 2009). The results in our study support this conclusion. The extracts from *Quercus robur* and *Castanea sativa* exhibited the highest antioxidant activity, correlating with their elevated total phenolic content. Conversely, the extract from *Cryptomeria japonica* displayed the lowest antioxidant activity, corresponding to its minimal total phenolic content.

Antimicrobial Activity Results

Agar well diffusion tests demonstrated positive activity for various samples, as depicted in Table 3. This study is not the initial exploration into this aspect. A study investigated by Omar et al., (2000) tested ethanolic extracts of the bark and the wood in many hardwood tree samples from North America, and good results were obtained. Between the samples, *Quercus robur's* ethanolic extracts were tested and positive antimicrobial activity was obtained with methicillin resistant *S. aureus, Bacillus subtilis, Mycobacterium phlei*, whereas no activity was observed over *E. faecalis, E. coli, P. aeruginosa, Salmonella typhimurium*, or *K. pneumonia* (Omar et al., 2000). Meanwhile our best results were obtained by the same species, *Quercus robur* with antimicrobial activity over *S. aureus* (15 mm), *K. pneumonia* (13 mm), *A. haemolyticus* (14 mm) and *C. albicans* (16 mm). Those results indicate a strong potential of this strain against *S. aureus* with taking in consideration the resistant strains. This same strain showed high total phenolic content, condensed tannin content and antioxidant activity of its ethanolic extracts, which may explain the observed antimicrobial activity.

Table 3. Antimicrobial activity results of the ethanolic extract evaluated by the measure of the inhibition zone diameter in mm

Sample	*S. aureus*	*E. coli*	*E. faecalis*	*P. aeruginosa*	*K. pneumoniae*	*A. haemolyticus*	*B. cereus*	*C. albicans*
S1 *Thuja plicata*	11	0	0	0	0	0	0	0
S2 *Sequoia sempervirens*	9	0	0	0	0	0	11	0
S3 *Eucalyptus globulus*	13	0	0	0	0	0	11	0
S4 *Pinus nigra*	0	0	0	0	0	0	0	0
S5 *Platanus orientalis*	NA	NA	NA	NA	NA	NA	NA	NA
S6 *Fagus orientalis*	0	0	0	0	0	0	0	0
S7 *Populus tremula*	0	0	0	0	0	0	0	0
S8 *Castanea sativa*	11	0	0	0	0	15*	0	17
S9 *Pinus sylvestris*	0	0	0	0	0	0	0	0
S10 *Pinus pinaster*	0	0	0	0	0	0	0	0
S11 *Picea orientalis*	0	0	0	0	0	0	0	0
S12 *Populus nigra*	0	0	0	0	0	0	0	0
S13 *Cryptomeria japonica*	0	0	0	0	0	0	9	0
S14 *Abies nordmanniana*	0	0	0	0	0	0	0	0
S15 *Quercus robur*	15	0	0	0	13	14*	0	16
DMSO (10%)	0	0	0	0	0	0	0	0
P+ (10µg/well)	33	25	22	20	24	23	20.5	23

* Heterogenic resistance profile, NA: not applied, P+: positive control

C. sativa is also known for its wealth in bioactive compounds such as phenolic acids, flavonoids and quercetin (Braga et al., 2015), and its extraction is mostly better with methanol, ethyl acetate or aqueous ethanol (Braga et al., 2015). In our study, *Castanea sativa*'s extract showed good antimicrobial activity with *S. aureus* (11 mm), *A. haemolyticus* (15 mm) and *C. albicans* (17 mm). Moreover, *Eucalyptus globulus* extracts showed remarkable activity with *S. aureus* and *B. cereus*. Eucalyptus species are famously known for their antimicrobial activity, mostly with their essential oils and non-polar components, whereas polar compounds are barely mentioned in the bibliographical resources. Nonetheless, the results we obtained may be due to a real inhibitory activity of the ethanolic extracts, or maybe to some residual oils that have exudate with the extracted compounds as artefacts. However, when applying the anti-QS activity tests, the plate containing eucalyptus samples did not show any bacterial growth for any of the samples included in the same plate (S1, S2, S3 and S4), not even in the positive control where no essential oil was added. This may indicate a powerful antibacterial activity of the essential oils of this sample, which may exert its bactericidal activity in its vaporous status during small laps of time. Ethanolic extracts obtained from the *Pinus* species did not show any antibiotic activity against the tested bacteria (samples S4, S9 and S10). Other study conducted by Yang and Jaakkola (2012) on Scots pines showed positive activity of different polar extracts and essential oils against many bacteria without mentioning any positive result concerning ethanolic compounds, particularly a positive activity with methanolic extracts from *Pinus sylvestris* was observed against *S. aureus.*

Anti-QS Activity Results

Anti-QS activity results of the essential oils showed three samples with statistically significant inhibitor activity of bacterial pigment production (*Populus nigra, Populus tremula* and *Platanus orientalis*). These results are expressed as charts in figures 1 - 3. The charts show clearly the inhibition of the pigment production by the bacteria revealed by the massive decrease of the Optical Density at 595 nanometers (OD595) in the presence of the EOs, in comparison with the bacterial growth in the absence of EOs (0%), meanwhile the bacterial count are still in the same average for the both situations. Figure 4 illustrates the anti-QS activity of vanilla at concentrations of 50 µg/ml and 100 µg/ml. Vanilla reduced pigment production to OD595 = 0.1 and 0.3, while bacterial growth remained unaffected (around 108-109 CFU/ml) compared to the control.

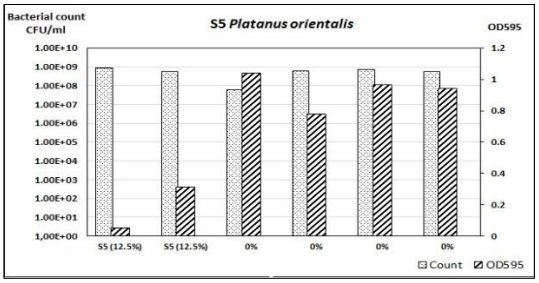

Figure 1. Anti-QS activity chart of *Platanus orientalis* oil sample (S5)

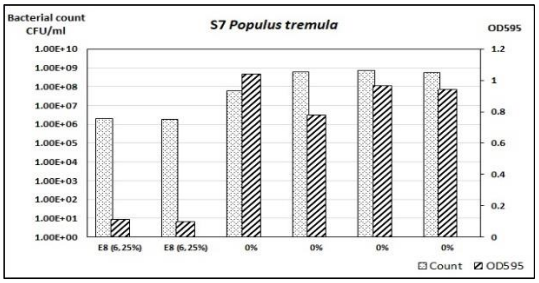

Figure 2. Anti-QS chart of *Populus tremula* oil sample (S7)

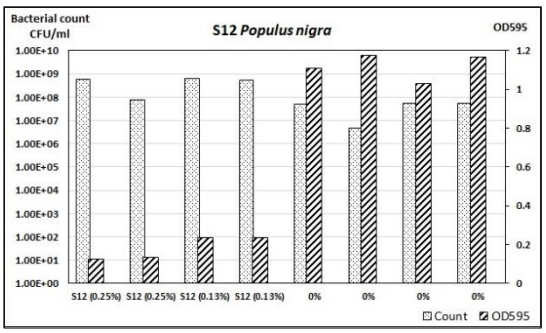

Figure 3. Anti-QS activity chart of *Populus nigra* bark essential oil sample (S12)

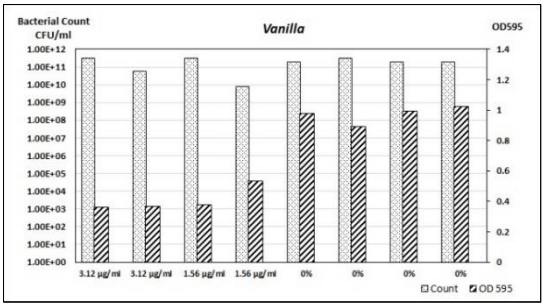

Figure 4. Anti-QS activity chart of Vanilla

Although EOs have been widely investigated for their anti-QS activity (Kerekes et al., 2013; Mokhetho et al., 2018; Poli et al., 2018), EOs of wood origins are barely presents in bibliography. Our results showed three positive anti-QS activity between 15 samples, which highlights the importance of these components and that wood and bark resources should be considered for further investigations in this purpose.

Conclusion

This study aimed to screen the bioactive potential of different wood species collected from Trabzon provinces. Many species showed high potential as mean of polyphenolic compounds, tannin content and antioxidant activity, which make them a potential bioactive resource for forest and pharmaceutical industry. Antimicrobial and anti-QS activity results also propose these species as promising nominate for pharmaceutical and antimicrobial purposes. In this prospect, *Q. robur* and *E. globulus* showed high bioactive potentials, which should be taken in consideration. Ethanol extracts seem to be insufficient and testing with other polar solvents, mainly water and methanol extraction is recommended. Identification of the bioactive molecules and their investigation with other bacterial species, particularly testing their effect on resistant bacterial strains is recommended. Essential oils seem promising agent for anti-QS activity, yet antimicrobial activity investigation is highly recommended.

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Author Contributions

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Conflict of Interest

The authors declare that they have no conflict of interest.

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