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Biological Activities of Trunk Bark Extracts of Five Tree Species from Anatolia, Turkey

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The antioxidant and antimicrobial capacity of the trunk bark extracts of five tree species, walnut (*Juglans regia* L.), pine (*Pinus brutia* Ten.), rhododendron (*Rhododendron ponticum* L.), juniper (*Juniperus oxycedrus* L.ssp.) and eucalyptus (*Eucalyptus globulus* L.), which were obtained from different regions of Anatolia, Turkey are investigated. Antioxidant-rich fractions were extracted from trunk-bark of the five species by using ethanol as solvent. Total antioxidant activity, ferric reducing/antioxidant power (FRAP), DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity and lipid peroxidation inhibitory activity of the extracts were studied. The total antioxidant activity of the extracts was evaluated by TLC-plate and ferric thiocyanate (FTC) methods. The total antioxidant activity and reducing power of the samples were concentration dependent. All the samples showed moderate to high antioxidant activity. The extracts also showed high radical scavenging activity. Pine and walnut extracts showed the highest inhibitory activity against lipid peroxide formation. Rhododendron was the least active in this respect. The antimicrobial activity of each extract was also studied with agar diffusion method using eight bacteria and two yeasts. Eucalyptus and walnut trunk-bark extracts showed considerably high antimicrobial activity against the microorganisms studied. All the extracts, especially those from walnut and juniper trunk bark proved to be a good source of antioxidants and to deserve further investigation as to their individual biologically active components, which may be an attractive source of nutraceutical supplements and of medicinal ingredients.

Key Words: *Juglans regia* (L.), Walnut, *Rhododendron ponticum* (L.), *Pinus brutia* (Ten.), Pine, *Juniperus oxycedrus* L. sp., *Eucalyptus globulus* L., Trunk bark extract, Antioxidant activity, Antimicrobial activity.

INTRODUCTION

Free radicals attracted physicists and radiologists much for long were much later found to be a product of normal metabolism. It is well known

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that radicals cause molecular transformations and gene mutations in many types of cells. Scientists report that the active compounds that are safe and necessary to scavenge free radicals, which are produced as a result of oxidative stress and are the main cause of many diseases, are mainly found in natural sources. The agents that scavenge or inhibit the formation of radicals are called antioxidants[1,2]. Antioxidants can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction, therefore, appear to be very important in the prevention of many diseases. Several methods have been developed in recent years to evaluate the total antioxidant capacity of biological samples[2-6]. The basis of many of these methods relies on linoleic acid used as the target lipid that is oxidized in the procedures and the peroxidation products are analyzed in different manner by various methods. Other widely used methods for measuring antioxidant activity involve the generation of radical species and the radical concentration is monitored as the present antioxidants scavenge them[7]. In TLC-plate and ferric thiocyanate (FTC) methods, inhibition or retardation of linoleic acid oxidation is monitored. Radical formation and the following scavenging are applied in another method of antioxidant activity measurement, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging. Ferric reducing power assay is another method of antioxidant capacity determination which utilizes the reduction of Fe(III) to Fe(II) by the active compounds and concomitant monitoring of colour intensity.

The number of antioxidant compounds identified in plants as secondary products, mainly phenolics, is currently estimated to be between 4000 and 6000 and increases every day. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives[8-11]. They are primarily synthesized by pentose phosphate, shikimate and phenylpropanoid pathways[12,13]. In many cases, these substances serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and protect molecular damage and predation by microorganisms, insects and herbivores[14,15]. A direct relationship has been found between the phenolic content and antioxidant capacity of different parts of plants. The antioxidant activity of phenolics is related to a number of different mechanisms as: free radical scavenging, hydrogen donation, singlet oxygen quenching, metal ion chelating and acting as a substrate for radicals such as superoxide and hydroxide[16]. Most parts of plants attracted interest of scientists for their biological and medicinal properties. Many scientists have investigated the chemical composition and antioxidant and antimicrobial properties of barks of several tree species[16-22]. Some trunk bark extracts were used as analgesic, anti-inflammatory, antibacterial, antiseptic and antifungal medicines[23-25].

Walnut tree (*Juglans regia* L.) is grown mainly in East Europe, Turkey, Iraq, east of Iran, Himalayas and Moldavia and most parts of it are used for many different purposes, such as food, medicine and cosmetics[26,27]. The seeds of walnut provide a highly nutritious food and walnut oil is rich in essential unsaturated fatty acids such as linoleic and linolenic acids[28]. In addition, walnut seeds contain α-tocopherol, polyphenols, and are widely used for treatment of cardiovascular diseases, common cold and rheumatisms[29-31]. The trunk bark of walnut tree is also used in dye and toothpaste production and cosmetics[32].

Pinus brutia Ten. is indigenous to Turkey. Its dried barks are rich in tannin and have been used as a tanning agent and to cause constipation. The leaves can be used as mucus remover and antiseptic[19]. Its resin is also used for medicinal purposes, for instance, to treat abscess or relieve pain[33]. Kaundun *et al.*[20] isolated antioxidant compounds, two proanthocyanidines, *i.e.* prodelphinidine and procyanidin and six flavonols, *e.g.*, myricetin, quercetin, larycitrin, kaempferol, isorhamnetin and syringetin from *P. brutia* Ten.

Juniperus species, among which *Juniperus oxycedrus* L. was of interest in the current study, are used as antiseptic, urine increaser and tar resource. Plants of this genus contain etheric oils, a tripentene known as resine cadinene and phenols, which are used in the treatment of some skin ailments[19]. Among this genus, *J. foetidissima* is used as menses remover, antiseptic and diuretic[23]. Fruit and tar of *J. oxycedrus* are widely used for medicinal purposes, such as in the treatment of cough and pain, hemorrhoids, eczema, sore throat and bronchitis and as insect repellant[31,33].

Rhododendron, also known as wild rose or forest rose, is a widely known plant especially with its nice flowers and with intoxications as a result of rhododendron honey consumption[34]. While it has many different sub-species, *Rhododendron ponticum* L. is endemic for Northeastern Black Sea region[35,36].

Eucalyptus (*Eucalyptus globulus* L.) is one of the most widely investigated trees in relation to their biological activities[2,37,38]. Eucalyptol, 1,8-cineol, a well-known anti-inflammatory agent, is a monoterpene obtained from eucalyptus leaves[39]. Eucalyptus leaf extracts are used as natural food additive in Japan. Eucalyptus oil and extracts posses lipid peroxidation inhibitory activity and eucalyptus oil is an intestine antiseptic and has antibacterial activity[40,41].

In the current study, total polyphenolics, total antioxidant activity with TLC-plate and ferric thiocyanate methods, radical scavenging activity and lipid peroxidation inhibitory activity of the ethanolic extracts from the trunk barks of five tree species grown in various regions of Turkey were investigated. The methods used in the study are the ones widely employed in

biological investigations and proved, in earlier studies, to be dependable analytical tools in assessing biological activity[4,6,41,43]. The objective of this study was to investigate the antioxidant and antimicrobial activities of the extracts from trunk barks of *J. regia*, *R. ponticum*, *P. brutia*, *J. oxycedrus*, and *E. globulus* in order to evaluate their medicinal value and to point out easily accessible sources of natural antioxidants that could be used as possible food supplement or in the pharmaceutical industry.

EXPERIMENTAL

All the reagents used were of analytical grade. L-Ascorbic acid, α-linoleic acid, ± catechin, 2-thiobarbituric acid, ferrous chloride, ammonium thiocyanate and DPPH stable radical were purchased from Sigma Chemical Co. Tween-20 and BHT were supplied by Applichem. TLC aluminum plates (Silica gel 60 F254) and Trolox® (6-hdroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were obtained from Merck Co. Folin-Ciocalteu's reagent was purchased from Fluka Chemie GmbH.

An ATI-Unicam UV-2 UV-Vis spectrophotometer was used in all absorbance measurements. Nuve EN 400 incubator was used for incubations at 35-40°C. Sanyo (CFC-free) deep-freezer was used for storing the extracts until tested. Denley BS400 Centrifuge was used for centrifugations.

Plant material: The trunk bark samples of *J. regia*, *R. ponticum*, *P. brutia*, *J. oxycedrus* and *E. globulus* were collected in Black Sea region of Turkey and authenticated by Mustafa Usta in Faculty of Forestry, Forest Industrial Engineering, Karadeniz Technical University, Trabzon, Turkey. All the samples were first sliced and dried and then ground to a fine powder in a mill before solvent extraction is applied.

Extraction: 5 g dried powder of each sample was extracted with 100 mL ethanol in a soxhlet apparatus until the extraction medium appeared which took 3 h and 5-6 cycles with 250 mL soxhlet system. Then the extracts were evaporated until dryness with rotary evaporator at 50°C under vacuum and the residues, after weight determination, were redissolved in ethanol to have stock solutions of 10 mg/mL concentration.

Determination of total phenolic contents: Total soluble phenolics in the ethanolic extracts of the samples were determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton[44] by using ± catechin as a standard. 0.1 mL sample solution (1.0 mg/mL) was diluted with 5.0 mL distilled water. 0.5 mL 0.2 N Folin-Ciocalteu reagent was added and the contents were vortexed. After a 3 min incubation, 1.5 mL Na_2CO_3 (2 %) was added and after vortexing, the mixture was incubated for 2 h at 20ºC with intermittent shaking. The absorbance was measured at 760 nm at the end of the incubation period. The concentration of total

phenolic compounds was determined as microgram of catechin equivalent by using a standard graph.

Determination of antioxidant activity: The antioxidant capacity of the trunk-bark extracts was examined by comparing to that of known antioxidants BHT, Trolox® and ascorbic acid by employing the following five complementary *in vitro* assays: TLC-plate method[45], ferric thiocyanate (FTC) method[46], ferric reducing/antioxidant power (FRAP) assay[47], DPPH free radical scavenging assay[48] and inhibition of lipid peroxidation[43]. A preliminary evaluation of antioxidant activity of the extracts was made using TLC-plate screening method and the extracts were then tested by the other methods.

Antioxidant activity by fluorescence persistence time: The method of Chang *et al.*[45] was slightly modified. A fluorescent coated TLC-plate was dried at 105°C for 0.5 h and a 5 mL extract (1.0 mg/mL) was spotted on the TLC plate twice with a 10 µL semi automatic pipette, drying in between the pipetings and at the end. The plate was then plunged into 3 % α-linoleic acid solution in hexane twice, drying in between. After complete dryness, the plate was placed 2.5 cm below a UV (254 nm) light source and the background of the spots appeared within first 10-15 min under continuous irradiation. The fluorescent spots on TLC-plate were observed every 10 min under continuous irradiation and the time each fluorescent spot disappeared was considered the induction period for lipid peroxidation. The antioxidant activities of the samples were evaluated by comparing their fluorescence disappearance times with those of the reference standards.

Ferric thiocyanate antioxidant activity method (FTC): The second antioxidant activity measurement assay employed is ferric thiocyanate (FTC) method[46]. Aliquots of 0.5 mL from each extract at 1.0, 2.5, 10 mg/mL concentrations were mixed with 2.5 mL α-linoleic acid emulsion (0.28 g linoleic acid, 0.28 g Tween 20 and 50 mL 0.02 M phosphate buffer, pH 7.0) and 2 mL 0.02 M phosphate buffer (pH 7.0). The mixture was incubated at 40°C in 10 mL test tubes. 0.1 mL 30% ammonium thiocyanate was added to each tube. The antioxidant activity was evaluated from the peroxide value, which was determined at various intervals during the incubation by measuring the absorbance at 500 nm 3 min after coloring with 0.1 mL 0.02 M $FeCl_2$.

Ferric reducing/antioxidant power assay (FRAP): The reducing power of the ethanolic extracts was determined according to a modified version of ferric reducing/antioxidant power (FRAP) assay of Oyaizu[47]. Different concentrations of extracts and ascorbic acid, for comparison, (1, 5 and 10 mg/mL) were mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL 1% potassium ferric-cyanide [$K_3Fe(CN)_6$]. The mixture

was incubated at 20°C for 20 min. After incubation period, the mixture was vortexed and 2.5 mL aliquots were mixed with 2.5 mL distilled water and 0.5 mL 0.1% $FeCl_3$ and the absorbance was measured at 700 nm. Higher absorbance value means higher reducing power of the sample.

Free radical scavenging activity: The free radical scavenging activity of the ethanolic extracts was measured by the method of Cuendet *et al.*[48]. Briefly, 50 μL extract of various concentrations was added to 5 mL 0.004 % ethanolic DPPH solution. After a 0.5 h incubation period at room temperature, the absorbance was read against a blank at 517 nm. Lower absorbance of the reaction mixture indicates higher DPPH radical scavenging activity.

Inhibition of lipid peroxide formation: In the determination of lipid peroxide formation inhibitory activity of the extracts, the reaction mixture contained 0.1 mL 25% (w/v) rat liver homogenate in 40 mM *tris*-HCl buffer (pH 7.0), 30 mM KCl, 0.16 mM ferrous iron, various concentrations of extract and positive controls and 0.06 mM ascorbic acid in a final volume of 0.5 mL. All the extracts and positive controls had their own control reactions, containing all related reagents except the test compounds. The mixture was then incubated at 37ºC for 1 h. The lipid peroxide formation was measured by the method of Ohkawa *et al.*[43]. For this, 0.4 mL of the reaction mixture was treated with 0.2 mL of sodium dodecyl sulfate (SDS) (8.1%), 1.5 mL of thiobarbituric acid (0.8%) and 1.5 mL of acetic acid solution (20 %) adjusted to pH 3.5 with NaOH. The total volume was then made up to 4 mL by adding distilled water and kept in a water-bath at 95°C for 1 h. After cooling, 1 mL of distilled water and 5 mL of *n*-butanol:pyridine mixture (15:1 v/v) were added and the mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was taken and its absorbance at 532 nm was measured. The per cent inhibition of lipid peroxidation was determined by comparing the results of the test compounds with those of controls not treated with the extracts. Per cent inhibition (I) of lipid peroxide formation was calculated according to the following equation:

$$I = (A_0 - A_1/A_0) \times 100$$

where A_0 is the absorbance of the control reaction containing all reagents except the test compound and A_1 is the absorbance of the test compound. The IC_{50} values represent the concentration of the compounds that cause 50 % inhibition of lipid peroxidation.

Determination of antimicrobial activity

Test microorganisms: All test microorganisms, eight bacteria and two yeasts, were obtained from Refik Saydam Hifzissihha Institute (Ankara, Turkey) and were as follow: *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212,

Moraxella catarrhalis ATCC 25238, *Escherichia coli* ATCC 35218, *Enterobacter cloacae* ATCC 13047, *Pseudomonas aeruginose* ATCC 10145, *Candida tropicalis* ATCC 13803 and *Candida albicans* ATCC 10231.

Agar-well diffusion method: Simple susceptibility screening test using agar-well diffusion method[49] as adapted earlier[50] was used. Each microorganism was suspended in Brain Heart Infusion (BHI) broth and diluted *ca.* 106 colony forming unit (cfu) per mL. They were flood-inoculated onto the surface of Brain Heart Infusion agar and Sabouraud Dextrose (Difco, Detriot, MI) agar (SDA) and then dried. For *C. albicans* and *C. tropicalis*, SDA was used. Seven millimeter diameter wells were cut from the agar using a sterile cork-borer and 50 µL of the solutions (1.0 mg/mL) were delivered into the wells. The plates were incubated for 18 h at 35°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganism. Ethanol served as negative control. The results were expressed in terms of diameter of inhibition zone: (-) no inhibition (5-7 mm in diameter), (+) very low inhibition (8-10 mm in diameter), (++) low inhibition (11-13 mm), (+++) average inhibition (14-6 mm) and (++++) high inhibition (> 17 mm).

RESULTS AND DISCUSSION

Many different parts of plants are known to contain various types of compounds possessing antioxidant capacities at different strengths[51]. Many natural antioxidants are found in wood, stem bark, trunk bark, leaf, fruit, root, flower and seed of trees[52]. Most of these compounds are normally phenolic or polyphenolic in nature. Some researches[53-55] have shown that stem bark and trunk bark of trees contain exceptionally large amounts of various phenolic substances. These substances also have antimicrobial and antioxidative properties.

Table-1 shows scientific, family, local and common names of the five tree species studied. The yields of extracts obtained using ethanol and their total phenolic contents are listed in Table-2. Since the per cent yield of the extracts and their polyphenolic contents did not correlated well, it may be assumed that the extracts contained compounds of non-phenolic nature. Among the samples, walnut showed the highest extraction yield whereas juniper had the lowest extractable components. In addition, juniper was found to have the highest amount of polyphenolics and rhododendron to have the lowest. Polyphenolic substances have an important role in suppressing lipid oxidation and are closely associated with antioxidant activity. Most phenolic compounds contribute to antioxidative action. Moreover, polyphenolic compounds have been suggested to have inhibitory effects on mutagenesis and carcinogenesis in humans when ingested up to 1.0 g daily from a diet rich in fruits and vegetables[8].

TABLE-1
FAMILY NAMES, LATIN NAMES, COMMON NAMES AND TURKISH (LOCAL) NAMES OF TREES STUDIED

Family name	Scientific name	Common name	Turkish name
Juglandaceae	*Juglans regia* L.	Walnut	Ceviz
Ericaceae	*Rhododendron ponticum* L.	Forest rose or wild rose	Orman gülü
Myrtaceae	*Eucalyptus globulus* L.	Eucalyptus	Ökaliptus
Pinaceae	*Pinus brutia* Ten.	Pine	Kizil çam
Cupressaceae	*Juniperus oxycedrus* L.ssp.	Juniper	Kokulu ardiç

TABLE-2
EXTRACTION YIELDS AND TOTAL PHENOLIC CONTENTS OF FIVE TRUNK BARK SAMPLES STUDIED

Sample	Extraction yield (g/100 g dry sample)	Total phenolics (g/100 g extract)
Walnut	8.0	58.4
Pine	4.8	63.0
Juniper	1.6	69.6
Rhododendron	2.8	34.8
Eucalyptus	2.8	52.2

Values are means of duplicate analyses.

The present study focussed mainly on the antioxidant activity of ethanolic extracts of the trunk barks of *J. regia*, *P. brutia*, *R. ponticum*, *J. oxycedrus* and *E. globulus*. The results of the TLC plate method are shown in Fig. 1. All of the extracts (1.0 mg/mL) showed considerable antioxidant activity for linoleic acid peroxidation. While BHT standard had the highest antioxidant activity. The five extracts showed antioxidant activities higher than that of other standards used, namely ascorbic acid and Trolox®, according to TLC plate method (Fig. 1). The order of the antioxidant activity of the extracts was juniper > walnut > eucalyptus > rhododendron > pine.

The amount of peroxides formed in linoleic acid emulsion during incubation in FTC method is determined spectrophotometrically by measuring absorbance at 500 nm (Fig. 2). In this method, higher the absorbance increase is, higher the concentration of peroxides formed and hence lower the antioxidant activity of the sample tested. The extracts showed considerable antioxidant activity according to FTC method though their activities were less than the reference standards used, BHT and Trolox®. The antioxidant activities of the ethanolic extracts were found in FTC method to be in the order of juniper > walnut > eucalyptus > rhododendron > pine (Fig. 2). High antioxidant activity of juniper and walnut may be correlated with their high polyphenolic contents. The antioxidant activity of the extracts increased linearly with the extract concentration (Figs. 3-7).

Burits *et al.*[18] and Cruz *et al.*[21] reported that extracts of various juniper species showed high hydroxyl radical scavenging and lipid peroxidation inhibitory activities. In addition, Topçu *et al.*[56] have isolated diterpenes from *Juniperus excelsa*, a different juniperus species and the compounds have also been found to be moderately active against *Mycobacterium tuberculosis*.

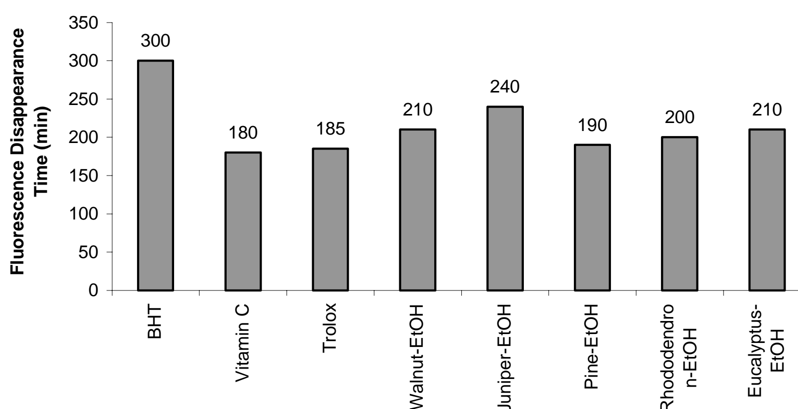

Fig. 1. Antioxidant activity of ethanolic extracts of trunk bark samples in TLC plate method (1.0 mg/mL) (BHT: butylated hydroxytoluene). All the values are average of duplicate measurements

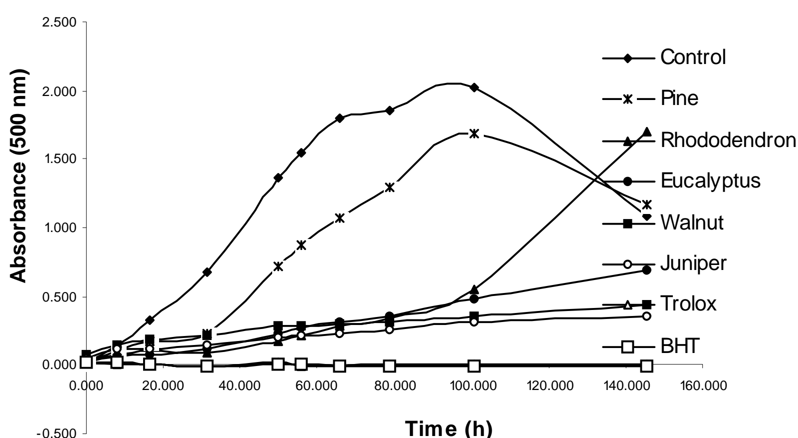

Fig. 2. Total antioxidant activity of the extracts of trunk-bark of the five species at 1.0 mg/mL concentration according to ferric thiocyanate (FTC) method. The results are the means of duplicate measurements. (BHT: butylated hydroxytoluene)

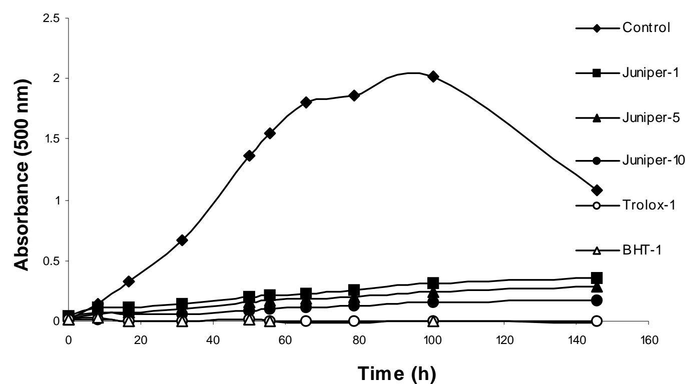

Fig. 3. Total antioxidant activity of trunk-bark extract of juniper (*J. oxycedrus*) and BHT at different concentrations according to ferric thiocyanate (FTC) method. The results are the means of duplicate measurements. Numbers 1, 5 and 10 next to the sample name on the graph represent mg/mL concentration

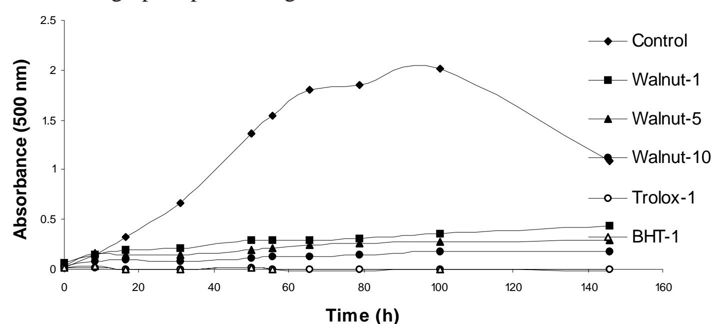

Fig. 4. Total antioxidant activity of trunk-bark extract of walnut (*J. regia*) and BHT at different concentrations according to ferric thiocyanate (FTC) method. The results are the means of duplicate measurements. Numbers 1, 5 and 10 next to the sample name on the graph represent mg/mL concentration

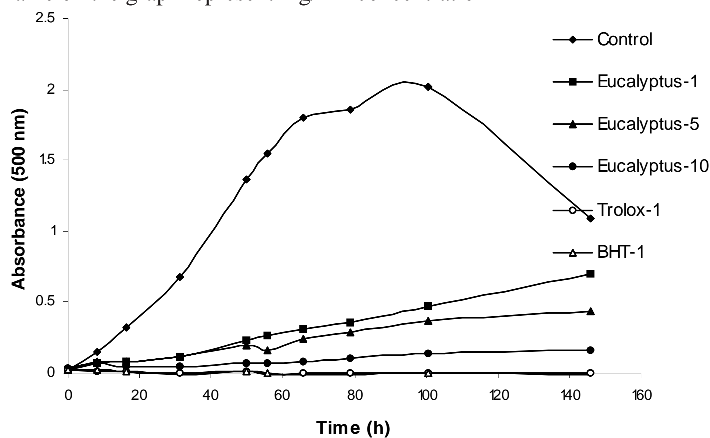

Fig. 5. Total antioxidant activity of trunk-bark extract of eucalyptus (*E. globulus*) and BHT at different concentrations according to ferric thiocyanate (FTC) method. The results are the means of duplicate measurements. Numbers 1, 5 and 10 next to the sample name on the graph represent mg/mL concentration

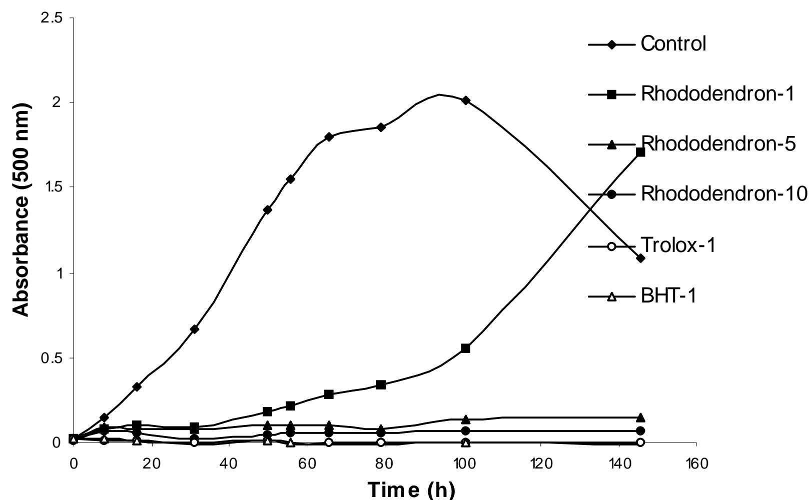

Fig. 6. Total antioxidant activity of trunk-bark extract of rhododendron (*R. ponticum*) and BHT at different concentrations according to ferric thiocyanate (FTC) method. The results are the means of duplicate measurements. Numbers 1, 5 and 10 next to the sample name on the graph represent mg/mL concentration

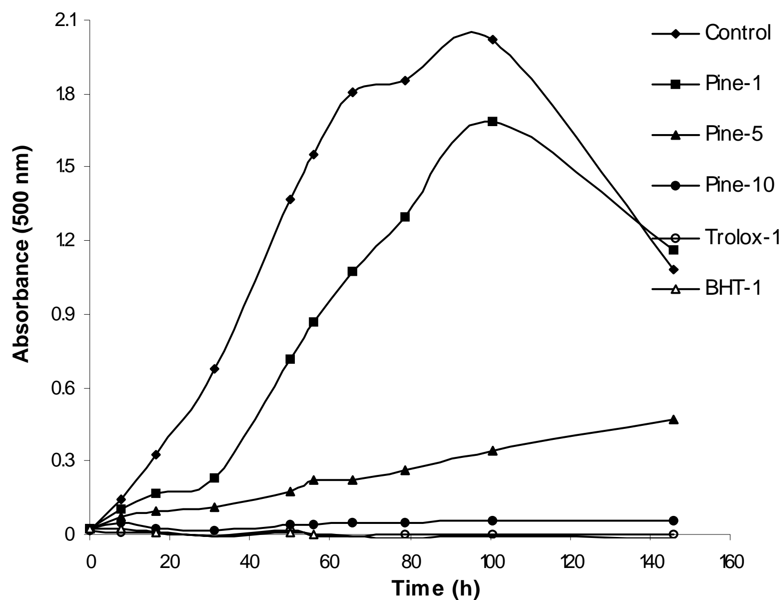

Fig. 7. Total antioxidant activity of trunk-bark extract of pine (*P. brutia*) and BHT at different concentrations according to ferric thiocyanate (FTC) method. The results are the means of duplicate measurements. Numbers 1, 5 and 10 next to the sample name on the graph represent mg/mL concentration

Reducing power test, in which the capacity of breaking radical chain reactions is reflected, is considered to be a good indicator of antioxidant capacity[42]. Thus, this method was employed to determine the antioxidant capacity and indirectly total reducing potentials of three different concentrations of the extracts and ascorbic acid (E_0 = 0.058 V), which was used as a reference standard. The reducing power measured for all five species showed a concentration dependent pattern (Fig. 8). Hence, the method proved to be applicable. The increased absorbance is an indication of higher reducing power in this method. Among the samples, eucalyptus showed the highest reducing power. The order of reducing power for the extracts and the standards is as follows: BHT > walnut > eucalyptus > pine > juniper > ascorbic acid > rhododendron. The total reducing power is the sum of the reducing powers of individual compounds present in a sample. The results of the total reducing power analyses did not coincide with that of total polyphenolics. The explanation of this may be that the type of polyphenols in the samples may vary highly as do their reducing powers. An example was reported about the reducing potentials of flavonoid derivatives quercetin, kaempferol and catechin, whose reducing potentials were reported to be 0.03, 0.12 and 0.16 V, respectively[3]. Amakura *et al.*[41] isolated gallic acid and ellagic acid from eucalyptus leaves and reported that both compounds possess antioxidant activity.

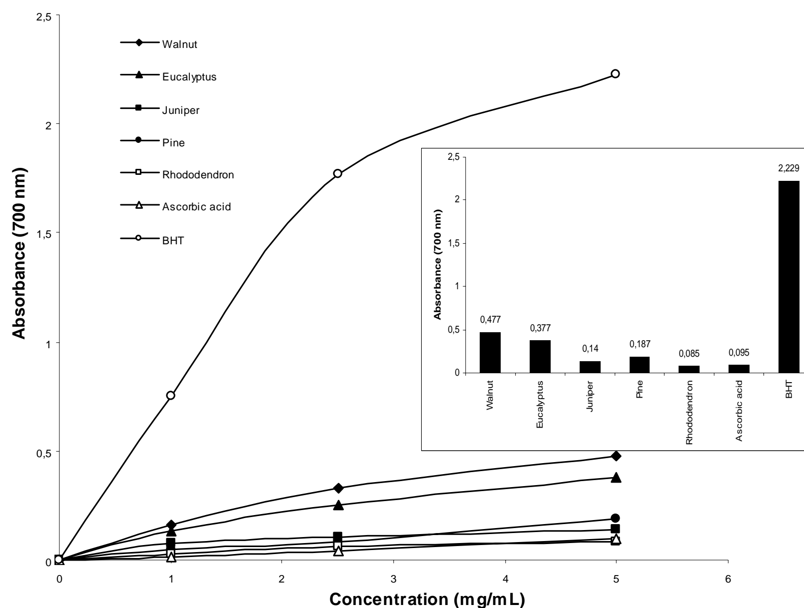

Fig. 8. Reducing power of the five trunk bark sample extracts. All the values are the means of duplicate analyses. The small graph on the right shows the absorbances at 700 nm for 5 mg/mL extract concentration of the samples and the standards BHT and ascorbic acid

2,2-Diphenyl-1-picrylhydrazyl (DPPH) can make stable free radicals in aqueous or ethanol solutions. It is well known that free radicals are able to induce lipid peroxidation. In order to evaluate the antioxidant potential of the trunk-bark samples through free radical scavenging, the DPPH free radical scavenging activity was determined. Fig. 9 illustrates 50% inhibitory concentrations (IC_{50}, mg/mL) of the samples for free radical formation. The radical scavenging activities of the samples and the standards were found to be in the order of ascorbic acid > pine > walnut > BHT > juniper > eucalyptus > rhododendron.

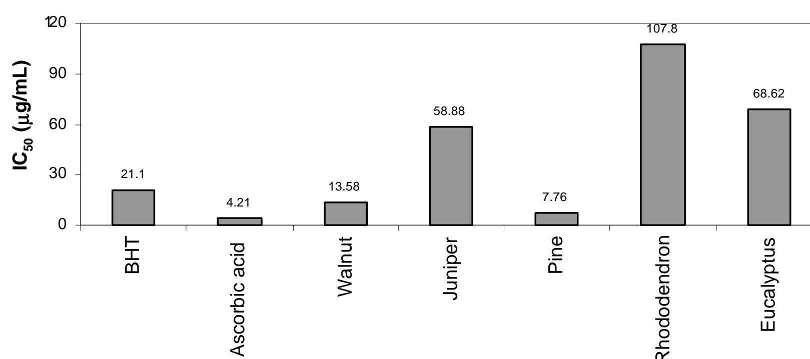

Fig. 9. Free radical (DPPH) scavenging activity of the trunk-bark extracts, BHT and ascorbic acid. IC_{50} represents the 50% inhibitory concentration of the samples against free radical formation. The values are the means of duplicate measurements

Inhibition of lipid peroxidation method was used to better identify antioxidant character of the samples. Lipid peroxidation was inhibited by all the samples at various extents (Table-3). The order of inhibitory activities, expressed as the concentration of the extract required to provide 50 % inhibition of peroxidation, was BHT > pine > walnut > eucalyptus > juniper > rhododendron. Extracts of eucalyptus leaves was reported[40,57] to inhibit the lipid peroxidation between 30 to 60 %.

TABLE-3
LIPID PEROXIDATION INHIBITORY ACTIVITIES OF THE EXTRACTS

Sample	IC_{50} (µg/mL)
Walnut	482
Pine	465
Juniper	881
Eucalyptus	778
Rhododendron	1245
BHT	13

All the values are the means of duplicate analyses.
IC_{50}: the extract concentration causing 50% inhibition of lipid peroxidation.

A good amount of research data about the antioxidant capacity of plants in the literature clearly show that the methods in many stages of research from sample preparation to antioxidant activity measurements vary highly and it is almost impossible to compare the results of one investigation with another. The trunk bark samples could be said to have quite a good level of antioxidant activity, it is not possible to compare our results with literature data due to the lack of standardization in the methods.

The test samples showed antimicrobial activity at various strengths towards ten microorganisms, eight bacteria and two yeasts. Among the active extracts, eucalyptus and walnut showed the highest antimicrobial activity (Table-4). This is in agreement with the findings of Cruz[21] and Potgieter *et al.*[57]. Walnut was especially effective against the pathogenic yeasts *C. albicans* and *C. tropicalis*. Parallel to our findings, Alkhawaja[32] reported that *J. regia* L. stem bark extracts inhibited Gram-positive bacteria *S. aureus* and Streptococcus mutants, Gram-negative bacteria *E. coli* and *P. aeruginosa* and its activity was lower than that of eucalyptus and walnut.

TABLE-4
ANTIMICROBIAL ACTIVITY OF THE EXTRACTS

Test Microorganism	Walnut	Juniper	Pine	Eucalyptus	Rhododendron
S. aureus ATCC 25923	+	-	-	++	++
E. faecalis ATCC 29212	+	-	-	+	+
B. subtilis ATCC 6633	++	++	-	+++	-
M. catarrhalis ATCC 25238	++	-	+	++	+
E. coli ATCC 35218	+	-	+	+	+
E. cloacae ATCC 13047	+	-	-	-	-
P. aeruginosa ATCC 10145	+	-	-	++	-
C. tropicalis ATCC 13803	+++	-	-	+	++
C. albicans ATCC 10231	+++	-	+	-	-

(-) no inhibition (5-7 mm in diameter), (+) very low inhibition (8-10 mm), (++) low inhibition (11-13 mm), (+++) average inhibition (14-16 mm), (++++) higher than average (17-20 mm).

In summary, several antioxidant and antimicrobial activity methods were utilized in order to determine the total antioxidant capacity and antimicrobial activity of the trunk bark extracts of five tree species. The trunk bark extracts studied showed antioxidant and antimicrobial activity

at various extents and the bark components could provide a valuable source of nutraceutical and pharmaceutical supplements and requires further investigation with regards to their individual antioxidant activities.

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