Antifungal and Antioxidant Activities of Heartwood, Bark, and Leaf Extracts of *Robinia pseudoacacia*

Seyyed Khalil Hosseinihashemi,[a] Sayed Khosrow HosseinAshrafi,[a] Abbas Jalali Goldeh,[a] and Mohamed Z. M. Salem [b,*]

Different solvent fractions (Fs) of water:methanol (1:1 *v/v*) of heartwood, bark, and leaf extracts of *Robinia pseudoacacia* were evaluated for their antioxidant activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method; the antifungal activity against the mycelial growth of *Trametes versicolor* fungus was also determined. The most active fractions were analyzed for their chemical composition using gas chromatography–mass spectrometry (GC/MS). At higher concentrations (0.016 mg/mL), the values of antioxidant activity were 92.3%, 92.5%, 50.6%, 93.4%, and 96.6%, for heartwood F7 (ethyl acetate fraction), bark F7 (ethyl acetate fraction), leaves F9 (methanol fraction), BHT, and vitamin C, respectively. Among the fractions and concentrations of extracts from heartwood, F7 at 12.5 ppm led to the lowest growth of *T. versicolor* (22.00 mm); F7 of the bark extract showed good antifungal activity, with lower mycelia growth values reached 11.33, 11.33, and 13.00 mm at concentrations of 12.5, 25, and 50 ppm, respectively. For leaf extracts, F9 showed good antifungal activity at all concentrations, where the values of mycelial growth were 26.00, 25.33, and 28.33 mm at concentrations of 12.5, 25, and 50 ppm, respectively. These results indicated that the fractions of *R. pseudoacacia* can be a valuable and economic resource for use in antioxidant activity or as an antifungal activity against the growth of *T. versicolor.*

Keywords: Robinia pseudoacacia; Heartwood; Bark; Leaves; Extracts; Antifungal activities; Antioxidant activities

*Contact information: a: Department of Wood Science and Paper Technology, Karaj Branch, Islamic Azad University, Karaj, Iran; b: Department of Forestry and Wood Technology, Faculty of Agriculture (EL-Shatby), Alexandria University, Alexandria, Egypt; *Corresponding author: zidan_forest@yahoo.com*

INTRODUCTION

Black locust (*Robinia pseudoacacia* L., Fabaceae) is known to be durable and resistant to the decay process; it produces high amounts of bioactive compounds (Putman *et al.* 1989; Smith *et al.* 1989) and has been used as a medicinal plant since ancient times (Rosu *et al.* 2012).

Extracts of *R. pseudoacacia* are known to have medicinal and poisonous uses (Zhang *et al.* 2008). Leaves of *R. pseudoacacia* contain tannins (Singh 1982). Phenolic compounds, in wood, bark, leaves, and especially flavonoids, are considered to have an important role in bioactivity (Putman *et al.* 1989; Nasir *et al.* 2005; Talas-Ogras *et al.* 2005; Zhang *et al.* 2008; Dünisch *et al.* 2009; Veitch *et al.* 2010).

It has been reported that acacia bark has an exceptional resistance to biodegradation, this property being assigned to its concentration of dihydrobinetin and robinetin (Rudman 1963). The extractives of highly durable heartwood possess fungicidal activity as well as being excellent free radical scavengers (Schultz and Nicholas 2000),

e.g., there is a significant relationship between decay resistance and the total amount of phenolics for pine (Harju *et al.* 2003). Secundiflorol, mucronulatol, isomucronulatol, and isovestitol, identified by spectral analyses, have been reported in an ethanolic extract of the *R. pseudoacacia* whole plant (Tian and McLaughlin 2000). Extracts from various parts of the plant have different antibacterial activities, *e.g.* flower and seed extracts, are efficient antibacterial agents for Gram-positive cocci, and extracts of bark and leaf are active against *Escherichia coli*, *Pseudomonas*, *Proteus*, *Salmonella choleraesuis*, and *Candida albicans* (Rosu *et al.* 2012).

There have been few reports on the antioxidant activity of extracts from *R. pseudoacacia*. The antioxidant activity of lyophilized extracts of acacia leaves had a lower antioxidant capacity (1940 μmol trolox equivalent g^{-1}) compared with *Rhus typhina* (4651), *Acer rubrum* (3805), and *Rosa multiflora* (2533) (Katiki *et al.* 2013). Recently, Marinas *et al.* (2014) found that the highest content of polyphenols (GAE) was found in the leaf extract (266.7 μg GAE mL^{-1} extract), followed by the extract of the seeds (232.2 μg GAE mL^{-1} extract). In addition, the content of polyphenols present in the flowers creates a strong antioxidant potential (Zhang *et al.* 2012).

Hosseinihashemi and Kanani (2012) and Hosseinihashemi *et al.* (2013), reported that the *n*-hexane extractives from the heartwood of *R. pseudoacacia* had hexadecanoic acid, trimethylsilyl ester, (Z,Z)-9,12-octadecadienoic acid, tetradecane, *bis*(2-ethylhexyl)phthalate, and hexadecane, while the major components in the ethanol extract are resorcinol, (Z,Z,Z)-9,12,15-octadecatrien-1-ol, hexadecanoic acid and (Z,Z)-9,12-octadecadienoic acid. Also, Mészáros *et al.* (2007) showed the chemical composition in the ethanol and acetone extracts by Py-GC/MS experiments; the contents found included volatile compounds such as: acids, fatty acids, aliphatic hydrocarbons, aromatic hydrocarbons, esters, fatty acids ester, alcohol aliphatic, *etc*.

This study aimed to determine the antioxidant activity of extracts from heartwood, bark, and leaves of *Robinia pseudoacacia* as well as the antifungal activity against the growth of wood-rotting fungus, *Trametes versicolor*. The chemical compositions of the most active fractions were also analyzed by means of GC/MS.

EXPERIMENTAL

Plant Materials

Fresh stems and leaves of *R. pseudoacacia* were collected from Karaj, Iran in October of 2011. The heartwood, bark, and leaves was separated from three trees and air-dried to achieve 8.0% moisture content.

Extraction and fractionation

The heartwood, bark of stems, and leaves were cut into small pieces and chopped using a laboratory electrical rotary mill to obtain bark flour. The flour size of heartwood, bark of stems, and leaves was between 40 and 60 mesh. Approximately 50 g of each flour material was placed into five extraction thimbles, and then five independent flours were extracted using pure acetone (300 mL in a 500-mL round-bottom flask) and a Soxhlet-type apparatus (Aldrich® Soxhlet extraction apparatus, USA) for 8 h. The combined extract was concentrated using a Heidolph Laborota 4001 rotary-evaporator apparatus (Sigma-Aldrich, USA) at 40 °C to reach a total solvent evaporation after approximately 15 min. Then, the extracts were collected, dried over anhydrous sodium sulphate, and

stored at 4 °C until further analysis. The solid extractive weights of heartwood, bark of stems, and leaves were 5.0, 4.7, and 4.5 g, respectively. Subsequently, 2.0 g of the solid extractives was dissolved in water:methanol (1:1 *v/v*), where the residue was discarded. Then the supernatant was poured into a separate funnel, followed by the addition of 50 mL of *n*-hexane. The mixture was shaken by hand for 10 min to afford two phases; water:methanol fraction and *n*-hexane fraction (discarded). Water:methanol fraction was subjected to rotary evaporator and half a gram of the solid water:methanol fraction was used for column chromatography with silica gel using a Merck KGaA 64271 (Darmstadt, Germany). The 12 fractions were labelled F1 to F12 (Fig. 1). An eluent volume of 3 × 10 mL was used in the chromatographic separation for each solvent.

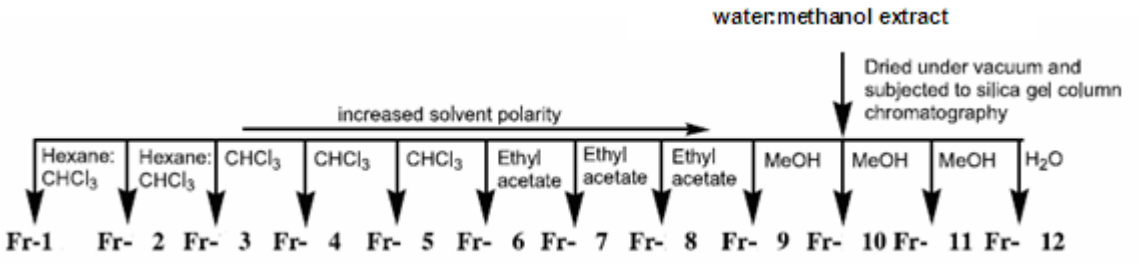

Fig. 1. Isolation scheme of active constituents of *R. pseudoacacia* from the water:methanol extracts of heartwood, bark, and leaves

Free radical scavenging activity by DPPH assay

The free radical scavenging activities of the acetone and water:methanol extracts, as well as the fractions from F1 to F12 of the heartwood, bark, and leaves powders, were determined using the 1,1-dipheny-2-picrylhydrazyl (DPPH, Sigma-Aldrich, Germany) method (Karau *et al.* 2013). For this method, a stock solution was prepared by dissolving 2.4 mg of DPPH free radicals in 100 mL of methanol. The stock solution was stored at 20 °C. The working solution was prepared by diluting the DPPH stock solution with methanol. Then, 1250 µL of the working solution was combined with 250 µL of the methanol extract from the medicinal plant (1 mg/mL). Serial dilutions were carried out with the stock solutions (1 mg/mL) of tested extract to obtain concentrations of 0.0005, 0.001, 0.002, 0.004, 0.008, and 0.016 mg/mL. The experiment was performed in triplicate, and the average absorbance was recorded for each concentration. The reaction mixture was mixed for 10 s and left to stand at room temperature in a dark place for 30 min. The absorbance was measured at 517 nm using a UV scanning spectrophotometer (Unico® 1200, USA-series). Ascorbic acid (AA) and butylated hydroxytoluene (BHT) were used as the reference standards and were dissolved in methanol to make stock solutions with the same concentration (1 mg/mL). The control samples were prepared with the same volume of solution, without test compounds and the referenced standards. Pure methanol (Sigma-Aldrich, USA) was used as a blank. The DPPH free radical scavenging activity (%) was calculated using the following equation,

$$\text{Inhibition} = 100(Ac - As)/Ac \quad (1)$$

where the percentage inhibition value was calculated from the absorbance of the control, *Ac*, and of the sample, *As*.

The controls contained all the reaction reagents except the extract or positive control substance. The values are presented as the means of triplicate analyses. The

antioxidant activity values were compared with BHT and vitamin C that were prepared with the same concentrations (0.0005, 0.001, 0.002, 0.004, 0.008, and 0.016 mg/mL).

Antifungal activity assay

To evaluate the antifungal properties of various *R. pseudoacacia* extracts, 25 mg of each extracts was dissolved into 2 mL of 10% dimethyl sulfoxide (DMSO, Sigma-Aldrich Co., USA) solvent as recommended by Mansour and Salem (2015) and Salem *et al.* (2016). The solvent was passed by syringe from a 0.45-µm Microsolve filter, and poured into a glass vial. The media were sterilized in an autoclave at 120 °C. Approximately 25 mL of the medium was poured into every Petri plate, and 25, 50, and 100 µL of extract solution was added by micro-sampler at various concentrations (12.5, 25, and 50 ppm on three antibiogram discs and three plates as replicates for three concentrations) to medium containing malt extract agar (MEA, 48g/L) and were poured into one of the Petri plates.

The plates were cooled in a sterile hood and inoculated with 0.50-cm plugs of *Trametes versicolor* fungus mycelia, introduced into the center of the Petri plate. Inoculated plates were incubated at 23 °C and 75% relative humidity without light. Three replicate antibiogram discs and three plates were used per treatment. Fungus was also grown on non-extract MEA as a control. Fungal growth was monitored daily by measuring length of radius by ruler that was covered by fungus in the plates. The fungal mycelial growth was plotted against the extract concentration, and the toxic level was determined by the extract concentration at which the fungal growth was completely inhibited, in accordance with the methods of Hosseini Hashemi *et al.* (2008) and Hosseini Hashemi and Jahan Latibari (2011). All fractions were prepared individually, at various concentrations (12.5, 25, and 50 ppm), to study their antifungal activity.

Analysis of extracts

Gas chromatography-mass spectrometry (GC/MS) analysis of the F7 (heartwood), F7 (bark), and F9 (leaf) extracts was performed using split mode (10:1) injection. One milligram of each solid extracts, was silylated with 30 µL of N,O-bis-(trimethylsilyl) triflouroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) reagent, and then approximately 30 µL of pyridine were run on a HP 6890 (Hewlett Packard, USA) gas chromatograph fitted with a cross-linked 5.0% PH ME siloxane HP-5 capillary column (dimensions: 30 m x 0.25 mm, 0.50 µm coating thickness) and coupled with a model 5975B mass detector. The GC/MS operation conditions were as follows: injector temperature 250 °C; transfer line 290 °C; oven temperature program 50 to 250 °C (5 °C/min); carrier gas: He at 1.4 mL/min; mass spectra: electron impact (EI+) mode 70 eV with a mass range of 40 to 450 m/z; and ion source temperature 250 °C. Individual components were identified using mass spectra with data from the literature and two mass spectrometric libraries (Wiley 275 L, 1998 and NIST-05) mass database matching and by comparing the retention times and mass spectra of constituents with published data (Julian and Konig 1988; Adams 1995, 2001). Retention indices (R_I) were determined with reference to a homologous series of normal alkanes, using the following formula (Kovats 1958),

$$R_I = 100\,[(n + (N\text{-}n) \times \log t_{1R}(x) - \log t_{1R}(C_n)/\log t_{1R}(C_N) - \log t_{1R}(C_n)] \quad (2)$$

where R_I is the retention index of the compound of interest, t_{1R} is the net retention time (t_R-t_0), t_0 is the retention time of solvent (dead time), t_R is the retention time of the compound of interest, C_n and C_N are the number of carbons in the n-alkanes eluting immediately before and after the compound of interest, respectively, and N and n are the number of carbon atoms in the n-alkane eluting immediately before and after the compound of interest, respectively.

Data analysis

Percentage of mycelial growth was calculated and an analysis of variance for the various treatments [extracts (tree part, bark, and heartwood), fractions, and concentrations] was conducted using the SPSS 17.0 software package (SPSS Inc., Chicago, IL, 2008). The 108 treatment designs, which are shown in Table 1, were all analyzed for variance using a complete randomized block design.

RESULTS AND DISCUSSION

After fractionation, the extracts from heartwood, bark, and leaves afforded 36 fractions. Figure 2 shows the weight of solid extracts of *R. pseudoacacia* for all the fractions (F1→F12). The weight of solid extracts ranged from 2 mg (F5) to 112 mg (F6), from 16.6 mg (F12) to 91.5 mg (F7), and from 1.3 mg (F1) to 115.7 mg (F10), in heartwood, bark, and leaves, respectively.

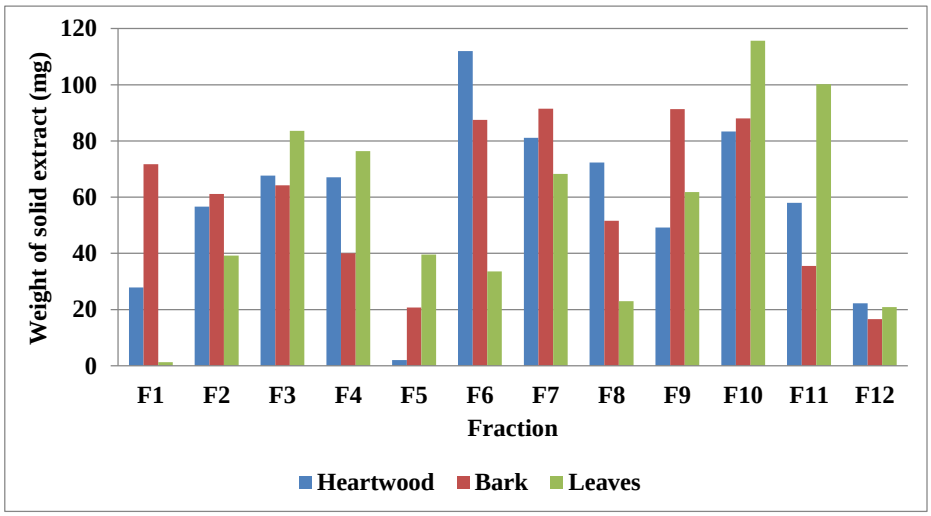

Fig. 2. The weight of solid extracts of heartwood, bark, and leaf of *R. pseudoacacia* after fractionation with different solvents

Antifungal Activity

The effects of treatments (extracts from different parts of the tree), fractions, and interaction between treatments and fractions were significant. However, the effects of concentrations, and the interactions (treatment*concentration, fraction*concentration, and treatment*fraction*concentration) were not significant relative to the growth of *Trametes versicolor*. Statistically, there was a significant difference among the extracts from heartwood, bark, and leaves relative to the mycelial growth of *Trametes versicolor*, where the highest growth was observed at the studied concentrations with a value of

43.00 mm from the F10 (heartwood extract), and fractions 5 and 6 from leaves extract in comparison with the mycelial growth values in control agar plates (43.00 mm).

Among the fractions and concentrations of extracts from heartwood, F7 with moderate activity at the concentration of 12.5 ppm observed the lowest growth of *T. versicolor* (22.00 mm) and the concentrations of 25 and 50 ppm showed mycelial growth values of 24.33 and 24.67 mm, respectively. Furthermore, F3 at 12.5 and 25 ppm led to mycelial growth values of 24.00 and 24.67 mm, respectively (Table 1).

Table 1. Antifungal Activity of Extracts from *R. pseudoacacia* against the Growth of *Trametes versicolor* at the 7th Day (mm)

Fs	Cons (ppm)	MG (mm)[a]	Fs	Cons (ppm)	MG (mm)[a]	Fs	Cons (ppm)	MG (mm)[a]
H1	12.5 25 50	31.67±11.504 33.33±11.930 32.00±12.767	B1	12.5 25 50	14.00±6.557 15.33±6.429 13.33±5.686	L1	12.5 25 50	30.33±11.015 22.67±2.517 27.33±13.650
H2	12.5 25 50	30.00±14.107 32.67±13.051 32.67±11.676	B2	12.5 25 50	14.33±4.041 16.00±5.292 14.00±1.732	L2	12.5 25 50	37.00±10.392 29.33±12.097 34.67±14.434
H3	12.5 25 50	24.00±6.000 24.67±7.024 25.00±6.000	B3	12.5 25 50	14.00±13.892 17.33±12.702 18.00±11.790	L3	12.5 25 50	40.33±4.619 24.33±2.082 40.33±4.619
H4	12.5 25 50	34.00±9.539 32.33±12.897 41.00±3.464	B4	12.5 25 50	35.33±11.240 35.00±11.790 30.67±12.503	L4	12.5 25 50	34.67±14.434 27.67±13.279 37.00±10.392
H5	12.5 25 50	32.00±0.000 32.00±0.000 32.00±0.000	B5	12.5 25 50	18.00±1.732 27.33±15.373 18.00±1.732	L5	12.5 25 50	43.00±0.000 43.00±0.000 43.00±0.000
H6	12.5 25 50	33.67±8.145 34.00±7.810 32.67±8.963	B6	12.5 25 50	35.67±16.166 35.00±17.321 36.00±13.892	L6	12.5 25 50	43.00±0.000 43.00±0.000 43.00±0.000
H7	12.5 25 50	22.00±0.000 24.33±7.095 24.67±5.686	B7	12.5 25 50	11.33±4.041 11.33±3.055 13.00±2.646	L7	12.5 25 50	33.33±16.743 33.67±16.166 34.67±14.434
H8	12.5 25 50	26.00±15.716 27.67±15.503 33.67±14.468	B8	12.5 25 50	28.00±8.718 28.33±8.505 29.00±7.810	L8	12.5 25 50	36.67±10.970 38.00±8.660 38.00±8.660
H9	12.5 25 50	33.33±9.074 33.33±9.074 34.33±8.505	B9	12.5 25 50	22.33±7.506 26.67±5.774 24.33±2.082	L9	12.5 25 50	26.00±12.530 25.33±12.583 28.33±9.074
H10	12.5 25 50	43.00±0.000 43.00±0.000 43.00±0.000	B10	12.5 25 50	33.67±16.166 32.67±17.898 33.00±17.321	L10	12.5 25 50	28.00±5.292 37.33±6.028 33.33±9.074
H11	12.5 25 50	33.33±9.074 30.00±11.790 31.00±10.392	B11	12.5 25 50	18.67±6.110 21.33±3.055 15.33±8.963	L11	12.5 25 50	29.33±12.097 29.33±12.662 32.33±18.475
H12	12.5 25 50	27.67±13.429 35.33±13.279 26.67±14.572	B12	12.5 25 50	27.67±14.189 33.67±16.166 26.00±16.523	L12	12.5 25 50	31.33±11.060 28.67±2.887 29.67±7.371

a: Values are mean± std. deviation; Fs: fractions; Cons: concentrations; MG; mycelial growth; H: heartwood; B: bark; L: leaves

The lowest growth, implying good activity, was observed by different extracts with different fractions. From the above results, F7 (ethyl acetate fraction) of the bark extracts showed the highest antifungal activity against *T. versicolor* at 12.5, 25, and 50 ppm, with mycelial growth of 11.33 mm, 11.33 mm, and 13.00 mm, respectively, followed by F1 at a concentration of 50 ppm (fungal growth with 13.33 mm). Also, even weak activity was observed from the extracts of the leaves; F1 at 25 ppm showed good activity and totally, the F9 showed good activity.

Further solvent partition assays showed that the most active compounds were in the water phase, not in the organic phase. Water-soluble extracts have a much higher antifungal efficacy in both the culture room and greenhouse conditions in a dose-dependent manner, as confirmed by *in vitro* bioassays (Zhang *et al.* 2008).

Figure 3 shows the antioxidant activity of different concentrations of F7 (heartwood extract), F7 (bark extract), and F9 (leaves extract) from the extracts of *R. pseudoacacia* heartwood, bark, and leaves, respectively. It can be seen that F7 from both heartwood and bark extracts were observed to have good antioxidant activities in comparison with BHT and vitamin C, where the values are closed or higher than the used standard compounds. For example, at the lowest concentration (0.0005 mg/mL), the values were 57.1%, 60.8%, 1.8%, 26.3%, and 89.9%, for heartwood F7, bark F7, leaves F7, BHT, and vitamin C, respectively. Also, at a higher concentration (0.016 mg/mL), the values were 92.3%, 92.5%, 50.6%, 93.4%, and 96.6%, for heartwood F7, bark F7, leaves F9, BHT, and vitamin C, respectively.

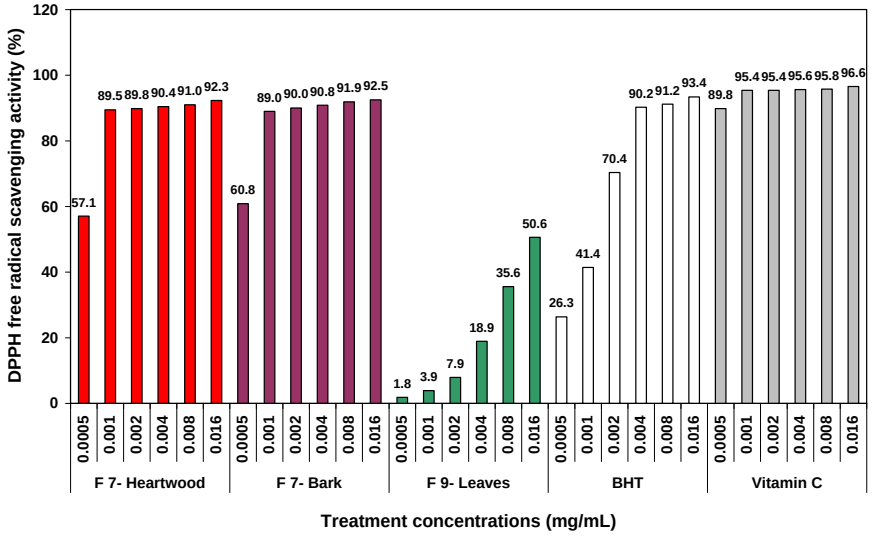

Fig. 3. Antioxidant activity of extracts from different parts of *R. pseudoacacia*

The antioxidant activity of different extracts from *R. pseudoacacia* was correlated with the phenolic content (Ji *et al.* 2012; Pasheva *et al.* 2013). The antioxidant activities of the extracts were possessed by condensed tannins (Katiki *et al.* 2013) and shown by flavonoids, but the antioxidant activity of lyophilized extracts of acacia leaves had a lower antioxidant capacity compared with some tested plants (Katiki *et al.* 2013).

The *R. pseudoacacia* leaf aqueous extract showed strong phytotoxicity in terms of radicle and hypocotyl growth inhibition when the aqueous extract was applied in five different concentrations (Nasir *et al.* 2005). A new geranyl flavonol, named robipseudin A, has been isolated from the leaves, along with another known geranyl flavone called

kuwanon S; these were shown to have moderate antioxidant activity in the DPPH radical scavenging assay (Zhang *et al.* 2013).

Chemical Constituents of Extracts

The investigated chemical parameters of F9 from the extracts of leaves of *R. pseudoacacia* are summarized in Table 2. The most abundant compounds are fatty acids; 1-eicosanol (13.50%), palmitic acid (13.42%), oleic acid (9.09%), and 9,17-octadecadienal, (Z)- (8.99). Loliolide (7.08%), a monoterpene lactone, was also found, which has been previously identified from S*argassum ringgoldianum* subsp. *coreanum* and was reported to have a moderate scavenging activity on both DPPH free radical and hydrogen peroxide (Yang *et al.* 2011). Our results have shown that neophytadiene, a branched hydrocarbon, was found in a percentage of 7.28%; this substance has been found in methanol and aqueous extracts of *Eupatorium odoratum* and showed significant antioxidant activity (Venkata Raman *et al.* 2012) and antibacterial activity (Zhang and Zhou 2011).

Table 2. The Identified Chemical Constituents in F9 of Leaf Extract

Leaf Component	Retention Time (min)	Retention Indices	Area (%)
Decane	10.514	990	4.13
Trimethylsilyl ether of glycerol	19.615	1288	0.75
(-)-Loliolide	31.699	1794	7.08
Neophytadiene	32.507	1834	7.28
Palmitic acid	35.134	1915	13.42
Oleic acid	38.361	2142	9.09
9,17-Octadecadienal, (Z)-	38.459	2147	8.99
Stearic acid	38.769	2165	4.87
Bis(2-ethylhexyl) phthalate	44.953	2543	1.99
1-Eicosanol	52.528	2890	13.50
Total	-	-	71.10

Most of the identified chemical constituents of F7 in bark (Table 3) were fatty acids; oleic acid (27.59%), heptadecene-(8)-carbonic acid-(1) (26.89%), palmitic acid (21.21%), and stearic acid (13.68%).

Table 3. The Identified Chemical Constituents in F7 of Bark Extract

Bark Component	Retention Time (min)	Retention Indices	Area (%)
Palmitic acid	35.134	1915	21.21
Heptadecene-(8)-carbonic acid-(1)	38.381	2143	26.89
Oleic acid	38.471	2148	27.59
Stearic acid	38.788	2166	13.68
Total	-	-	89.37

Table 4 presents the chemical constituents found in F7 of heartwood extract. Fatty acids; oleic acid (19.42%), palmitic acid (12.66%), and stearic acid (6.74%); decane (12.52%), a hydrocarbon compound, derivative of resorcinol (2.05%), a phenolic compound; and N,N-bis(trimethylsilyl)-2-(2-thienyl)quinolone-4-amine (5.76), an alkaloid compound, were identified in F7 of heartwood extract.

Table 4. The Identified Chemical Constituents in F7 of Heartwood Extract

Wood Component	Retention Time (min)	Retention Indices	Area (%)
Decane	10.235	982	12.52
Dodecane	16.950	1195	3.42
Resorcinol, *o*-bis(trimethylsilyl)	22.267	1269	2.05
Tetradecane	22.507	1271	1.52
N,N-Bis(trimethylsilyl)-2-(2-thienyl)quinolone-4-amine	32.307	1824	5.76
Palmitic acid	35.121	1966	12.66
Oleic acid	38.355	2141	19.42
Stearic acid	38.769	2165	6.74
Bis(2-ethylhexyl) phthalate	44.959	2543	3.71
Total	-	-	67.80

From our literature survey, the extracts of the plant have been shown to have a high content of volatile oil phenolic compounds, flavonoids, and tannins with antimicrobial properties (Rosu *et al.* 2012). From the ethanolic extract of the whole plant, secundiflorol, mucronulatol, isomucronulatol, and isovestitol were identified by spectral analyses (Tian and McLaughlin 2000). The 3,3',4',5'-pentahydroxy flavone isolated from the leaf extract was found to be highly inhibitory and inhibited the growth of lettuce root and shoot growth at all applied concentrations. The EC_{50} of this compound was found to be 10μg/g FW (Nasir *et al.* 2003). *R. pseudoacacia* contain polyphenolic compounds, such as tannins (Rakesh *et al.* 2000) and robinlin, a novel bioactive homo-monoterpene (Tian *et al.* 2001). HPLC analysis showed the presence of catechin (0.925 μg mL^{-1}), rutin (0.831 μg mL^{-1}), resveratrol (0.664 μg mL^{-1}) and quercetin (0.456 μg mL^{-1}) in the leaf extract (Marinas *et al.* 2014). In addition, heartwood contains *β*-resorcylic acid and methyl *β*-resorcylate (Hosseinihashemi *et al.* 2013). Four flavone glycosides have been isolated from extracts of *R. pseudoacacia* leaves: 7-O-β-d-glucuronopyranosyl-(1→2)[α-l-rhamnopyranosyl-(1→6)]-β-d-glucopyranosides of acacetin (5,7-dihydroxy-4′-methoxyflavone), apigenin (5,7,4′-trihydroxyflavone), diosmetin (5,7,3′-trihydroxy-4′-methoxyflavone), and luteolin (5,7,3′,4′-tetrahydroxyflavone) (Veitch *et al.* 2010).

CONCLUSIONS

1. Heartwood, bark, and leaf extracts from *R. pseudoacacia* were evaluated to determine their antioxidant and antifungal activities. The results of the percentage of mycelial growth of *T. versicolor* caused by different fractions from different parts of *R. pseudoacacia* found that F7 (ethyl acetate fraction) of the bark extract showed the

highest antifungal activity against *T. versicolor* at concentrations of 12.5, 25, and 50 ppm, followed by F1 at 50 ppm.

2. Moderate activity was found for F7 (ethyl acetate fraction) of heartwood extracts at 12.5 ppm. Also, even weak activity was observed from the extracts of leaves: F1 at 25 ppm showed good activity and totally, the F9 showed good activity.
3. The ethyl acetate fraction (F7) from both heartwood and bark extracts was observed to have good antioxidant activities in comparison with BHT and vitamin C, where the values are similar to or higher than the standard compounds.

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