

Essential Oils from Wood, Bark, and Needles of *Pinus roxburghii* Sarg. from Alexandria, Egypt: Antibacterial and Antioxidant Activities

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The essential oils extracted by hydro-distillation of *Pinus roxburghii* wood, bark, and needles were analyzed by GC/MS, and their antibacterial and antioxidant activities were evaluated. Twenty-two, thirty-one, and twenty-eight compounds were identified in the essential oils of wood, bark, and needles, respectively. The major chemical constituents of wood's essential oil were caryophyllene (16.75%), thunbergol (16.29%), 3-carene (14.95%), cembrene (12.08%), α -thujene (10.81%), and terpinolen (7.17%). In bark, they were α -pinene (31.29%) and 3-carene (28.05%), and in needles, they were α -pinene (39%) and 3-carene (33.37%). Almost all of the essential oils were active against human pathogen bacteria, and the essential oils from bark and needles were active against the plant pathogen bacteria *Ralstonia solanacearum* and *Pectobacterium carotovorum*. Alternatively, *Erwinia amylovora* was resistant to all tested oils. The total antioxidant activities (TAA%) of the essential oils from wood ($82 \pm 2.12\%$), and bark ($85 \pm 1.24\%$) were higher than that of tannic acid ($81 \pm 1.02\%$), and the TAA% from the essential oil of needles ($50 \pm 2.24\%$) was lower than that of tannic acid.

Keywords: *Pinus roxburghii*; Essential oil; Antibacterial activity; Antioxidant activity; Wood; Bark; Needles

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INTRODUCTION

Currently, there is a large, sustainable supply of foliage, twigs, bark, and wood biomass residues that are suitable for the manufacture of products including particleboard and medium density fiberboard (MDF). Moreover, chemical substances useful in pharmaceutical applications can be economically produced from these biomass sources. There are many socially and environmentally beneficial approaches to integrating essential oil recovery into existing forestry and manufacturing operations (Kelkar *et al.* 2006).

Chir pine, *Pinus roxburghii* Sarg. (Pinaceae), is used in the production of timber, paper, pulp, turpentine, resin, and many traditional medicines (Siddiqui *et al.* 2009). The plant is cultivated for ornamental purposes and has long been known for its medicinal value (Shah 2006). Extracts from needles of *P. roxburghii* were reported to contain an intestinal antiseptic, antidyslipedemic, spasmolytic, and antioxidant properties (Puri *et al.* 2011). Pine rosin is used to make adhesives, paper, varnish, printing ink, synthetic rubber, chewing gums, and other products (Wiyono *et al.* 2006; Shuaib *et al.* 2013). The

needles and stems of *P. roxburghii* have been reported as rich in essential oils, vitamin C, tannins, and alkaloids.

Wood is the world's major source of turpentine oil (Vallejo *et al.* 1994; Asta *et al.* 2006). Several studies have revealed that monoterpenes and sesquiterpenes are the dominant components of pine needles, and because of characteristics such as their odor, pine needle oil can be used in the manufacture of cosmetic products and perfumes (Dormont *et al.* 1998; Pagula and Baeckstrom 2006; Dob *et al.* 2007).

According to the review reported by Shuaib *et al.* (2013), the oleoresin of *P. roxburghii* contains α -pinene (20 to 30%), β -pinene (5 to 10%), β -3-carene (55 to 65%), longifolene, and other terpenes (2 to 10%), as well as β -carene, β -longifolene, and longicycline. The analysis of wood oil from *P. roxburghii* revealed the presence of triterpenes and steroids, which are known for their hepatoprotective activity (Khan *et al.* 2012).

The major compounds in the needles essential oil of *P. roxburghii* were identified as caryophyllene, 3-carene, α -humulene, α -pinene, β -pinene, α -terpeniol, car-3-ene, longifolene, camphene, limonene, α -terpinene, α -terpineol, *d*-borneol, and *dl*-camphor (Swales and Dev 1979; Mishra *et al.* 1988; Hassan and Amjid 2009; Willför *et al.* 2009). The chemical components of pine oil can be used in the manufacture of various products (Mirov 1961) including β -pinene (pharmaceutical), α -pinene (fragrance), 3-carene (manufacture of menthol), myrcene (insect attractant), α -terpineol (perfume and household products), and terpinolene (flavoring food and enhancing fragrance).

Phytochemical investigations indicate that the methanolic extract of the stem bark contains 1,5-dihydroxy-3,6,7-trimethoxy-8-dimethylallyloxy-xanthone and 1-hydroxy-3,6-dimethoxy-2- β -D-glucopyranoxanthone (Rawat *et al.* 2006). Longifolene was first isolated from Indian turpentine oil by Jadhav and Nayak (1980), and β -pinene was found to be a major component in the oil obtained from the trees of Rawalpindi seed (Verma and Suri 1978). The major constituents of the essential oil of the needles and stems of Chir pine from Pakistan were α -pinene and were found to be active against *Staphylococcus aureus* and *Bacillus subtilis* but inactive against *Escherichia coli*, *Salmonella typhi*, and *Enterobacter aerogenes* (Hassan and Amjid 2009; Zafar *et al.* 2010). Additionally, the stem oil significantly inhibited the growth of all fungi tested, namely *Aspergillus terreus*, *Aspergillus flavus*, *Aspergillus candidus*, *Aspergillus versicolor*, *Aspergillus niger*, and *Trichoderma viride* (Hassan and Amjid 2009). The essential oils from the cones, needles, and stem bark of *P. roxburghii* from Nepal were dominated by sesquiterpenes, particularly (E)-caryophyllene (26.8 to 34.5%) and α -humulene (5.0 to 7.3%), as well as monoterpene alcohols including terpinen-4-ol (4.1 to 30.1%) and α -terpineol (2.8 to 5.0%) (Satyal *et al.* 2013).

The aim of this investigation was to determine the chemical constituents, antibacterial properties, and antioxidant activities of *P. roxburghii* wood, bark, and needle essential oils from trees found in Alexandria, Egypt.

EXPERIMENTAL

Materials

Plant materials and oil extraction

Samples of *P. roxburghii* wood, bark, and needles were supplied by the Antoniadis Garden, Horticultural Research Institute, Alexandria, Egypt, during the month

of August 2013. The plant was kindly identified at the Department of Forestry and Wood Technology, Faculty of Agriculture, Alexandria University. The samples were cut into small pieces weighing 100 g and hydro-distilled for 3 h in a Clevenger apparatus (Salem *et al.* 2014a). The extracted oils were dried over anhydrous Na₂SO₄, and the yield was measured with respect to the fresh weight of the sample (0.30 mL, 0.75 mL, and 1.25 mL per 100 g fresh weight of wood, bark, and needles, respectively). The oil was kept dry in sealed Eppendorf tubes with aluminum sheets and stored at 4 °C prior to chemical analysis (Salem *et al.* 2013).

Bacterial strains

The Gram-positive bacteria *Bacillus subtilis* ATCC 6633, *Sarcina lutea* ATCC 9341, and *Staphylococcus aureus* ATCC 6538, and the Gram-negative bacteria *Escherichia coli* ATCC 8739, *Ralstonia solanacearum*, *Erwinia amylovora* ATCC 49946, and *Pectobacterium carotovorum* subsp. *carotovorum* (strain No. ippbc038) were provided by the Laboratory of the Plant Pathology Department, Faculty of Agriculture, Alexandria University, Egypt.

Methods

GC/MS analysis of the essential oil

The chemical constituents of essential oils of wood, bark, and needles from *P. roxburghii* were analyzed using the Trace GC Ultra/Mass Spectrophotometer ISQ (Thermo Scientific) (GC/MS) apparatus (Institute of Graduate Studies and Researches - Alexandria University, Alexandria, Egypt). The GC-MS was equipped with a ZB-5MS Zebron capillary column (95% dimethyl polysiloxane + 5% diphenyl, length 30 m × 0.32 mm internal diameter, 0.25 µm film thickness). Helium (average velocity 39 cm/s at constant flow rate of 1 mL/min) was used as carrier gas, and the oven temperature was held at 45 °C for 2 min and increased from 45 °C to 165 °C at 4 °C/min, and 165 °C to 280 °C at 15 °C/min and held for 5 min. Samples (2 µL) were injected at 250 °C with a splitless setting for 1 min then with split at flow ratio of 1:10.

All mass spectra were recorded using electron impact ionization (EI) at 70 eV with ion source temperature of 220 °C. The mass spectrometer was scanned from *m/z* 40 to 500 (mass ratio) at five scans per second. Peak area percentage was used to obtain quantitative data using the GC with HP-ChemStation software (Elansary and Ashmawy 2013) without correction factors. Identification of the constituents was performed on the basis of MS library searches (NIST and Wiley) (Davies 1990; Adams 2007; McLafferty 2009). Retention indices (RIs) were calculated using a generalized equation for all components using a mixture of aliphatic hydrocarbons (C₈-C₃₂; Sigma-Aldrich, USA) which were co-injected at the temperature program mentioned above used in GC/MS for the essential oils samples and computer matching with Wiley 7 n.L library.

Antibacterial activities of essential oils

The essential oils of the wood, bark, and needles of *P. roxburghii* were prepared by dissolution in 10% dimethyl sulfoxide (DMSO; E. Merck, Germany) and Tween[®] 80 (Sigma-Aldrich, USA) (10:1 v/v) at a concentration of 2000 µL/mL. The antibacterial activities against the growth of the Gram-positive bacteria *B. subtilis*, *S. lutea*, and *S. aureus*, as well as the Gram-negative bacteria *E. coli*, *R. solanacearum*, *E. amylovora*, and *P. carotovorum* subsp. *carotovorum* (strain No. ippbc038) were evaluated using the Kirby-Bauer disc diffusion method (Bauer *et al.* 1966). Control discs were impregnated

with 20 μL of DMSO solution, and tetracycline (20 $\mu\text{g}/\text{disc}$) was used as a positive control with the tested bacteria. The experiment was performed in triplicate, and the data expressed as mean values \pm standard deviation (SD). Minimum inhibitory concentrations (MICs) were determined by serial dilutions (8, 16, 32, 64, 126, 250, 500, 1000, 2000, 4000, and 5000 $\mu\text{L}/\text{mL}$) of essential oils using 96-well micro-plates (Eloff 1998).

Antioxidant activities of the essential oils

The total antioxidant activities (TAA%) of the tested essential oils from the wood, bark, and needles of *P. roxburghii* were measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method according the recommendations of Salem *et al.* (2014b) and Elansary *et al.* (2012). The percentage inhibition of the DPPH radical was measured using the following equation:

$$\text{TAA (\%)} = (A_0 - A_s / A_0) \times 100 \quad (1)$$

where TAA is the total antioxidant activity, A_0 (control) is the absorbance of DPPH solution in methanol, and A_s is the absorbance of a DPPH solution containing the essential oil (test) or tannic acid (positive control) solution.

Statistical analysis

In the present study, the values from the inhibition zones and from the antioxidant activity were reported as mean values of three replicates \pm the standard deviation.

RESULTS AND DISCUSSION

Essential Oils Composition

The essential oils from the wood, bark, and leaves obtained by hydro-distillation were characterized visually by their transparent color and fresh pine odor. Figure 1 and Table 1 show the variations in the chemical constituents identified in the essential oils from the different parts (wood, bark, and needles) of *P. roxburghii*. The identified essential oils consisted of 22, 31, and 28 compounds in the wood, bark, and needles, respectively. The identified compounds represented 99.15%, 99.76, and 98.8% of the total essential oils from wood, bark, and needles, respectively.

The major chemical constituents of wood essential oil are caryophyllene (16.75%), thunbergol (16.29%), 3-carene (14.95%), cembrene (12.08%), α -thujene (10.81%), terpinolen (7.17%), α -pinene (4.8%), α -caryophyllene (3.7%), sabinene (3.59%), verticiol (1.84%), 4-terpineol (1.79%), and myrcene (1.28). Hassan and Amjid (2009) reported that the major component in the essential oil of stem was α -pinene (41.9%) followed by 3-carene (16.3%) and caryophyllene (12.3%).

The main components in the essential oil from bark are α -pinene (31.29%), 3-carene (28.05%), cembrene (4.86%), longifolene (4.42%), thunbergol (4.11%), β -pinene (2.99%), sylvestrene (2.4%), terpineol (2.05%), terpinolen (2.03%), terpinyl acetate (1.56%), elemol (1.46%), methyl dehydroabietate (1.37%), myrcene (1.36%), bornyl acetate (1.1%), α -cadinol (1.08%), and phenethyl isovalerate (1%). Satyal *et al.* (2013) reported that the essential oil from bark was dominated by sesquiterpenes, particularly (*E*)-caryophyllene and α -humulene as well as monoterpene alcohols including terpinen-4-ol and α -terpineol.

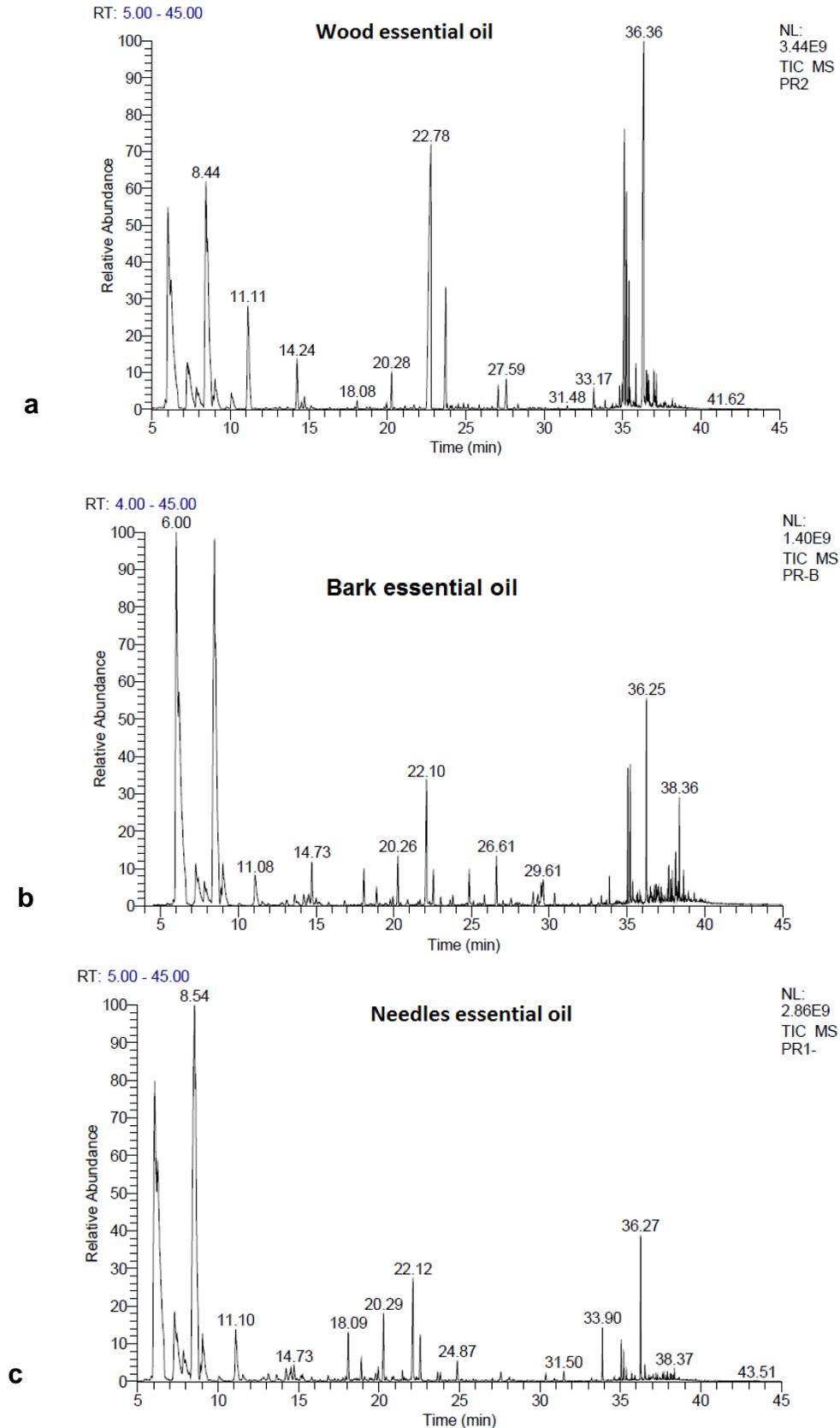


Fig. 1. GC/MS chromatograms of the essential oil from the (a) wood, (b) bark, and (c) needles of *P. roxburghii*

The major chemical constituents found in the essential oil from the needles are α -pinene (39%), 3-carene (33.37%), β -pinene (4.6%), longifolene (2.76%), sylvestrene (2.73%), terpinolen (2.61%), terpinyl acetate (2.02%), thunbergol (1.75%), bornyl acetate (1.18%), caryophyllene (1.07%), and cembrene (1.05%). Zafar *et al.* (2010) reported that the major component in the essential oil of needles was α -pinene (29.3%), followed by caryophyllene (21.9%), 3-carene (14.2%), α -terpineol (4.5%), caryophyllene oxide (3.1%), borneol acetate (2.2%), α -longipinene (1.2%), β -myrcene (1.1%), and terpinyl acetate (1.0%).

The present study and other research regarding the essential oils of different parts of *P. roxburghii* show a marked variation in the essential oil composition, perhaps due to climatic, seasonal, time of collection, geographic, or genetic variations (Oluwadayo and Olakunle 2008). β -caryophyllene was found to be a dominant compound in the essential oil of *P. pinaster* (Mimoune *et al.* 2013). The principle constituents of the fruit oil of *P. roxburghii* from India were α -pinene (60.8%) and β -pinene (30.2%) (Qadir and Shah 2014).

Table 1. Essential Oil Composition of Wood, Bark, and Needles of *P. roxburghii*

| Constituent | RI ^a | Percentage in Oil ^b | | |
|----------------------------|-----------------|--------------------------------|-------|---------|
| | | Wood | Bark | Needles |
| α -Thujene | 930 | 10.81 | | |
| α -Pinene | 939 | 4.8 | 31.29 | 39 |
| Sabinene | 958 | 3.59 | | |
| β -Pinene | 979 | | 2.99 | 4.6 |
| Myrcene | 980 | 1.28 | 1.36 | 1.35 |
| 3-Carene | 1011 | 14.95 | 28.05 | 33.37 |
| o-Cymene | 1019 | 0.25 | 0.42 | 0.35 |
| Sylvestrene | 1020 | | 2.40 | 2.73 |
| γ -Terpinene | 1067 | 0.96 | | 0.23 |
| Terpinolene | 1098 | 7.17 | 2.03 | 2.61 |
| Linalool | 1100 | | | 0.27 |
| α -Phellandren-8-ol | 1135 | | 0.48 | 0.23 |
| 4-Terpineol | 1175 | 1.79 | | 0.45 |
| p-Cymen-8-ol | 1179 | 0.21 | 0.61 | 0.55 |
| Terpineol | 1182 | 0.34 | 2.05 | 0.43 |
| Verbenone | 1183 | | 0.56 | 0.52 |
| Eucarvone | 1287 | | | 0.18 |
| Bornyl acetate | 1289 | 0.22 | 1.1 | 1.18 |
| Terpinyl acetate | 1334 | 0.15 | 1.56 | 2.02 |
| Geraniol acetate | 1359 | | | 0.22 |
| Longifolene | 1389 | | 4.42 | 2.76 |
| Caryophyllene | 1425 | 16.75 | 0.98 | 1.07 |
| α -Caryophyllene | 1437 | 3.7 | | 0.2 |
| α -Himachalene | 1447 | | | 0.17 |
| Phenethyl isovalerate | 1468 | | 1 | 0.42 |
| β -Farnesene | 1493 | | | 0.18 |
| Farnesol, acetate | 1510 | 0.15 | 0.48 | 0.68 |
| Elemol | 1523 | | 1.46 | |
| Nerolidol | 1540 | 0.5 | | |

| | | | | |
|--------------------------|------|-------|-------|------|
| Caryophyllene oxide | 1549 | 0.88 | | 0.23 |
| γ-Eudesmol | 1606 | | 0.43 | |
| tau.-Muurolol | 1626 | | 0.36 | |
| α-cadinol | 1629 | | 1.08 | |
| β-Eudesmol | 1647 | | 0.68 | |
| Cembrene | 1932 | 12.08 | 4.86 | 1.05 |
| Thunbergol | 2032 | 16.29 | 4.11 | 1.75 |
| Verticiol | 2187 | 1.84 | 0.49 | |
| Geranylgeraniol | 2190 | 0.44 | | |
| Dehydroabietal | 2241 | | 0.52 | |
| Methyl sandaracopimarate | 2250 | | 0.88 | |
| Methyl isopimarate | 2284 | | 0.52 | |
| Methyl palustrate | 2290 | | 0.83 | |
| Methyl dehydroabietate | 2293 | | 1.37 | |
| Abalyn | 2369 | | 0.39 | |
| Total | | 99.15 | 99.76 | 98.8 |
| Unidentified | | 0.85 | 0.24 | 1.2 |

^a Essential oil components were identified by comparison of mass spectra and RIs obtained in both columns with those of reference compounds and those reported in literature or with those of mass spectra libraries (Adams 2007; McLafferty 2009)

^b Percentage of the total FID area obtained on an HP-5 capillary column

Antibacterial Activity of Essential Oils

Table 2 shows the antibacterial activity of the essential oils from the wood, bark, and needles of *P. roxburghii*. The results show that the inhibition zones (IZ) at an oil concentration of 2000 µL/mL ranged between 8 and 17 mm. All the essential oils demonstrated good antibacterial activity against the studied bacterial strains except *E. amylovora*, which was resistant to all tested oils. In the present study, almost all of the essential oils were active against the human pathogen bacteria (*B. subtilis*, *S. lutea*, *E. coli*, and *S. aureus*), whereas the essential oils from bark and needles were active against the plant pathogen bacteria *R. solanacearum* and *P. carotovorum*.

The essential oil from wood had the highest IZs against the growth of *B. subtilis* (15 ± 0.5 mm) and *S. aureus* (13 ± 0.8 mm), with MIC values of 64 µL/mL and under 250 µL/mL, respectively. On the other hand, the lowest value was found against the growth of *R. solanacearum* (8 ± 1.89 mm, with an MIC value of 1000 µL/mL). From other studies, antibacterial activity of stem essential oil against *S. aureus* and *B. subtilis* has been observed, while no activity has been observed against *E. coli* or *Enterobacter aerogenes* (Hassan and Amjid 2009). However, in the present study, this oil proved active against *E. coli* (IZ value of 12 ± 0.6 mm with an MIC value of 500 µL/mL). Other studies have also reported that the oil is not active against *E. coli* (Hong *et al.* 2004).

Bark essential oil had the highest activity against the growth of *E. coli* (17 ± 1.3 mm), followed by *R. solanacearum* (14 ± 1.2 mm), with MIC values of under 250 µL/mL and 126 µL/mL, respectively, whereas the lowest value was found against *B. subtilis* (8 ± 0.70 mm with an MIC value of 2000 µL/mL).

The essential oil from needles showed good antibacterial activity against *S. lutea* (17 ± 1.2 mm), *S. aureus* (14 ± 1.2 mm), *R. solanacearum* (14 ± 1.3 mm), *B. subtilis* (14 ± 1.8 mm), and *P. carotovorum* (12 ± 1.2 mm), with MIC values of 1000, 500, 500, 500,

and 500 $\mu\text{L}/\text{mL}$, accordingly. On the other hand, the oil from needles did not show activity against the growth of *E. coli* or *E. amylovora*.

Previously, the antibacterial activity of the essential oil of the needles indicated that this oil showed maximum activity against *S. aureus* and *B. subtilis*, while no activity was observed against *E. coli*, *Salmonella typhi*, or *Enterobacter aerogenes* (Zafar *et al.* 2010). In other findings, it was observed that leaf extract from *P. roxburghii* inhibited the growth of *E. coli* (Parihar *et al.* 2006). Barjaktarović *et al.* (2005) reported that cadinene, caryophyllene, myrcene, terpinene, and sabinene have pronounced anti-inflammatory and antibacterial properties.

Aqueous and alcoholic extracts of the stem, leaves, bark, female cone, and male cone from *P. roxburghii* had inhibitory activity against *A. tumefaciens*, while all the extracts except stem extract showed inhibitory activity against *E. coli* (Bissa *et al.* 2008). In other work, essential oil, and chloroform extracts of *P. roxburghii* wood showed significant antibacterial and antifungal activities against *Pseudomonas aeruginosa*, *E. coli*, *S. aureus*, *Streptococcus pyogenes*, *B. subtilis*, *Candida albicans*, *A. niger*, and *Aspergillus clavatus* activities rather than the methanolic extract (Chaudhary *et al.* 2012).

Table 2. Antibacterial Activity of Essential Oils from the Wood, Bark, and Needles of *P. roxburghii*

| Bacterial strain | Essential oils | | | | | | Negative control (mm) | Positive control* (mm) |
|------------------------|----------------|-------|--------|-------|---------|-------|-----------------------|------------------------|
| | Wood | | Bark | | Needles | | | |
| | IZ | MIC | IZ | MIC | IZ | MIC | | |
| <i>B. subtilis</i> | 15±0.5 | 64 | 8±0.70 | 2000 | 14±1.8 | 500 | n.a. | 15 |
| <i>S. aureus</i> | 13±0.8 | < 250 | 12±1.0 | < 250 | 14±1.2 | 500 | n.a. | 20 |
| <i>E. coli</i> | 12±0.6 | 500 | 17±1.3 | < 250 | na | >5000 | n.a. | 21 |
| <i>S. lutea</i> | 12±0.8 | 4000 | 10±1.1 | < 250 | 17±1.2 | 1000 | n.a. | 25 |
| <i>R. solanacearum</i> | 8±1.89 | 1000 | 14±1.2 | 126 | 14±1.3 | 500 | n.a. | n.t. |
| <i>E. amylovora</i> | na | >5000 | na | >5000 | na | >5000 | n.a. | n.t. |
| <i>P. carotovorum</i> | na | >5000 | 12±1.2 | 500 | 12±1.2 | 500 | n.a. | n.t. |

IZ: Diameter of inhibition zone (mean \pm SD, mm) including disc diameter of 5 mm at 2000 $\mu\text{L}/\text{mL}$

MIC: Minimum inhibitory concentration; values are given as $\mu\text{L}/\text{mL}$

n.a. Not active

n.t. Not tested

*Tetracycline (20 $\mu\text{g}/\text{disc}$)

An interesting feature was observed using the essential oil from wood and bark as antibacterial agent in the case of *E. coli*. According to previous work, this strain is highly resistant to the oil from needles and wood (Hassan and Amjid 2009; Zafar *et al.* 2010). In the present study, however, the oils from wood and bark were observed to have good activity against *E. coli*. The present study indicated that wood essential oil of *P. roxburghii* contains active components which showed greatest activity against the tested bacteria. For example, this study found α -thujene only in wood oil, and previously this component was shown to inhibit yeast growth (Delaquis *et al.* 2002). In addition, Sabinene was found only in the oil of wood, at a concentration of 3.59%. Glišić *et al.* (2007) concluded that sabinene had a significant influence on fungal growth, which is in accordance with data obtained by Santoyo *et al.* (2006). Additionally, Glišić *et al.* (2007) observed that the essential oil fractions of *Juniperus communis* L. containing the highest concentrations of α -pinene and sabinene effectively inhibited the growth of all bacteria (*B. cereus*, *E. coli*, *Listeria monocytogenes*, *Corynebacterium* sp., *Pseudomonas*

aeruginosa, and *S. aureus*), yeast (*Candida albicans*), and fungi (*Alternaria* sp., *Aspergillus nidulans*, and *Aspergillus niger*) studied.

Total Antioxidant Capacity

The percentages of the free radical scavenging activities of wood, bark, and needle essential oils from *P. roxburghii* (Table 3) were $82 \pm 2.12\%$, $85 \pm 1.24\%$, and $50 \pm 2.24\%$, respectively. It can be observed that the total antioxidant activities of the essential oils from wood and bark was higher than that of tannic acid ($81 \pm 1.02\%$), whereas the value of TAA% from the essential oil of needles was lower than the value observed by tannic acid.

Table 3. Total Antioxidant Activities of the Essential Oils from Wood, Bark, and Needles from *P. roxburghii*

| Total antioxidant activity (%) | | | |
|--------------------------------|-----------|-------------|---------------------|
| Wood E.O. | Bark E.O. | Needle E.O. | Tannic Acid control |
| 82±2.12 | 85±1.24 | 50±2.24 | 81±1.02 |

E.O.: Essential oil

Previous studies found that the extracts of *P. roxburghii* including essential oils, phenolic compounds, resin, and sterols had remarkable antioxidant, anti-inflammatory, and antidyslipidemic properties (Abbasi *et al.* 2010; Puri *et al.* 2011). On the other hand, terpenoid and steroid compounds are known for their antioxidant and hepatoprotective activities, and these compounds are found in wood oil (Di Carlo *et al.* 1999). Furthermore, the study by Puri *et al.* (2011) showed that extracts of the needles possessed significant antioxidant qualities. The essential oil from fresh fruits of *P. roxburghii*, however, showed only negligible radical scavenging activity (Qadir and Shah 2014).

The main oil compositions of plant essential oils are monoterpene and sesquiterpene hydrocarbons and their derivatives. These derivatives act as antibacterial and antifungal substances, the most well-known of which being terpenes and phenolics in general (Gülten *et al.* 2012). It has been reported that there is a correlation between the antioxidant capacity and the antibacterial activity of medicinal plants such as *Juglans regia* (Sharafati-Chaleshtori *et al.* 2011). On the basis of the above result, it can be concluded that essential oils from *P. roxburghii* wood, bark, and needles have significant potential as antibacterial and antioxidant substances.

CONCLUSIONS

1. Twenty-two, thirty-one, and twenty-eight compounds were identified in the essential oils of wood, bark, and needles, respectively. The major chemical constituents of wood essential oil were caryophyllene (16.75%), thunbergol (16.29%), 3-carene (14.95%), cembrene (12.08%), α -thujene (10.81%), and terpinolen (7.17%). In bark they were α -pinene (31.29%) and 3-carene (28.05%) and in needles α -pinene (39%) and 3-carene (33.37%).
2. All the essential oils showed good activity against human pathogen bacteria, whereas the essential oils from bark and needles showed good activity against plant pathogen

bacteria *Ralstonia solanacearum* and *Pectobacterium carotovorum*. *Erwinia amylovora* was resistant to all tested oils.

3. The total antioxidant activities (TAA%) of the essential oils from wood and bark were higher than that of tannic acid, whereas the value of TAA% from the essential oil of needles was lower than that of tannic acid.

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