

Efficacy of Oily and Resinous Fractions of Cone Extracts from *Pinus roxburghii* Sarg

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In the present study the oily and resinous fractions of cone from *Pinus roxburghii* Sarg., was evaluated at different concentration (4000, 2000, 1000 and 500 µg/mL) against the growth of some plant and human pathogenic bacteria namely; *Bacillus subtilis*, *Escherichia coli*, *Ralstonia solanacearum*, *Erwinia amylovora* and *Pectobacterium carotovorum* subsp. *carotovorum* using the Kirby-Bauer disc diffusion susceptibility test. The resinous fraction showed the highest antibacterial activity against the growth of *Erwinia amylovora* with inhibition zones 35 mm, 30 mm, 30 mm, and 22.33±2.51 mm, with the concentrations of 4000 µg/mL, 2000 µg/mL, 1000 µg/mL, and 500 µg/mL, respectively, and *Bacillus subtilis* at the concentration of 4000 µg/mL (Inhibition zone value of 13.33±1.52 mm) and at 2000 µg/mL (12 mm). The oily fraction showed good activity against *Bacillus subtilis*, and *Ralstonia solanacearum* at the concentrations of 4000 µg/mL with Inhibition zone values of 14.33±1.15 mm, and 13±1.73 mm, respectively. The resinous fraction from cone extract of *Pinus roxburghii* could be useful as a natural biocide tool for controlling the growth of *Erwinia amylovora*.

Key words: *Pinus roxburghii*; Cone; Oily fraction; Resinous fraction; Antibacterial activity.

Pinus roxburghii Sarg., belongs to Family *Pinaceae* is commonly known as “chir pine” and has a long history of medicinal use (Qadir *et al.*, 2014). Turpentine oil is produced from wood of *P. roxburghii* (Vallejo *et al.*, 1994; Asta *et al.*, 2006). Monoterpenes and sesquiterpenes are the main groups presented in the essential oils from pine needles which can be used in the manufacture of perfumes and cosmetic materials (Dormont *et al.*, 1998; Pagula and Baeckstrom 2006; Dob *et al.*, 2007). The essential oil composition of cones was reported from Egypt by Islam (2006). Needles extracts were reported to have an antioxidant

activity (Puri *et al.*, 2011). The tree produces rosin material which can be used to manufacture adhesives, varnish, printing ink, synthetic rubber and chewing gums (Wiyono *et al.*, 2006; Shuaib *et al.*, 2013). Satyal *et al.*, (2013) reported that the essential oils from different parts of *P. roxburghii* including cones were dominated by sesquiterpenes, particularly (E)-caryophyllene, and α -humulene, and the monoterpene alcohols including terpinen-4-ol and α -terpineol. Cone extracts showed good antibacterial activity, moreover, Δ -3-carene (2.3%-6.8%) was only found in cone oil (Bissa *et al.*, 2008). The aqueous and alcoholic extracts from stem, leaves, bark, female cone and male cone of *P. roxburghii* were assayed for their antibacterial activity against the growth plant pathogen *Agrobacterium tumefaciens* and

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against four human pathogens, *E. coli*, *Salmonella arizonae*, *S. typhi* and *S. aureus*. All the extracts showed inhibitory activity against *A. tumefaciens* and the extracts showed inhibitory activity against *E. coli* and *S. typhi* except stem extract and against *S. aureus* except the alcoholic extract of bark and aqueous extract of male cone. Leaves and female cones alcoholic extracts were observed good activity against *S. arizonae* (Parihar *et al.*, 2006).

In the present study the oily and resinous fractions of Cone from *Pinus roxburghii* was evaluated at different concentration (4000, 2000, 1000, and 500 µg/mL) against the growth of some plant and human pathogenic bacteria namely; *Bacillus subtilis*, *Escherichia coli*, *Ralstonia solanacearum*, *Erwinia amylovora* and *Pectobacterium carotovorum* subsp. *carotovorum* using the Kirby-Bauer disc diffusion method.

MATERIALS AND METHODS

Plant material and preparation of the extract

Pinus roxburghii tree was supplied from Antoniadis Garden, Horticultural Research Institute, Alexandria, Egypt. The plant was identified at the Department of Forestry and Wood Technology, Faculty of Agriculture, Alexandria University. The mature cones are collected from the trees during March-April, 2014. Cones material was separated from the stem. One cone was macerated in 200 mL of a solvents mixture of acetone: diethyl ether (1:1 v/v) for two weeks. The mixture was filtered through filter paper (Whatman No. 1) (Ali *et al.*, 2014). The extract was appeared as oily and resinous fractions, and the two layers was separated using funnel separator. The solvents were evaporated using a rotary vacuum evaporator at 45 °C and the oily and resinous fractions (Figure 1) were dried and concentrated and stored in sealed vials at 4 °C until further use. The concentrated oily and resinous layers were prepared at a concentrations of 4000 2000 µg/mL, 1000 µg/mL, and 500 µg/mL by diluting the two fractions in 10% Dimethylsulfoxide (DMSO, Sigma-Aldrich, USA) and distilled water (1:1 v/v). The prepared concentrations were stored at 4°C in the refrigerator until further use (Salem *et al.*, 2013).

Antibacterial activity

The antibacterial activity of oily and resinous fractions of Cone from *Pinus roxburghii*

was evaluated against the growth of *Bacillus subtilis* ATCC 6633 (human bacterial pathogen), *Escherichia coli* ATCC 8739 (plant bacterial pathogen), *Ralstonia solanacearum* (plant bacterial pathogen), *Erwinia amylovora* (plant bacterial pathogen) and *Pectobacterium carotovorum* subsp. *carotovorum* (strain No. ippbc038) (plant bacterial pathogen) using the Kirby-Bauer disc diffusion method (Bauer *et al.*, 1966). The bacterial strains were supplied by the Laboratory of Plant Pathology Department, Faculty of Agriculture, Alexandria University, Egypt. The plates of Mueller Hinton Agar (MHA) media were prepared by pouring about 15 mL of the media into sterile Petri dishes to a depth of 5.0 mm and allowed to solidify for 5 min. A freshly 24-hour's old bacterial suspension (0.5 mL) was spread over the surface of MHA plates by using sterile cotton swabs. Subsequently, the surface of the plated was allowed to dry for half hour and sterile discs (4 mm diameter) of Whatman filter paper no. 1 were stacked on the surface of the inoculated media and each disc was received 20 µL of the concentrated oily and resinous fractions (4000, 2000, 1000 and 500 µg/mL) dissolved in 10% DMSO and distilled water (1:1 v/v). The inhibition zones around the discs were recorded in millimeters with three measurements (Ali *et al.*, 2013a,b).

RESULTS AND DISCUSSION

Results of the inhibition zones reported by the resinous fraction from the extract of *Pinus roxburghii* cone are shown in Table 1. The resinous fraction showed good activity against the growth of *Bacillus subtilis* at the concentration of 4000 µg/mL (Inhibition zone value of 13.33±1.52 mm) and at 2000 µg/mL (12 mm). The resinous fraction showed the highest antibacterial activity against the growth of *Erwinia amylovora* (Fig. 2). in the present study we kept the Petri dish at the laboratory room for three months and the dish was checked again to know if the bacteria regrowth or no and we found that the inhibition zone was stilled as it is, and only small contamination with fungi was appeared at the side of the Petri dish (Fig. 3) and it could be concluded that the resinous fraction had a bactericidal effect.

All the concentration were shown highly

Table 1. Antibacterial activity of resinous fraction of *Pinus roxburghii* against the growth of some plant and human bacterial pathogens

Bacterial strains	Inhibition zones of stem bark extract (mm)*				DMSO
	4000 µg/mL	2000 µg/mL	1000 µg/mL	250 µg/mL	
<i>Bacillus subtilis</i>	13.33±1.52	12	na	na	na
<i>Escherichia coli</i>	na	na	na	na	na
<i>Ralstonia solanacearum</i>	na	na	na	na	na
<i>Erwinia amylovora</i>	35	30	22.33±2.51	30	na
<i>Pectobacterium carotovorum</i>	na	na	na	na	na

*: Diameter of inhibition zone (mean ± SD mm) including disc diameter of 4 mm.

Table 2. Antibacterial activity of oily fraction of *Pinus roxburghii* against the growth of some plant and human bacterial pathogens

Bacterial strains	Inhibition zones of stem bark extract (mm)*				DMSO
	4000 µg/mL	2000 µg/mL	1000 µg/mL	250 µg/mL	
<i>Bacillus subtilis</i>	14.33±1.15	12	11.33±1.15	8.66±0.57	na
<i>Escherichia coli</i>	na	na	na	na	na
<i>Ralstonia solanacearum</i>	13±1.73	12.33±0.57	9	6	na
<i>Erwinia amylovora</i>	na	na	na	na	na
<i>Pectobacterium carotovorum</i>	na	na	na	na	na

*: The Inhibition zones values are presented as mean ± SD.

activity with inhibition zones 35 mm, 30 mm, 30 mm, and 22.33±2.51 mm, with the concentrations of 4000 µg/mL, 2000 µg/mL, 1000 µg/mL, and 500 µg/mL, respectively. The following bacterial strains *Escherichia coli*, *Ralstonia solanacearum*, *Pectobacterium carotovorum* were showed a resistance to all the studied concentrations of resinous fraction.

Results presented in Table 2, showed that the oily fraction observed good activity against the growth of *Bacillus subtilis* at the concentrations of 4000 µg/mL, 2000 µg/mL, 1000 µg/mL, and 500 µg/mL, with Inhibition zone values of 14.33±1.15 mm, 12 mm, 11.33±1.15 mm, and 8.66±0.57 mm, respectively.

The inhibition zone values observed against the growth of *Ralstonia solanacearum* were 13±1.73 mm, 12.33±0.57 mm, 9 mm, and 6 mm, with the concentrations of 4000 µg/mL, 2000 µg/mL, 1000 µg/mL, and 500 µg/mL, respectively. *Escherichia coli*, *Erwinia amylovora*, and *Pectobacterium carotovorum* showed resistance to all the oily fraction concentrations of cone extract from *Pinus roxburghii*.

Overall, the resinous fraction from cone extract of *Pinus roxburghii* had good antibacterial activity against the growth of *Bacillus subtilis*, and *Erwinia amylovora*. The resinous fraction showed good activity against the growth of *Bacillus subtilis*, and *Ralstonia solanacearum*. Parihar *et al.*, (2006) reported that the aqueous and alcoholic extracts were showed inhibitory activity against *A. tumefaciens* and the extracts showed inhibitory activity against *E. coli* and *S. typhi* except stem extract and against *S. aureus* except the alcoholic extract of bark and aqueous extract of male cone. Leaves and female cones alcoholic extracts were observed good activity against *S. arizonae*. These results not in consist with the present study, where, the oily and resinous fraction did not show any activity against *E. coli*. Recently, Satyal *et al.*, (2013) reported that the bioactivity assays of cone essential oil was showed remarkable cytotoxic activity (with 100% killing of MCF-7 cells at 100 µg/mL, and remarkable Brine shrimp lethality with LC₅₀=11.8 µg/mL and antifungal activity against *Aspergillus niger* with minimum inhibitory concentration of 39 µg/mL. Additionally, (Bissa *et*

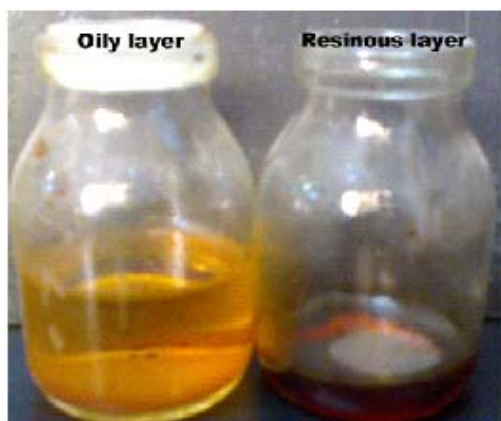
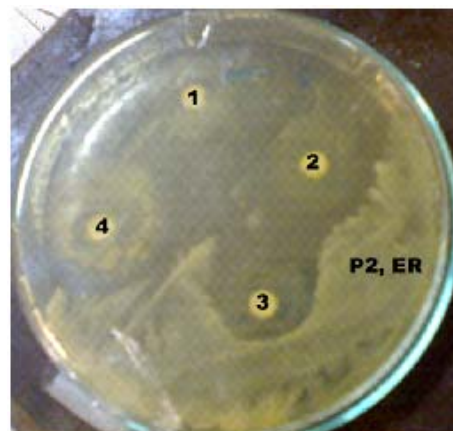
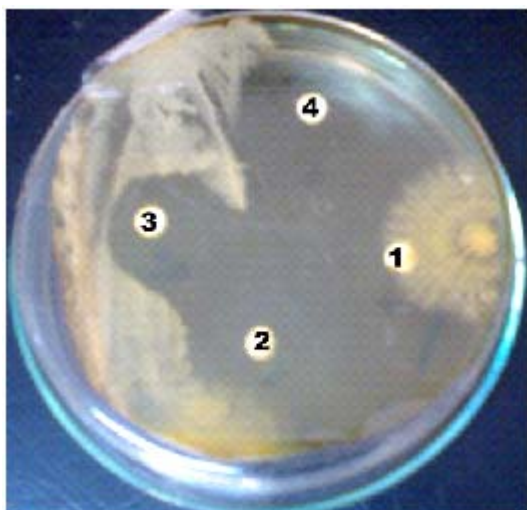


Fig. 1. Oily and resinous fractions from the cone extract



1: 4000 µg/mL; 2: 2000 µg/mL; 3: 1000 µg/mL; 4: 500 µg/mL

Fig. 2. The inhibition zones observed after 48 hours of the incubation by the resinous fraction (P2) from *Pinus roxburghii* cone against the growth of *Erwinia amylovora* (ER).



1: 4000 µg/mL; 2: 2000 µg/mL; 3: 1000 µg/mL; 4: 500 µg/mL

Fig. 3. The inhibition zones, observed after 3 months of the incubation by the resinous fraction from *Pinus roxburghii* cone against the growth of *Erwinia amylovora*

al., 2008) reported that the cone extracts showed good antibacterial activity.

CONCLUSION

The oily and resinous fractions of cone from *Pinus roxburghii* was evaluated at different concentration (4000, 2000, 1000 and 500 µg/mL) against the growth of some plant and human pathogenic bacteria namely; *Bacillus subtilis*, *Escherichia coli*, *Ralstonia solanacearum*, *Erwinia amylovora* and *Pectobacterium*

carotovorum subsp. *carotovorum*. The resinous fraction from cone extract of *Pinus roxburghii* had good antibacterial activity against the growth of *Bacillus subtilis*, and *Erwinia amylovora*. The oily fraction showed good activity against the growth of *Bacillus subtilis*, and *Ralstonia solanacearum*. The resinous fraction from cone extract of *Pinus roxburghii* could be useful as a natural biocide tool for controlling the growth of *Erwinia amylovora*.

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