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Antibacterial Activity of Extract from the Stem Bark of *Schinus terebinthifolius*

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In the present study the methanol extract of stem bark of *Schinus terebinthifolius* 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 methanol stem bark extract presented a moderate activity against the studies bacterial pathogens *B. subtilis*, *E. coli* and *R. solanacearum*, where other bacterial strains (*E. amylovora* and *P. carotovorum* subsp. *carotovorum*) were showed resistances to the methanol extract.

Key words*: Schinus terebinthifolius*; Methanol extract; Stem bark; Antibacterial activity.

Schinus terebinthifolius Raddi (Anacardiaceae) is found in the Brazilian coast, and is distributed from the northeast to the south part of Brazilian coast (Corrêa, 1974; Carvalho *et al.*, 2013) and has been introduced and naturalized in many countries of the world (Taylor, 2005). Typically, *Schinus terebinthifolius* has been shown a multiple-stemmed trunk with most stems less than 4 inches (<10 cm). The biological activates of have been studied for many years; have been described since the first edition of the Brazilian Pharmacopoeia (Carvalho *et al.*, 2013). The plant is producing a resin-like material which contains monoterpenes to protect the tissues of the plant against the penetrations of the predictor's pathogens (Byers, 1995). Molina-Salinas *et al.*, (2006) reported that *Schinus terebinthifolius* is used as antibacterial and antiviral activity. Almost all parts of *S. terebinthifolius*, including leaves, bark, fruit, seeds, resin, and oleoresin (or balsam), have been used medicinally by indigenous peoples throughout the tropical regions. In South Africa, a leaf tea is used to treat colds, and a leaf decoction is inhaled for hypertension, depression, and irregular heart beat (El-Massry *et al.*, 2009). The extracted oil from bark has been used for the treatment of tumors and corneal diseases (Bornhausen, 2010). In rats, the extracts from stem bark were showed good protective effect against gastric ulcerations induced by immobilization stresses at low temperature (Carlini *et al.*, 2010). Johann *et al.*, (2010) isolated the following chemical constitutes; Schinol, and biphenyl 4'-ethyl-4-methyl-2,2',6,6'-tetrahydroxy[1,1'-biphenyl]-4,4'-dicarboxylate from the stem, and showed good antifungal activity. Stem bark of *Schinus terebinthifolius* was reported as rich in tannins and essential oils; also the stem bark ethanol extract

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was showed the presence of anthraquinones, phenols, and triterpenes; and phenols, flavones, flavonoids, xanthones, leucoanthocyanidins, flavanones and free steroids in leaves (Jorge and Markmann, 1996; Lima *et al.*, 2006). The plant has been specially evidenced in flavonoid-enriched fractions (Varela-Barca *et al.*, 2007). Also, stem bark was reported to have the monoterpenes, sesquiterpenes, triterpene, ketones and acids (Lloyd *et al.*, 1977). On the other hand, Lima *et al.*, (2009) reported that the oral administration of dried extracts of stem bark in Wistar rats did not induce any toxic effect. In the present study the methanol extract of stem bark of *Schinus terebinthifolius* was evaluated at different concentration 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.

MATERIALS AND METHODS

Plant material and preparation of the extract

Schinus terebinthifolius plants were provided from Antoniadis Garden, Horticultural Research Institute, Alexandria, Egypt, during the month of April 2014. The plant was identified at the Department of Forestry and Wood Technology, Faculty of Agriculture, Alexandria University. The stem bark material was separated from the stem and air-dried at room temperature for one week, milled into powder to obtain a 40-60-mesh ground material and stored in paper bags until use. The pulverized stem bark (50 g) was macerated in 200 mL of methanol (80%) for 14 days, filtered through a cotton plug, followed by Whatman filter paper number 1 (Ali *et al.*, 2014). The methanol solvent was evaporated using a rotary vacuum evaporator at 45 °C and the extract was then dried and concentrated and stored in sealed vials at 4 °C until further use. The percentage yields of the methanol extract of stem bark from *Schinus terebinthifolius* was 12.14% (Fig. 1).

The weighed methanolic crude extract was prepared for stock solution at a concentration of 4000 µg/mL by diluting the crude extracts in 10% Dimethylsulfoxide (DMSO, Sigma-Aldrich) and distilled water (1:1 *v/v*). Different concentrations were prepared from the stock solution to obtain 2000 µg/mL, 1000 µg/mL, and 500 µg/mL. All the prepared concentrations were stored at 4°C in the refrigerator until further use (Salem *et al.*, 2013).

Antibacterial activity

The antibacterial activity of methanol extract from stem bark of *Schinus terebinthifolius* was evaluated against the growth of the Gram-positive bacteria *Bacillus subtilis* ATCC 6633 (human bacterial pathogen), as well as the Gram-negative bacteria *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 susceptibility test (Bauer *et al*., 1966). The bacterial strains were provided by the Laboratory of Plant Pathology Department, Faculty of Agriculture, Alexandria University, Egypt. Nutrient agar medium was used for maintenance of the bacterial test organisms. Mueller Hinton Agar (MHA) medium was used for screening the antibacterial activities applying the disc diffusion method and then it was prepared and autoclaved. The MHA plates were prepared by pouring about 15 mL of melted media into sterile Petri dishes to a depth of 5.0 mm and allowed to solidify for 5 min. Using sterile cotton swabs, 0.5 mL of fresh 24- hour's old bacterial suspension were spread over the surface of MHA plates, the surface allowed drying. Sterile plain discs of 4 mm diameter (Whatman filter paper no. 1) were placed on the surface of agar plates and each disc was loaded with 20 µL of the concentrated stem bark extract (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 triplicate measurements.

RESULTS AND DISCUSSION

Results of the antibacterial evaluation of stem bark extract from *S. terebinthifolius* are presented in Table 1. At the concentration of 4000 µg/mL, the methanol extract of the stem bark showed good antibacterial activity against the growth of

Bacillus subtilis with an IZ value of 26±6.08 mm, and at 2000 µg/mL (13±0.57 mm), whereas the other concentration did not show any activity. The bark methanol extract showed good activity against the growth of *Escherichia coli* at 4000 µg/mL (15.66±0.57 mm) and 2000 µg/mL (12±0.57 mm). The methanol extract at 4000 and 2000 µg/mL was presented moderate activity against the growth of the plant bacterium *Ralstonia solanacearum* with the inhibition zones values of 9.66±0.57 mm and 7.66±0.57 mm, respectively. Alternatively, the methanol extract of stem bark did not present any activity against the growth of the bacteria *Escherichia coli*, *Erwinia amylovora* and *Pectobacterium carotovorum* at all the studied concentrations.

Table 1. Antibacterial activity of stem bark extract of *S. terebinthifolius* against the growth of some plant and human bacterial pathogens

Bacterial strains	Inhibition zones of stem bark (mm) *				DMSO
	4000 µg/mL	2000 µg/mL	1000 µg/mL	250 µg/mL	
Bacillus subtilis	26±6.08	13±0.57	na	na	na
Escherichia coli	15.66±0.57	12±0.57	na	na	na
Ralstonia solanacearum	9.66±0.57	7.66±0.57	na	na	na
Erwinia amylovora	na	na	na	na	na
Pectobacterium carotovorum	na	na	na	na	na

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

Generally, the methanol stem bark extract presented a moderate activity against the studies bacterial pathogens *Bacillus subtilis*, *Escherichia coli* and *Ralstonia solanacearum*, where other bacterial strains (*Erwinia amylovora* and *Pectobacterium carotovorum* subsp. *carotovorum*) were shown a resistance to the extract.

Previously, the activity of stem bark extracts (anti-inflammatory, antibacterial, antifungal and anticancer could be related to the tannins which the major compounds of stem bark extract of *S. terebinthifolius* (Matos, 1994). The flavonoid fractions of stem bark were capable of breaking phosphodiester bonds in DNA (Varela-Barca *et al.*, 2007). The combination of the stem bark powder of *Schinus terebintifolius* with other natural products were showed antifungal against *A. brasilienses* (Machado *et al.*, 2012).

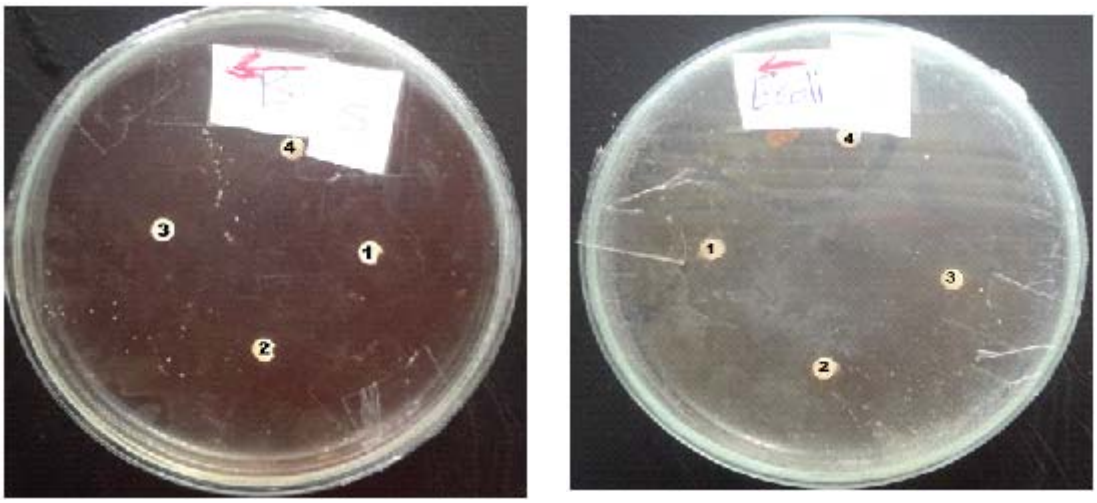
A hydroalcoholic stem bark extract of *Schinus terebinthifolius* in a dose of 0.1 g/kg had presented a positive effect on the healing process of colon anastomoses (Coutinho *et al.*, 2006), as well as on healing surgical bladder wounds (Lucena *et al.*, 2006) and on the back wounds of rats via percutaneous route (Castelo-Branco Neto *et al.*, 2006). Also, several recent reports found that the following chemical groups fatty acids, alkaloids, flavonoids, polyphenols, tannins, coumarins, glucosides, terpenes, as well as the essential oils were responsible for the bioactivity of the extracts from the plant tissues (Ali *et al.*, 2013a; Ali *et al.*, 2013b; Salem *et al.*, 2013). Salem *et al.*, (2013) reported that the main methyl esters of fatty acids reported in the stem bark were tridecanoic, tetradecenoic, myristic, erucic, 14- pentadecenooic, pentadecanoic, and hexadecenoic acids.

The aqueous and alcoholic extracts were evaluated for its antibacterial activity (Lima *et al.*, 2004) which was showed that only the alcoholic

Fig. 1. Methanol crude extracts of bark from *Schinus terebinthifolius*

extracts observed good inhibitory effect on the growth of *Staphylococcus aureus* and *Bacillus cereus* and no inhibitory effect was shown against the growth of *Candida albicans*, *Aspergillus niger*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella choleraesuis* were observed for both extracts (Degáspari *et al.*, 2005). Lima *et al.*, (2009) suggested that acute toxicity of the bark extract of *Schinus terebinthifolius* was practically null via oral route of Wistar rats of both sexes, with similar results was observed by Pires *et al.*, (2004) in mice.

The stem bark ethanolic extract of *Schinus terebinthifolius* at a concentration of 1 mg/mL), was observed excellent activity against the resistant strains of *Staphylococcus aureus*, with minimum inhibitory concentration (MIC) value of ≤100 mg/mL (Lima *et al.*, 2006).



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

Bacillus subtilis *Escherichia coli*

Fig. 2. Antibacterial activity of methanol bark extract of *S. terebinthifolius* against the growth of *Bacillus subtilis* and *Escherichia coli*

CONCLUSION

Stem bark methanol extract of *Schinus terebinthifolius* 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 extract was presented a moderate activity against the studies bacterial pathogens *Bacillus subtilis Escherichia coli*, and *Ralstonia solanacearum*, where the other bacterial strains (*Erwinia amylovora* and *Pectobacterium carotovorum* subsp. *carotovorum*) were showed resistances to the methanol extract.

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