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Evaluation of Extracts from Different Parts of Some Tree Species against the Growth of Human Bacterial Pathogens

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In the present study the methanolic extracts from *Ficus retusa* L. (fruits), *F. benghalensis* L. (fruits), *Schinus terebinthifolius* Raddi (fruits), *Erythrina humeana* (bark), *Delonix regia* (bark), *Lantana camara* L. (leaves), *Syzygium cumini* L. (leaves), and *Cupressus sempervirens* L. (leaves), and the water soluble exudate gum from *Meryta sinclairii* (wood exudate gum) were evaluated for their antibacterial against four human pathogenic bacteria namely; *Micrococcus luteus*, *Staphylococcus aureus*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. The bioassay was evaluated at a concentration of 2000 µg/mL using the disc-diffusion method. The extracts were shown a variation in the inhibition zones ranges from 7-22 mm. The present results could be useful for further work related to the bioactivity agents for controlling the infectious diseases caused by human bacterial pathogens.

Key words: Antibacterial; Methanolic extract; Tree species.

Trees are sources of renewable feedstock for medicinal compounds which plays a dominant role in maintenance of human health. Recently, over 50 % of all modern clinical drugs are of natural product origin which plays an important role in drug development programs of the pharmaceutical industry (Ali *et al.*, 2013a; Abdel-Megeed *et al.*, 2013; Salem, 2013; Ahmad *et al.*, 2008).

The search for new drugs of natural products from the higher plants (trees) are takes good roles for controlling the infectious diseases caused by clinical pathogen (Aly *et al.*, 2012). The most important of these bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds contained saponins, flavonoids, alkaloids, ß-sitosterol, carotene, hydrocarbons phytotoxins 2-carotene and zeaxanthein (Abdel-Megeed *et al.*, 2013; Fatmawaty *et al.*, 2013; Parekh and Chanda, 2007; Guerere *et al.*, 1986; Jungalwala and Cama, 1962; Subramanian *et al.*, 1966). Phenolic compound like; gallic acid, was found in the bark and other phenolic acids such as sorbic, sinapic, p-coumaric, m-coumaric, ferulic, caffeic, 3-hydroxybenzoic, 4-hydroxycinnamic and 4-hydroxybenzoic acids (Shabir *et al.*, 2011). Plants rich in flavonoids like kaempferal were rported to exhibit different antimicrobial activities (Goel *et al.*, 1998).

In the present study different parts of some tree species were used to evaluate their extracts as antibacterial activity against the growth of four human pathogenic bacteria; *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter baumannii* and *Micrococcus luteus*.

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MATERIALS AND METHODS

Plant material

Different parts from *Ficus retusa* L. (fruits), *Ficus benghalensis* L. (fruits), *Schinus terebinthifolius* Raddi (fruits), *Erythrina humeana* (bark), *Delonix regia* (bark), *Lantana camara* L. (leaves), *Syzygium cumini* L. (leaves), *Cupressus sempervirens* L. (leaves), *Meryta sinclairii* (Hook.f.) Seem. (Wood exudate gum) were collected in August 2013 from different regions at Alexandria city, Egypt. The tree species were identified at the Department of Forestry and Wood Technology, Faculty of Agriculture, Alexandria University. All the materials (fruits, leaves, bark) were air-dried at room temperature and ground into small particles with small laboratory mill. The exudate gum from the wood of *M. sinclairii* was collected directly from the wood after cutting the stems.

Preparation of the extracts

The ground materials (100 g from each of them) were macerated with 200 mL of methanol (80%). After one week of maceration the materials were filtrated (Salem *et al.*, 2013) and the residues were discarded. The methanoilc crude extracts (MCE) were concentrated using a rotary evaporator under reduced pressure at 45°C. The extract was lyophilized, weighed and stored at 4°C in the refrigerator until further uses. The concentrated extracts were prepared for a stock solution at concentration of 2000 µg/mL by diluting the crude extract in 10% Dimethylsulfoxide (DMSO, Sigma-Aldrich) and distilled water (1:1 *v*/*v*). For the exudate gum from the wood *M. sinclairii* that was collected directly from the wood after cutting the stems, the concentrated solution was done by using autoclaved distilled water. We would like to inform here that the methanolic extracts from *D. regia*, S. *terebinthifolius*, *E. humeana* and *F. benghalensis* were used in the present study form the stored previous extracts (Ali *et al.*, 2013b; Salem, 2013).

Antibacterial activity

The antibacterial activity of the extracts was employed using the following human pathogenic bacteria; *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter baumannii* and *Micrococcus luteus*. The previous human pathogenic bacteria strains were provided by Botany Department, Microbiology Section, Faculty of Science, Alexandria University, Egypt. The extracts were prepared at a concentration of 2000 µg/mL. Nutrient agar (NA) medium was used for maintenance of the tested bacterial organisms. Mueller Hinton agar (MHA) was used in all bioassays applying the disc diffusion method. The antibacterial activity was carried out using the Kirby-Bauer disc diffusion method (NCCLS, 1997). Filter paper discs (5 mm in diameter) were suspended with 20 µL of the concentrated extracts and placed on the inoculated plates and incubated at 37°C for 24 hrs. The diameters of the inhibition zones were recorded in millimeters.

RESULTS AND DISCUSSION

Table 1 is presenting the quantities of the weighted MCE extracts from the different parts of the studied trees.

According to the results presented in Table 2, the inhibition zones found against the growth of *M. luteus* were present by *F. retusa*, *F. benghalensis*, *S. terebinthifolius*, *E. humeana*, *S. cumini*, *C. sempervirens*, *M. sinclairii* with values of 17mm, 11mm, 14mm, 20mm, 12mm, 17m and 11mm, respectively. With *S. aureus*, the inhibition zones values 8mm, 12mm, 7mm, 22mm and 12mm were observed by the extracts from *F. retusa*, *S. terebinthifolius*, *E. humeana*, *L. camara* and *S. cumini*, respectively. The extracts from *S. terebinthifolius*, *S. cumini* and *C. sempervirens* were showed activity against the growth of *A. baumannii* with the following inhibition zones values; 17mm, 13mm and 23mm, respectively. The

Table 1. The quantities of the weighted extracts

Tree species	Part	g extract/100 g oven dry sample
F. retusa	Fruits	10.45
F. benghalensis	Fruits	15.7[a]
S. terebinthifolius	Fruits	15.9[a]
E. humeana	Bark	10.26[b]
D. regia	Bark	8.23[b]
L. camara	Leaves	14.12
S. cumini	Leaves	10.67
C. sempervirens	Leaves	6.13
M. sinclairii	Wood	10 g were taken

a: data from Ali *et al.*, (2013a); b: data from Salem (2013)

extracts from *F. retusa*, *F. benghalensis*, *E. humeana*, *L. camara*, *S. cumini*, *C. sempervirens* and *M. sinclairii* with inhibition zones values of 16mm, 11mm, 9mm, 14mm, 8mm, 9mm and 13mm, respectively, were observed against the growth of *P. aeruginosa*. It can be concluded that the extracts from the bark, fruits and leaves of *E. humeana*, *C. sempervirens* and *F. retusa* had the highest effect against the growth of *M. luteus*. The highest effects against the growth of *S. aureus* were shown by the extracts from *L. camara* (leaves), *S. cumini* (leaves) and *S. terebinthifolius* (fruits). The growth of *A. baumannii* was affected the extracts of *C. sempervirens* (leaves), *S. cumini* (leaves) and *F. benghalensis* (fruits). The extracts of *F. retusa* (fruits), *L. camara* (leaves) and *M. sinclairii* (wood gum exudate) were showed good activity against the growth of *P. aeruginosa*.

Aly *et al.,* (2012) reported that the plants having antibacterial constituents suggested to have enormous therapeutic potential as they could act without any side effect as often found with synthetic antibacterial products. On the other hand, Scrinivasan *et al.,* (2001) found that most antibacterial medicinal plants are more effective against Gram-positive than Gram-negative bacteria. The antimicrobial activities of extracts of *Ficus* have been recently reviewed by (Salem *et al.,* 2013). reported that the methanol extracts from *F. retusa* have been shown moderate activity against *Bacillus cereus*, *Bacillus subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *Serratia marcescens* and *Agrobacterium tumefaciens* Salem (2005) and the extracts was contained high antibacterial properties towards Gram-positive and Gram-negative bacteria (Ao *et al.,* (2008). The extracts of the aerial parts of *F. retusa* "variegata" showed mild antimicrobial activity against *Candida albicans*, *Mucor* spp. *Salmonella typhi*, *E. coli* and *Bacillus* spp. (Sarg *et al.*, 2011). The golden yellow leaves of *F. microcarpa* was reported to have a high amounts of flavonoids, carotenoids, triterpenoids, fatty alcohol, steroids, coumarins, flavane-4-hydroxybenzoate and isoflavones (Chiang and Kuo, 2003; Takahashi *et al.,* 2002; Li and Kuo, 1998). It was reported that the extracts from *F. retusa* had a good antibacterial activity (Aly *et al.,* 2012), and the GC/MS analysis of ethyl acetate fractions from the methanol extract of leaves from *F. retusa*, showed the presence of 1,2-benzenedicarboxylic acid-dibutyl ester (15.19%), phenol,4-(2-aminopropyl)-, (+/-) (9.27%) and R-(2,2,3,3-2H4) butyrolactone (13.24%) (Aly *et al.,* 2013).

The different solvents extracts from different parts of *F. benghalensis* exhibited significant anti- bacterial activity (Koona and Rao, 2012; Manimozhi *et al.,* 2012; Bhangale *et al.,* 2010; Singh and Watla, 2010; Gayathri and Kannabiran, 2009; Uma *et al.,* 2009; Salem, 2005; Mousa *et al.,* 1994). For example, the bark extracts showed significant antibacterial activity against *S. aureus*, *P. aeruginosa* and *K. pneumoniae* (Gayathri *et al.,* 1998), the aerial root extracts exhibited activity against bacterial strains especially against *S.*

Table 2. Antibacterial activity (inhibition zones in mm) of extracts from different parts of tree species against the growth of some human bacterial pathogenic

Tree species	Part	Extract	*M. luteus*	*S. aureus*	*A. baumannii*	*P. aeruginosa*
F. retusa	Fruits	MCE	17	8	na	16
F. benghalensis	Fruits	MCE	11	na	na	11
S. terebinthifolius	Fruits	MCE	14	12	17	na
E. humeana	Bark	MCE	20	7	na	9
D. regia	Bark	MCE	na	na	na	na
L. camara	Leaves	MCE	na	22	na	14
S. cumini	Leaves	MCE	12	12	13	8
C. sempervirens	Leaves	MCE	17	na	23	9
M. sinclairii	Wood	Exudate gum	11	na	na	13
Tetracycline *			22	20	21	25
DMSO			na	na	na	na

MCE: methanolic crude extract
na: Not active. * Tetracycline (20 µg/disc).
Inhibition Zone (mm) including disc diameter of 5 mm at 2000 µg/mL.

aureus (Singh and Watal, 2010) and was more potent towards *B. subtilis* (Jagtap *et al.,* 2012), and found that fruit extracts had significant antibacterial activity (Mousa *et al.*, 1994) as well other parts (Ahmad *et al.,* 2011). On the other hand, the ethanolic extract at different concentrations of roots showed moderate anti-bacterial activity against *S. aureus*, *P. aeruginosa* and *K. pneumonia* (Murti and Kumar, 2011) while the hexane extract of leaves found to be resistance against *K. pneumoniae*, *P. aeruginosa* and *M. luteus* at low concentration, and *K. pneumoniae* showed intermediate activity to its high concentration (Koona and Rao, 2012). The acetone, methanol and ethyl acetate extracts of bark were observed good antibacterial activity against *P. aeruginosa*, *E. coli*, *P. vulgaris*, *B. subtilis*, and *S. aureus* (Manimozhi *et al.,* 2012).

Leaf extracts of *C. sempervirens* have been shown a remarkable effect in enhancing liver and kidney functions (Ali *et al.,* 2010). *S. cumini* has long been considered to have medicinal properties to treat inflammation, diabetes mellitus, constipation, leucorrhoea, stomachalgia, fever, gastropathy, trangury and dermopathy and to inhibit blood discharges in the faces (Shafi *et al.,* 2002). The extracts from leaves, fruit, root-bark and stem-bark showed antifungal activity (Jabeen and Javaid, 2010).

Many chemical components extracted from different parts of *S. terebinthifolius* like phenols, terpens, steroids, tannins, flavonoids, anthraquinones, xanthones, schinol and biphenyl 4'-ethyl-4-methyl-2,2',6,6'-tetrahydroxy[1,1'-biphenyl]-4,4'-dicarboxylate have been identified (Ceruks *et al.,* 2007; Lima *et al.,* 2006; Degáspari *et al.,* 2005; Kassem *et al.*, 2004). Most of them were found to have a good antifungal, antibacterial and antioxidant properties (Johann *et al.,* 2010; Báidez *et al.,* 2006).

Pharmacologically, *Delonix regia* was reported to have rich content of flavonoids like kaempferal that was exhibited antiulcer effect (Goel *et al.,* 1998). Previously, the extracts of bark contained saponins, flavonoids, alkaloids, β-sitosterol, carotene, hydrocarbons phytotoxins 2-carotene and zeaxanthein (Fatmawaty *et al.,* 2013; Parekh and Chanda, 2007; Guerere *et al.,* 1986; Jungalwala and Cama, 1962; Subramanian *et al.,* 1966) were exhibited antimicrobial activities. Also, the main phenolic compound, gallic acid, was found in the bark and other phenolic acids such as sorbic, sinapic, p-coumaric, m-coumaric, ferulic, caffeic, 3-hydroxybenzoic, 4-hydroxycinnamic and 4-hydroxybenzoic acids (Shabir *et al.,* 2011).

The leaf oil of *L. camara* showed strong antibacterial activity (Saikia and Sahoo, 2011). The plant had antimycobacterial (Beguma *et al.,* 2008), antifungal (Sonibare and Effiong, 2008) and nematicidal effect (Qamar *et al.,* 2005). The gum exudate of *M. sinclairii* is composes mainly of polysaccharides. The extracts of the bark and leaves of *E. humeana* have been reported to have an antibacterial activity (Pillay *et al.,* 2001). The extracts have revealed the presence of terpenes in (Nkengfack *et al.,* 1994), a bioactive alkaloid-rich plants (Barakat *et al.,* 1977) and isoflavones, pterocarpanes, flavanones and isoflavanones (Chacha *et al.,* 2005).

CONCLUSIONS

The extracts from the bark, fruits and leaves of *E. humeana*, *C. sempervirens* and *F. retusa* had the highest effect against the growth of *M. luteus*. The highest effects against the growth of *S. aureus* were shown by the extracts from *L. camara* (leaves), *S. cumini* (leaves) and *S. terebinthifolius* (fruits). The growth of *A. baumannii* was affected the extracts of *C. sempervirens* (leaves), *S. cumini* (leaves) and *F. benghalensis* (fruits). The extracts of *F. retusa* (fruits), *L. camara* (leaves) and *M. sinclairii* (wood gum exudate) were showed good activity against the growth of *P. aeruginosa*. The present results could be useful for further work related to the bioactivity agents for controlling the infectious diseases caused by human bacterial pathogens.

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