JOURNAL OF PURE AND APPLIED MICROBIOLOGY, May 2014. Vol. **8**(Spl. Edn. 1), p. 105-109

Evaluation of Extracts from Leaves of *Brachychiton diversifolius* R.Br against the Growth of Some Clinical Pathogens

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(Received: 08 January 2014; accepted: 24 March 2014)

In the present study the acetone, methanol, chloroform, ethanol and distilled water extracts of leaves from *Brachychiton diversifolius* R.Br were evaluated for their antibacterial activity against four clinical pathogens namely; *Micrococcus luteus*, *Staphylococcus aureus*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. The inhibition zones (IZs) were ranged between 7-17 mm at a concentration of 2000 μg/mL. Methanol and ethanol extracts showed good activity against the growth of *M. luteus* (IZ value of 17 mm). Chloroform extract presented moderate activity against *S. aureus* (IZ value of 8 mm). The methanol extract presented good activity against *A. baumannii* (IZ value of 15 mm) followed by the chloroform extract (IZ value of 12 mm). Furthermore, the distilled water extract was showed a selectively active against the growth of *P. aeruginosa* with IZ value of 10 mm, while the other extracts did not show any activity. The present results could be promise a basis for further work related to the bioactivity agents from *B. diversifolius* against the growth of human bacterial pathogens.

Key words: *Brachychiton diversifolius R.Br*; Extracts; Antibacterial activity; Clinical pathogens.

Brachychiton diversifolius R.Br., belongs to Family Sterculiaceae and now within Malvaceae, is originated in Northern Australia (Brock 2001). Seeds of *B. diuersifolius* are indigenous food eaten by Australian Aborigines (James and Forbes-Ewan, 1982). The extracts of different parts from *B. diversifolius* were previously evaluated against some laboratory bacterial and fungal pathogens and it was suggested that *B. diversifolius* is a great potential source of antibacterial and antioxidant compounds useful for pharmaceutical and plant health application (Abdel-Megeed *et al*., 2013). Also, the essential oils from the seeds of different species including *B. diversifolius* were reported to have some compounds as such, α-pinene, β-pinene, linalool, hexadecanol (Rao *et al*. 1989). Furthermore, fatty acids like malvalic acid of *B. diversifolius* seeds were present in higher amounts than sterculic acid, and dihydrosterculic acid (Rao *et al*. 1989). Cyclopropene fatty acids were presented in seed oils of families of Sterculiaceae, Malvaceae, and Bombacaceae, which have been shown to own good biological effects in animal feeding trials (Lee *et al*. 1971; Smith 1970; Phelps *et al*. 1965). Different extracts of wood branches were shown good activity against some pathogenic

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bacteria were ranged from 7±0.7 to 17±1.4 mm (Abdel-Megeed *et al.* 2013; Ngomdir *et al*. 2007). There are many variations in the susceptibility of bacteria to extracts according to strains and species in the basis of inhibition zones (Karou *et al.*, 2006).

The cultivated medicinal trees in Egypt were received good attention to the knowledge in the field of medicinal plants (Aly *et al.*, 2012). The reports about the antimicrobial within timber trees have become valuable and a very good tool and with possibly novel mechanisms of action, since the microorganisms have been observed resistance for many commercial antibiotics. Therapy of bacterial infections is a frequent problem due to the emergence of bacterial strains resistant to numerous antibiotics (Cowan, 1999). *S. aureus* were reported to cause of hospital-acquired infections (Richards *et al.*, 1999) and is difficult to treat because of evolved resistance to antimicrobial drugs (Klein *et al.*, 2007). *P. aeruginosa* causes chronic obstructive pulmonary disease (Adonizio *et al.*, 2008). *M. luteus* is not a severe pathogenic bacterium and it has served as the model system for bacterial cell wall study (Deng *et al.*, 2010). *Acinetobacter* cause suppurative infection in any organ or tissue and any human body (Urban *et al.*, 2003). In the present study, different solvents extract of leaves from *Brachychiton diversifolius* R.Br were evaluated for their antibacterial against four clinical pathogens namely; *Micrococcus luteus*, *Staphylococcus aureus*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Plant material

Leaves of *Brachychiton diversifolius* were collected in August 2013 from Antoniadis Garden, Horticultural Research Institute, Alexandria, Egypt. The plant was kindly identified and at the Department of Forestry and Wood Technology, Faculty of Agriculture, Alexandria University. The leaves were air-dried at room temperature and pulverized into powder (40-60 mesh) with small laboratory mill and stored in paper bags until uses.

Preparation of the extracts

The ground leaves were divided into five groups (each group contained 100 g). Subsequently, each group was soaked with 200 mL of acetone, methanol, chloroform, ethanol and distilled water. After one week of soaking, the solutions were passed through activated charcoal to remove the chlorophyll and filtrated (Salem *et al.*, 2012). The crude extracts (acetone, methanol, chloroform, ethanol and distilled water) 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 99.5% Dimethylsulfoxide (DMSO, Sigma-Aldrich) and distilled water (1:1 v/v).

Antibacterial activity

To evaluate the activity of extracts from leaves of *B. diversifolius*, the human pathogenic bacteria; *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter baumannii* and *Micrococcus luteus* were used. 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.

Kirby-Bauer disc diffusion method

The Kirby-Bauer disc diffusion susceptibility test method was used for the antibacterial activity (NCCLS, 1997). Filter paper discs (5 mm in diameter) were suspended with 20 µL of the 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

The extracts were quantified by weight from acetone, methanol, chloroform, ethanol and distilled water and were presented the following quantities; 4.30, 8.23, 4.50, 8.14 and 6.42 g/100 dry leaves of *B. diversifolius*, respectively. In the present study, as a preliminary evaluation for further studies, the antibacterial activities of different solvent extracts of *B. diversifolius* leaves were measured by the diameters of the inhibition zones of the growth of some human pathogenic

Table 1. Antibacterial activity of different extracts from leaves of *Brachychiton diversifolius* against the growth of some pathogenic bacteria

Extract	Inhibition zones (mm)			
	M. luteus	*S. aureus*	*A. baumannii*	*P. aeruginosa*
Acetone	10	7	na	na
Methanol	17	na	15	na
Chloroform	11	8	12	7
Ethanol	17	na	na	na
Distilled water	na	na	na	10
Tetracycline *	22	20	21	25
DMSO	na	na	na	na

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

bacteria at a concentration of 2000 µg/mL (Table 1).

In accordance to results presented in Table 1, acetone extract showed moderate activity against *M. luteus* (IZ value 10 mm), weak active against *S. aureus* (IZ value 7 mm) and did not show any activity against the growth of *A. baumannii* and *P. aeruginosa*. Methanol extract was presented high antibacterial activity against *M. luteus* (IZ value 17 mm) and *A. baumannii* (IZ value 15 mm) and did not show any activity against the growth of *P. aeruginosa*, *S. aureus*. Chloroform extracts are presented activities against all the four studied bacterial strains with different degrees. The observed zones of inhibitions were 11 mm, 8 mm, 12 mm 7 mm, were recorded against the growth of *M. luteus*, *S. aureus*, *A. baumannii* and *P. aeruginosa*, respectively. Ethanol extract was presented high and selectively antibacterial activity against *M. luteus* (IZ value 17 mm) and did not show any activity against the growth of *S. aureus*, *A. baumannii* and *P. aeruginosa*. Distilled water showed moderate and selectively antibacterial activity against *P. aeruginosa* (IZ value 10 mm) and did not show any activity against the growth of *M. luteus*, *S. aureus* and *A. baumannii*.

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 (Aly *et al.*, 2012). Additionally, the most antibacterial medicinal plants are more effective against gram-positive than gram-negative bacteria (Scrinivasan *et al.*, 2001).

The search for new drugs from natural products as extracted from the higher plants (plants) for controlling the infectious diseases caused by clinical pathogen could be partially achieved (Aly *et al.*, 2012). The most important of these bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds (Cowan, 1999). Abdel-Megeed *et al*. (2013) reported that the different fractions of wood extracts have been shown a high amount of flavonoids and tannins.

In the present study the chloroform extract have been shown moderate activity against the growth of *M. luteus*, *S. aureus*, *A. baumannii* and *P. aeruginosa*. Previously, the chloroform fraction or extracts is composed mainly in the alkaloids compounds (Aly *et al.*, 2012; Harborne, 1973). Additionally, the precipitated alkaloids from *Conocarpus lancifolius* have antimicrobial and antioxidant activities (Ali *et al.*, 2013; Salem *et al.*, 2013; Erdemoglu *et al.*, 2007). The toxicity of phenolic compounds includes enzyme inhibition by the oxidized compounds, which could be presented in the methanol and ethanol extracts, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Roedig-Penman and Gordon 1998; Balandrin *et al.*, 1987; Mason and Wasserman, 1987). Tsuchiya, (1996) reported that the Gram-negative bacteria had less sensitivity to plant extracts possibly as a result of their extra

lipopolysaccharide and protein cell wall that provides a permeability barrier to the antibacterial agent. The Gram-positive bacteria are more sensitive to the extracts because of the single layer of their cell wall (Kaur and Arora, 2009). Flavonoids have been reported to form complex with extracellular, soluble proteins and bacterial cell walls and possess the antibacterial activity (Kaur and Arora, 2009; Meli *et al.*, 1990) and the alkaloids extracts are used as an antibacterial activity (Evan, 2002). Tannins were reported to have good antimicrobial activities (Ojo *et al.,* 2007). Additionally, in the present study the distilled water showed only activity against *P. aeruginosa*, and previously the aqueous fraction exhibited no activity against the tested bacteria (Salem *et al.,* 2013; Ahmadv and Sariri, 2008; Parekh and Chanda, 2007).

By using the crude extracts from leaves of *B. diversifolius* as an antibacterial material could be of great value for pharmaceutical industry. But indeed further work should be done to know the main chemical compounds responsible for the activity and its value for human and plant health.

CONCLUSIONS

In the present study the different solvent extracts namely; acetone, methanol, chloroform, ethanol and distilled water extracts of leaves from *Brachychiton diversifolius* R.Br were evaluated for their antibacterial against four clinical pathogens namely; *Micrococcus luteus*, *Staphylococcus aureus, Acinetobacter baumannii* and *Pseudomonas aeruginosa*. The inhibition zones were ranged between 7-17 mm at a concentration of 2000 µg/mL. Methanol and ethanol extracts showed good activity against the growth of *M. luteus*. Chloroform extract presented moderate activity against *S. aureus*. The methanol extract presented good activity against *A. baumannii* followed by the chloroform extract. The distilled water extract was showed a selectively active against the growth of *P. aeruginosa*, while the other extracts did not show any activity. The present results could be promise a basis for further work related to the bioactivity agents from *Brachychiton diversifolius* against the growth of human bacterial pathogens.

ACKNOWLEDGMENTS

This project was supported by King Saud University, Deanship of Scientific Research, College of Science Research Center.

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