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Brachychiton diversifolius as a Source of Natural Products: Antibacterial and Antioxidant Evaluation of Extracts of Wood Branches

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In this study, different extracts of wood branches of *Brachychiton diversifolius* were evaluated for their antibacterial and antioxidant activities with respect to the total phenolic and flavonoid contents. The antibacterial activity was assayed against the growth of some plant and human pathogenic bacteria using the agar disc-diffusion and minimum inhibitory concentrations methods The antioxidant activity was measured by 1,1,-diphenyl-2-picryl-hydrazyl (DPPH) method. The different extracts of *B. diversifolius* are obtained by successive solvent extraction with methanol and its fractions; ethyl acetate, chloroform, *n*-butanol and aqueous. The methanol and ethyl acetate extracts showed significant effective against the tested bacteria. The wood branches methanol extract exhibited the highest amount of total phenolics (40.3±3.00 mg Tannin acid equivalents/ g extract) and flavonoids (30.76±2.12 mg Catechin equivalents/g extract) and the highest total antioxidant activity (%) with 85.6±2.22%. It can be suggested that *B. diversifolius* is a great potential source of antibacterial and antioxidant compounds useful for pharmaceutical and plant health application. These findings provide scientific evidence to support the traditional biocide uses of these extracts and indicate a promising potential of these plants for agricultural purposes as antimicrobial agents. Thus they can be used in the treatment of infectious diseases caused by pathogenic bacteria. Further *in vivo* studies are necessary to substantiate our findings. More importantly there is need for detailed scientific study of traditional practices to ensure that valuable mode of action knowledge for controlling these pathogenic bacteria to provide scientific evidence for their efficacy.

Key words: *Brachychiton diversifolius*, wood branches, antioxidant activity, plant pathogens, antibacterial activity, total phenolics, total flavonoids.

Higher plants are renewable sources of antimicrobial agents and medicinal compounds which play a dominant role in maintenance of human and plant health since antiquities and an important role in drug development programs of the pharmaceutical industry and integrated pest management (IPM) (Baker *et al*., 1995; Stuffness and Douros, 1982).

Brachychiton diversifolius R.Br., is found in northern Australia and originally classified in the family Sterculiaceae, which is now within

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Malvaceae (Brock, 2001). The essential oils of *B. discolor*, *B. diversifolius*, and *B. acerifolius,* species of the Sterculiaceae family, have some compounds as such, ±-pinene, ±-pinene, linalool, hexadecanol (Rao *et al*. 1989). Cyclopropene fatty acids (CPFA) are constituents of seed oils in the botanical families Sterculiaceae, Malvaceae, and Bombacaceae (Smith, 1970), which have been shown in animal feeding trials to have adverse biological effects (Phelps *et al*., 1965; Lee *et al*., 1971). However, seeds of one *Brachychiton* species, *B. diversifolius*, are indigenous food eaten by Australian Aborigines (James and Forbes-Ewan, 1982). Fatty acid and amino acid compositions of *B. diversifolius*, *B. discolor*, and *B. acerifolius* seeds were observed (Rao *et al*. 1989). Malvalic acid was present in greater amounts than sterculic acid, an unexpected finding since *Brachychiton* species being members of the family Sterculiaceae may be expected to have more sterculic than malvalic acid. Dihydrosterculic acid was found only in very small amounts (0.3-0.7%). The presence of CPFA in these three seeds is consistent with their general distribution in Malvaceae Bombacaceae, and Sterculaceae families (Smith, 1970).

In the present work the methanol extract and its fraction in different solvents of wood branches from *B. diversifolius* was evaluated for its antibacterial and antioxidant activities against some plant and human pathogenic bacteria which cause a high level of wilting in potato and dianthus crops.

MATERIALS AND METHODS

Preparation of extracts

Wood branches (8 cm in diameter) of *B. diversifolius* were collected in August 2012 from Antoniadis Garden, Horticultural Research Institute, Alexandria, Egypt. Wood branches were debarked and air-dried at room temperature for one week. The dried wood were ground and sieved at approximately 0.2–0.4 mm and extracted by soaking of 100g by 150 mL of 90% methanol. The methanol extract (MeOH extract) resulted after filtration was concentrated to dryness under reduced pressure at 45°C with a rotary evaporator, lyophilized and stored at 4°C until further use. The MeOH extract was fractionated by successive solvent extraction with ethyl acetate (EtOAc fraction), chloroform ($CHCl_3$ fraction) and *n*-butanol (*n*-BuOH fraction) saturated with water (Aq fraction) using funnel separator (Salem *et al*. 2013a). The lyophilized extracts were subjected for phytoconstituents using standard methods by dissolving it in specific reagents (Harborne, 2005).

Determination of total phenolics

The total phenolics content was determined with the Folin-Ciocalteau assay as described by Marinova *et al*. (2005) with minor modification. Where, 1 mL of the MeOH extract and EtOAc, $CHCl_3$, *n*-BuOH and Aq and the standard solution of Tannic acid (20, 40, 60, 80 and 100 mg/L) was added to 9 mL of distilled deionized water (dd H_2O). Folin-Ciocalteu's phenol reagent (1 mL) was added to the mixture and shaken and 10 mL of 7% Na_2CO_3 solution was added to the mixture after 5 min. The solution was diluted to 25 mL with dd H_2O and mixed and the absorbance against the prepared reagent blank was determined at 750 nm with a UV scanning spectrophotometer after incubation for 90 min at room temperature. The results were expressed as milligrams of Tannic acid equivalents (TAE) per gram extract (mg TAE/g dry extract) (Salem *et al*. 2013b).

Determination of total flavonoids

The aluminum chloride colorimetric assay (Marinova *et al*. 2005) was used to measure the total flavonoids content. Briefly, 1 mL of extracts or a standard solution of (+)-catechin (20, 40, 60, 80 and 100 mg/L) was added to 4 ml of dd H_2O and 0.3 mL of 5% $NaNO_2$ was added and after 5 min, 0.3 ml of 10% $AlCl_3$ was added. 2 mL of 1 M NaOH was added and the total volume was made up to 10 mL with dd H_2O at the sixth minute. The absorbance was measured at 510nm with a UV scanning spectrophotometer. The results were expressed as milligrams of (+)-catechin equivalents (CE) per gram extract (mg CE/ g dry extract).

DPPH radical-scavenging assay

Free radical scavenging activity of the samples was determined using the 1,1,-diphenyl-2-picryl-hydrazyl (DPPH) method (Elansary *et al*. 2012). Two mL of 0.1 mM DPPH (Sigma-Aldrich) reagent were dissolved in pure methanol and added to a test tube with 2 mL of the sample solution in methanol (200 µg/L) and the reaction mixture was shaken vigorously for 10 s. After incubation at room temperature (28±2°C) for 30 min, the absorbance (A) of DPPH for control and samples

was measured at 517 nm, using a UV scanning spectrophotometer. Total antioxidant activity (TAA %) was expressed as the percentage inhibition of the DPPH radical and was determined by the following equation:

$$TAA\,(\%) = (A_0 - A_s / A_0) \times 100$$

where TAA is the total antioxidant activity, A_0 is the absorbance of DPPH solution in methanol and A_s is the absorbance of a DPPH solution with a tested fractions solution (test) or Tannic acid (positive control) solution.

Antibacterial activity test

The antibacterial activity was carried out on the extracts with concentration of 2000 µg/mL against the Gram positive bacteria; *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 14579, *Micrococcus luteus* ATCC 4698, *Sarcina lutea* ATCC 9341 and *Staphylococcus aureus* ATCC 6538 and the Gram negative bacteria; *Escherichia coli* ATCC 8739, *Serratia marcescens* ATCC 13880, *Salmonella typhi* ATCC 6229, *Proteus vulgaris* ATCC 6509 and *Pseudomonas aeruginosa* ATCC 9027. The previous plant and human pathogenic bacteria strains were provided from the Botany Department, Microbiology Section, Faculty of Science, Alexandria University, Egypt.

The plant pathogens bacteria (Salem, 2013), namely *Dickeya dianthicola* (host; Dianthus), *Pectobacterium carotovorum* subsp. *Wasabiae* (host; Potato), *Pectobacterium carotovorum* subsp. *Carotovorum* (host; Potato), *Pectobacterium carotovorum* subsp. *Atrosepticum* (host; Potato) and *Dickeya chrysanthemi* (host; Potato), were provided from the Laboratory of Plant Pathology Department, Faculty of Agriculture, Alexandria University, Egypt. Nutrient agar (NA) medium was used for maintenance of the tested bacterial organisms. Mueller Hinton agar (MHA) was used in all bioassays.

Disc diffusion method assay and minimum inhibitory concentrations

The agar disc-diffusion method was used for the determination of antibacterial activities of the extracts (NCCLS, 1997). A suspension of the bacteria (0.1 mL of 10^8 cells/mL) was put on the solid media plates. Filter paper discs with 5 mm in diameter were loaded with 20 µL of the extract and placed on the inoculated plates. The plates were incubated at 37°C for 24 h. The diameters of the inhibition zones (IZs) were measured in millimeters. Negative control was prepared using respective solvent. Minimum inhibitory concentrations (MICs) were determined by serial dilution of extracts (100, 250, 500, 1000, 2000 and 4000 µg/mL) (Eloff, 1988).

Statistical analysis

The results of antibacterial activity, total phenolic, total flavonoids and DPPH radical scavenging activity were expressed as mean values ± standard deviation (SD).

RESULTS AND DISCUSSION

Phytochemical constituents of extracts

The wood branches MeOH extract of *B. diversifolius* yielded 20% based on oven dry weight of the wood branches. The percentage quantities of *B. diversifolius* methanolic extracts with different solvents were EtOAc (5.16%), *n*-butanol (10.37%), $CHCl_3$ (5%) and aqueous with 13%. The phytochemical screenings of *B. diversifolius* wood branches (Table 1) were observed that the extracts contain tannins, flavonoids, saponins, phenolics, steroids and traces of alkaloids.

Total phenolic and flavonoid contents and antioxidant activity of extracts

The measurements of total phenolics, total flavonoids and antioxidant activity of *B. diversifolius* wood branched extracts are presented in Table 2. The amount estimated in the MeOH extract and EtOAc, $CHCl_3$, *n*-BuOH, and Aq fractions had 40.3±3.00, 60.1±3.50, 20±1.67, 14.32±1.2 and 10.21±1.1 mg TAE/g extract, respectively.

Total flavonoids amount found in wood branched extracts were 30.76±2.12, 27.34±2.06, 5.90±1.89, 12.80±1.33 and 17.50±1.36 mg CE/g extract with MeOH extract and EtOAc, $CHCl_3$, *n*-BuOH, and Aq fractions, respectively. The TAA % was 85.6±2.22, 80.15±1.12, 20.90±1.13, 10.50±1.40 and 5.50±1.14% for MeOH extract and EtOAc, $CHCl_3$, *n*-BuOH, and Aq fractions, respectively. The methanloic extract is nearly equivalent to the antioxidant activity of TA (90±5.12%).

Flavanoids have been reported to own the ability to form complex with extracellular, soluble proteins and bacterial cell walls and possess the antibacterial activity (Kaur and Arora,

2009; Lin *et al*., 2008; Meli *et al*. 1990) and the purified alkaloids are used as bactericidal activity (Evan, 2002). The higher plants have alkaloids and flavonoids which control the growth of microbial pathogen. The toxicity of phenolic compounds includes enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Roedig-Penman and Gordon 1998; Mason and Wasserman, 1987; Balandrin *et al*., 1987).

In general, the Gram-negative bacteria have shown 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 (Tsuchiya, 1996). Furthermore, the Gram-positive bacteria are more sensitive to the extracts because of the single layer of their cell wall while the double membrane of Gram-negative bacteria should make them less sensitive (Kaur and Arora, 2009). Tannins also were shown good antimicrobial activities (Ojo *et al*., 2007).

Antibacterial activity of wood branches extracts

The *in vitro* antibacterial activity of different solvent extracts of wood branches from *B. diversifolius* at a concentration of 2000 µg/mL and the MICs values were given in Table 3. The inhibition zones of MeOH extract were highest against the growth of *S. lutea* and *P. aeruginosa* (15±1.5 and 15±1.2 mm) with MICs of 500 and < 250 µg/mL, respectively, and the *P. vulgaris* showed resistance to the extract at 2000 µg/mL. The inhibition zones of EtOAc fraction ranged between 17±0.4 mm to 6±0.7 mm showed and the highest activity against *B. subtilis* (17±0.4 mm) with MIC of 500 µg/mL. On the other hand, the *M. luteus, S. aureus and P. aeruginosa* were shown resistance to the extract at 2000 µg/mL level of concentration. Additionally, the EtOAc fraction of *B. diversifolius*

Table 1. Phytochemical analysis of extracts from *B. diversifolius* wood branches.

Extract	Tannins	Flavonoids	Alkaloids	Saponins	Phenols	Steroids
MeOH	+++	+++	+	+	+++	++
EtOAc	++	+++	-	-	++	-
$CHCl_3$	++	+++	+	+	-	++
n-BuOH	+	+	-	++	+	+
Aq	+	+	-	+	+	-

+++ Strong; ++ medium; + poor; - absence. MeOH- methanol crude extract; EtOAc- ethyl acetate fraction; $CHCl_3$-chloroform fraction; *n*-BuOH- *n*-butanol fraction; Aq- aqueous fraction. The measurements were repeated three times and the classification based on the color intensity of the precipitate.

Table 2: Total phenolics and flavonoids contents and antioxidant activity of different extracts from *B. diversifolius* wood branches

Extract	Total phenolic (mg TAE/g extract)	Total flavonoids (mg CE/g extract)	TAA%
MeOH	40.3±3.00	30.76±2.12	85.6±2.22
EtOAc	60.1±3.50	27.34±2.06	80.15±1.12
$CHCl_3$	20±1.67	5.90±1.89	20.90±1.13
n-BuOH	14.32±1.2	12.80±1.33	10.50±1.40
Aq	10.21±1.1	17.50±1.36	5.50±1.14
TA			(90±5.12)

All values are mean±SD of three replicates; TAA%: Total antioxidant activity; TAE: Tannin acid equivalents; CE: (+)-catechin equivalents; TA: Tannin acid; MeOH- methanol crude extract; EtOAc- ethyl acetate fraction; $CHCl_3$- chloroform fraction; *n*-BuOH- *n*-butanol fraction; Aq- aqueous fraction

Table 3. Antibacterial activity of extracts from *B. diversifolius wood branches* using agar-disc diffusion and minimum inhibitory concentration assays

Bacterial strain	IZ (mm), MIC (µg/mL)											
	MeOH extract		EtOAc		$CHCl_3$		*n*-BuOH		Aq		Negative control	Positive control*
	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC		
Gram-positive												
B. subtilis	10±1.1	500	17±0.4	500	12±0.6	1500	8±0.5	1000	R	>5000	R	18
B. cereus	12±1.8	500	12±1.2	250	R	>5000	R	>5000	R	>5000	R	17
M. luteus	12±1.3	500	R	>5000	7±0.7	1500	R	>5000	R	>5000	R	19
S. lutea	15±1.5	500	15±1.3	< 250	17±1.4	< 250	R	>5000	R	>5000	R	20
S. aureus	10±0.2	1000	R	>5000	7±0.9	500	7±0.7	500	R	>5000	R	23
Gram-negative												
E. coli	12±1.8	< 250	15±1.2	< 250	R	>5000	12±0.9	< 250	R	>5000	R	18
S. marcescens	12±1.2	1500	12±1.3	1000	R	>5000	R	>5000	R	>5000	R	20
S. typhii	10±0.8	1000	6±0.7	1000	8±0.6	1500	R	>5000	R	>5000	R	20
P. vulgaris	R	>5000	12±1.2	1500	R	>5000	13±1.7	1000	R	>5000	R	23
P. aeruginosa	15±1.2	< 250	R	>5000	8±1.0	1000	13±0.6	500	R	>5000	R	22

MeOH- methanol crude extract; EtOAc- ethyl acetate fraction; $CHCl_3$- chloroform fraction; *n*-BuOH- *n*-butanol fraction; Aq- aqueous fraction; IZ: Diameter of inhibition zone (mm)±SD including disc diameter of 5 mm at 2000 µg/mL for three replicates.
MIC: Minimum inhibitory concentration; values given as µg/mL.
R: Resistance at 2000 µg/mL.
*: Tetracycline (20 µg/disc) (Salem *et al.* 2013b).

wood branches showed good activity against the other studied bacteria especially the Gram-negative bacteria. The inhibition zones of $CHCl_3$ fraction ranged between 17±1.4 mm (*S. lutea* with MIC of < 250 µg/mL) and 7±0.7 mm (*M. luteus* with MIC of 1500 µg/mL) where the *B. cereus, E. coli, S. marcescens* and *P. vulgaris* were resistance to the extract at a concentration of 2000 µg/mL. Most of the studied bacteria (*B. cereus*, *M. luteus*, *S. lutea*, *S. marcescens* and *S. typhii*) were observed a resistance the *n*-BuOH fraction at a concentration of 2000 µg/mL. The highest was observed against *P. aeruginosa* (Inhibition zone 15±0.6 mm with MIC value of 500 µg/mL) and the lowest against *S. aureus* with inhibition zone 7±0.7 mm and MIC of 500 µg/mL (Ngomdir *et al*., 2007). On the other hand, all the bacterial strains under this study were shown a resitsnce to the Aq fraction at a concentration of 2000 µg/mL. The inhibition zones were found to be above the value of 15 mm were most effective.

The results of the antibacterial activity of extracts against the growth of the previous human pathogenic bacteria revealed that MeOH extract and EtOAc fraction showed good antibacterial activity against the tested bacteria. The *n*-BuOH and $CHCl_3$ fractions showed weakly activity against the tested bacterial strains. On the other hand, the Aq fraction exhibited no activity against the tested bacteria (Salem *et al*., 2013 a,b; Parekh and Chanda, 2007; Ahmadvand and Sariri, 2008).

Table 4 presents the antibacterial activities of different extracts of *B. diversifolius* wood branches against the growth of some plant pathogenic bacteria at 4000 mg/L. The zones of inhibition were ranged between 16-6 mm. The most

Table 4. Antibacterial activity of different extracts of *B. diversifolius* wood branches against the growth of some plant pathogenic bacteria

Pathogenic bacteria	Inhibition zone (mm)[1] Extract concentration (mg/L)					DMSO
	MeOH	EtOAc	$CHCl_3$	*n*-BuOH	Aq	
P. carotovorum subsp. *wasabiae*	13±1.2	16±0.67	7±0.4	R	R	n.a.
P. carotovorum subsp. *carotovorum*	13±1.5	13±0.8	7±0.4	R	R	n.a.
P. carotovorum subsp. *atrosepticum*	12±0.9	10±0.8	6±0.2	13±1.3	R	n.a.
D. dianthicola	8±0.7	12±1.02	6±0.3	R	R	n.a.
D. chrysanthemi	R	R	R	R	R	n.a.

[1]: Diameter of inhibition zone (mm) including disc diameter of 5 mm; R: Resistance; n.a. Not active; DMSO: Dimethyl sulfoxide $[(CH_3)_2SO]$ as a negative control; Inhibition > 15 mm (strong inhibition), 15 – 10 mm (moderate), and <10 mm (weak).

inhibition was observed against the growth of *P. carotovorum subsp. wasabiae* (16±0.67 mm from EtOAc fraction). The resistance bacterium was *D. chrysanthemi* to all the studied extracts at 4000 mg/L. The results showed that the MeOH extract and EtOAc fraction of wood branches from *B. diversifolius* had the highest antibacterial activity against the studied bacteria.

The $CHCl_3$ fraction showed a weak activity against the growth of the studied bacterial strains and these results in agree with the previous report (Pillay *et al*. 2001) against the growth of other bacterial strains. No inhibition was observed in the case of *n*-BuOH and Aq fractions as well as the negative control (DMSO). It could be concluded that the alcoholic extract showed the highest antibacterial activities (Dhanaraj *et al*., 2012).

CONCLUSION

The results revealed that the wood branches of *B. diversifolius* had moderate antibacterial and antioxidant activities. Since it is the first report about the effect of extracts from *B. diversifolius* and against the growth plant pathogens (*Dickeya dianthicola*, *Pectobacterium carotovorum* subsp. *wasabiae*, *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium carotovorum* subsp.

atrosepticum and *Dickeya chrysanthemi*) which cause a high level of wilting in potato and dianthus crops, the methanolic extracts and EtOAc fraction observed an remarkable effect against the growth of these bacteria. The extracts also showed good antibacterial activities against the growth of some plant and human pathogenic bacteria strains. The results could be helpful for extraction the new natural chemical compounds which will be useful against the plant pathogens and human bacteria. More importantly there is need for detailed scientific study of traditional practices to ensure that valuable mode of action knowledge for controlling these pathogenic bacteria to provide scientific evidence for their efficacy.

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