Evaluation of the Antibacterial and Antioxidant Activities of Stem Bark Extracts of Delonix Regia and Erythrina Humeana Grown in Egypt

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(Received: February 01, 2013; Accepted: February 23, 2013)

Abstract— Antibacterial and antioxidant activities of the methanolic extracts from Delonix regia and Erythrina humeana barks were evaluated in the present study. Different concentrations of methanol extract were tested for the antibacterial activity by using the disc diffusion and minimum inhibitory concentration (MIC) methods. The antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The methanolic stem bark extract of D. regia and E. humeana have a moderate antibacterial activity. The inhibition zones of the stem bark methanolic extracts of D. regia and E. humeana were ranged between 6-15 mm and 6-12 mm, respectively. The total antioxidant activity (TAA %) of bark extract was 78.35±1.45 % and 73.40±2.67 % and these values are close to the TAA % found in Gallic acid (80±2.12 %). The extracts of D. regia and E. humeana stem bark may be valuable for functional applications against bacteria causing a high level of wilting in potato and dianthus crops.

Index Terms—Antioxidant activity, Antibacterial activity, Bark extract, Delonix regia, Erythrina humeana

I. INTRODUCTION

Higher plants, as sources of medicinal compounds continue to play a dominant role in maintenance of human health since antiquities. 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 [1,2]. Delonix regia (Boj. ex Hook.) Raf. (syn. Poinciana regia Bojer ex. Hook) is a flamboyant tree, planted as an avenue tree in gardens and also on roadsides throughout Egypt. D. regia is reported to contain rich content of flavonoids like kaempferal exhibit antiulcer effect [3]. Quercetin; a flavonoid has been reported to lengthen the small intestinal transit time in the gastrointestinal system [4]. The literature survey reveals that D. regia bark contains β-sitosterol, saponins, alkaloids, carotene, hydrocarbons phytotoxins and flavonoids [5,6].

Phenolic compounds with high concentration found in the bark and flower and they are sufficiently distributed in leaves [6]. The flowers of D. regia have broad spectrum anti-bacterial and anti-fungal activity [7]. Bark and flower showed significant analgesic and anti-inflammatory activity [8]. Five compounds, lupeol, epilupeol, β-sitosterol, stigmasterol and p-methoxybenzaldehyde were isolated from the petroleum ether and dichloromethane fractions of a methanolic extract of the stem bark of D. regia [9]. The zones of inhibition demonstrated by the petroleum ether, carbon tetrachloride and dichloromethane fractions ranged from 9-14 mm, 11-13 mm and 9-20 mm, respectively, compared to kanamycin as a standard antibiotic with zones of inhibition ranged between 20 mm and 25 mm [9].

Gallic acid in bark as the main phenolic acids was found in an amount > 1.50 mg/100 g of dry weight (DW). Besides, small amounts (< 1.50 mg/100 g DW) of some other phenolic acids such as sorbic, sinapic, p-coumaric, m-coumaric, ferulic, caffeic, 3-hydroxybenzoic, 4-hydroxycinnamic and 4-hydroxybenzoic acids were also detected [10]. The extract yields from leaves, flowers and bark ranged from 10.19 to 36.24, 12.97 to 48.47 and 4.22 to 8.48 g/100 g dry weight (DW), respectively [10]. Other results showed significant antibacterial activity at the concentration of 200 mg/ml. The pharamcognistical studies like total ash, acid insoluble ash, water soluble ash was found 8.5%, 1.6%, 2.8%, respectively [11]. Plant extract exhibited antibacterial, anti-malarial and anti-fungal properties [12].

The genus Erythrina includes approximately 120 species which occur in tropical and subtropical regions. The name coral tree which is frequently used for these plants refers to the red seeds and the brilliant red flowers produced [13]. The traditional usage indicates that Erythrina species could have analgesic, anti-inflammatory and antibacterial activity [13]. Erythrina humeana Spreng is an ornamental tree and medicinal plant native to South Africa, and the roots have been used for sprains, tuberculosis and bronchitis [14]. The bark and leaves extracts of have been reported to have an antibacterial activity [13]. Phytochemical analysis has revealed the presence of terpenes in plants from the Erythrina genus [15], which are also recognized as bioactive alkaloid-rich plants [16] and flavonoids, especially, isoflavones, pterocarpanes, flavanones and isoflavanones [17].
In the present work the methanol extract of barks from *D. regia* and *E. humeana* was evaluated for its antibacterial and antioxidant activities against some potential bacteria causing a high level of wilting in potato and dianthus crops.

II. MATERIALS AND METHODS

**Plant material**
The bark tissues of *D. regia* and *E. humeana* belonging to family Fabaceae were collected from Antoniadis Garden, Horticultural Research Institute, Alexandria, Egypt, during the month of January 2013. The plants were kindly identified and obtained voucher number at Egypt barcode of life project, Faculty of Agriculture, Alexandria University.

**Extraction of plant material**
The bark materials were shade air-dried at room temperature for one week and pulverized. About 50 g of the air-dried bark from each tow species were macerated in 80% methanol (1 l) for 72 h under room temperature [18]. The extracts were filtered, concentrated, lyophilized and stored in a dark vials at 4°C for further use. The crude extract was dried and weighted.

**Source of test organisms and growth conditions**
The pure cultures of test bacteria, namely *Dickeya dianthicola, Pectobacterium carotovorum* subsp. *wasabiae, Pectobacterium carotovorum* subsp. *carotovorum, Pectobacterium carotovorum* subsp. *atrosepticum* and *Dickeya chrysanthemi* were obtained from the Laboratory of Plant Pathology Department, Faculty of Agriculture, Alexandria University, Egypt. Table 1 presents the types of the bacteria used in this study. 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.

**Antibacterial Activity**
Antibacterial assay of bark extracts was carried out at a concentration of 1000 µg/ml by using the Kirby-Bauer disc diffusion susceptibility test [19]. A suspension of the tested bacteria [1 ml of 10³ colony forming units (CFU/ml)] was spread on the surface of solid media plates. Filter paper discs (4 mm in diameter) were soaked with 20 µl of the tested extract.

The sterile impregnated disc with plant extract were placed on the agar surface with flamed forceps and gently pressed down to ensure complete contact of the disc with the agar surface. The plates were incubated at 37°C for 24 h. After the incubation period the inhibition zones around the discs were measured and recorded. The inhibition zones obtained were compared with the positive control (25 µl of tetracycline 10 µg/disc). For the negative control, discs were saturated with 20 µl of dimethyl sulfoxide (DMSO, Sigma-Aldrich) solution [20]. The results are expressed as mean values ± standard deviation (SD).

**Antioxidant activity**
Antioxidant activity of extract was done using the 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich) method [21]. An aliquot of 2 ml of stock solution of 0.1 mM DPPH reagent dissolved in pure methanol was added to a test tube with 2 ml of the sample solution in methanol (200 µg/l). The reaction mixture was mixed for 10 s and left to stand in fibre box at room temperature in the dark for 30 min. The absorbance was measured at 517 nm, using a UV scanning spectrophotometer (Unico® 1200, Alexandria, Egypt). The decrease of the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity. Total antioxidant activity (TAA %) was expressed as the percentage inhibition of the DPPH radical and was determined by the following formula: [TAA (%) = (Acontrol−A sample/A control) × 100], where TAA is the total antioxidant activity, A control is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound. The control contained 2 ml of DPPH solution and 2 ml of methanol. The measurements of DPPH radical scavenging activity were carried out for three replicates. Gallic acid was used as a positive control solution or a standard antioxidant.

**Minimum inhibitory concentrations**
Minimum inhibitory concentrations (MICs) were determined by serial dilution of extracts (100, 250, 500, 1000, 2000 and 4000 µg/ml). This was performed in 96-well micro-plates [22] by filling all wells, with 50 µl sterile Mueller Hinton Broth (MHB) with minor modification. After adding 50 µl of the bacterial suspension (10⁵ CFU/ml) to each row (except for the sterility control) the micro-plate was covered and incubated at 37°C at 100 % relative humidity overnight. 0.2 mg/ml solution of p-iodonitrotetrazolium violet (Sigma-Aldrich) was added to each well. Inhibition of the growth was indicated by a clear solution or a definite decrease in color reaction. The results are expressed as mean values ± standard deviation (SD).
The bacterial strains used in the present study

<table>
<thead>
<tr>
<th>Number</th>
<th>Strain No.</th>
<th>Bacterial strain</th>
<th>Geographical origin</th>
<th>Host</th>
<th>Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2114</td>
<td>Dickeya dianthica</td>
<td>Type strain</td>
<td>Dianthus</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>ippb041</td>
<td>Pectobacterium carotovorum subsp. wasabiae</td>
<td>Hamedan, Iran</td>
<td>Potato</td>
<td>HQ424871.1</td>
</tr>
<tr>
<td>3</td>
<td>ippb038</td>
<td>Pectobacterium carotovorum subsp. carotovorum</td>
<td>Tehran, Iran</td>
<td>Potato</td>
<td>HQ424870.1</td>
</tr>
<tr>
<td>4</td>
<td>PCA 1007</td>
<td>Pectobacterium carotovorum subsp. atrosepticum</td>
<td>Type strain</td>
<td>Potato</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>DSM4610</td>
<td>Dickeya chrysanthemi</td>
<td>Geographical origin</td>
<td>Host</td>
<td></td>
</tr>
</tbody>
</table>

### III. RESULTS AND DISCUSSION

The % of bark methanol extract was 8.23 % and 10.26 % for *D. regia* and *E. huneana*, respectively. The antibacterial activities of bark methanolic extract of *D. regia* and *E. huneana* were evaluated by measuring the zones of inhibitions (IZs) and MICs values on some plant pathogenic bacteria. The zones of inhibition were ranged between 6-15 mm (Table 2). The most inhibition was observed against the growth of *D. chrysanthemi* (15 mm at 4000 mg/l) and the MIC of <500>100 mg/l. The resistance bacterium was *P. carotovorum* subsp. *atrosepticum* (6 mm at 4000 mg/l) with MIC value of 4000 mg/l.

The antibacterial activity of bark methanolic extract of *D. regia* may be referred to the presence of flavonoids, alkaloids and phenolic compounds. Previous phytochemical investigations of *D. regia* revealed the presence of auroxanthin, mutatochrome and pyruvic acid [23]. Recently, five compounds, lupeol, epilupeol, *b*-sitosterol, stigmasterol and *p*-methoxybenzaldehyde were isolated from the petroleum ether and dichloromethane fractions of stem bark extract of *D. regia* as well as preliminary antimicrobial and toxic activities of the various extracts [9]. The literature survey reveals that *D. regia* bark contains *b*-sitosterol, saponins, alkaloids, carotene, hydrocarbons phytoxins and flavonoids [5,6]. In the previous studies the flavonoids of the plant extracts have been exhibited antimicrobial and antioxidants properties [24,25].

The zones of inhibition were ranged between 6-12 mm for the methanolic extract of *E. huneana* bark against the growth of some plant pathogenic bacteria (Table 3). The highest inhibition zones were found against the growth of *P. carotovorum* subsp. *wasabiae* (12 mm at 4000 mg/l) with MIC value of 2000 mg/l. The growth of *P. carotovorum* subsp. carotovorum (9 mm), *P. carotovorum* subsp. *atrosepticum* (10 mm) and *Dickeya chrysanthemi* (10 mm) was moderately inhibited. On the other hand, *D. dianthica* didn’t show any inhibition zones. The results showed that the bark extract of *E. huneana* had a weak activity against the growth of the studied bacterial strains and these results in agree with the previous report [13] against the growth of other bacterial strains. No inhibition was observed in the case of negative control (Tables 2 and 3).

The total antioxidant activity (TAA %) of bark extract was 78.35±1.45 % and 73.40±2.67 % and these values are close to the TAA % showed by Gallic acid (80±2.12 %). In *D. regia*, the mostly secondary metabolites such as flavonoids [26] sterols [27] tannins and phenol compounds [28] were found. Some carotenoids like 2-carotene, zeaxanthin etc. [23] also exhibits antimicrobial activities. Gallic acid in the bark as the main phenolic compound was found and other phenolic acids such as sorbic, sinapic, *p*-coumaric, *m*-coumaric, ferulic, caffeic, 3-hydroxybenzoic, 4-hydroxycinnamic and 4-hydroxybenzoic acids were also presented [10].

High phenolic compounds in methanol extracts play the role of these activities and possess potent antioxidants and antimicrobial. Flavonoids have been proven to display a wide range of pharmacological and biochemical actions, such as antimicrobial and antitumorigenic activities [29]. Flavonoids can act as free radical scavengers and terminate the radical chain reactions that occur during the oxidation of triglycerides in the food systems [30]. Moreover, the presence of tannins in the extracts may explain its potent bioactivities are known to possess potent antioxidants [31].
Antibacterial activity of bark methanolic extract of *D. regia* observed against the growth of some plant pathogenic bacteria

<table>
<thead>
<tr>
<th>Pathogenic bacteria</th>
<th>Inhibition zone (mm)</th>
<th>MIC (mg/l)</th>
<th>Tetracycline (10µg/disc)</th>
<th>DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extract concentration (mg/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4000 2000 1000 500</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. carotovorum</em> subsp. wasabiae</td>
<td>11 n.a. n.a. n.a.</td>
<td>4000</td>
<td>25</td>
<td>n.a.</td>
</tr>
<tr>
<td><em>P. carotovorum</em> subsp. carotovorum</td>
<td>10 8 n.a. n.a.</td>
<td>2000</td>
<td>18</td>
<td>n.a.</td>
</tr>
<tr>
<td><em>P. carotovorum</em> subsp. atrosepticum</td>
<td>6 n.a. n.a. n.a.</td>
<td>4000</td>
<td>20</td>
<td>n.a.</td>
</tr>
<tr>
<td><em>D. dianthica</em></td>
<td>13 12 6 6</td>
<td>&lt;500&gt;100</td>
<td>23</td>
<td>n.a.</td>
</tr>
<tr>
<td><em>D. chrysanthemi</em></td>
<td>15 8 7 6</td>
<td>&lt;500&gt;100</td>
<td>21</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

1: Diameter of inhibition zone (mm) including disc diameter of 4 mm.

2: Inhibition zones (mm) observed by tetracycline as a positive control (10µg/disc).

n.a. Not active; MIC: Minimum inhibitory concentration (mg/l).

DMSO: Dimethyl sulfoxide [(CH₃)₂SO] as a negative control.

Inhibition > 15 mm (strong inhibition), 15 – 10 mm (moderate), and <10 mm (weak).

Antibacterial activity of bark methanolic extract of *E. humeana* observed against the growth of some plant pathogenic bacteria

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<tr>
<td><em>P. carotovorum</em> subsp. carotovorum</td>
<td>9 8 n.a. n.a.</td>
<td>2000</td>
<td>18</td>
<td>n.a.</td>
</tr>
<tr>
<td><em>P. carotovorum</em> subsp. atrosepticum</td>
<td>10 9 9 n.a.</td>
<td>1000</td>
<td>20</td>
<td>n.a.</td>
</tr>
<tr>
<td><em>D. dianthica</em></td>
<td>n.a. n.a. n.a. n.a.</td>
<td>&gt;4000</td>
<td>23</td>
<td>n.a.</td>
</tr>
<tr>
<td><em>D. chrysanthemi</em></td>
<td>10 8 6 6</td>
<td>&lt;500&gt;100</td>
<td>21</td>
<td>n.a.</td>
</tr>
</tbody>
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Inhibition > 15 mm (strong inhibition), 15 – 10 mm (moderate), and <10 mm (weak).

VI. CONCLUSION

The results revealed that the bark extracts of *Delonix regia* and *Erythrina humeana* had potent antioxidant activities and possessed a moderate antibacterial activity. Since it is the first report about the effect of extracts from *Delonix regia* and *Erythrina humeana* against the growth of *Dickeya dianthica*, *Pectobacterium carotovorum* subsp. *wasabiae*, *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium carotovorum* subsp. *atrosepticum* and *Dicieka chrysanthemi* which causing a high level of wilting in potato and dianthus crops, the methanolic extracts of the stem bark may be valuable for functional applications against the growth of these bacteria. These activities could be due to strong presence of compounds such as flavonoids, tannins, alkaloids and saponins.

ACKNOWLEDGEMENTS

The author would like to thank Dr. Nader A. Ashmawy (Plant pathology Department, Faculty of Agriculture (EL-Shatby), Alexandria University, Egypt), for providing the bacterial cultures.

REFERENCES


