In vitro comparative study of antimicrobial activity of whole plant and root’s bark of Delonix regia (Bojer Ex. Hook)

Musaddique Hussain1, 2*, Shahid Masood Raza2, Khalid Hussain Janbaz1, Muhammad Razi Ullah Khan2, Abdul Aziz1 and Abdul Majeed1

1 Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan.
2 School of Pharmacy, The University of Faisalabad, Faisalabad, Pakistan

* Corresponding author: Musaddique Hussain; e-mail: musaddique.ph@gmail.com

ABSTRACT
Medicinal plants are the potent source of biologically active compounds and have always been of field of interest for the effective chemotherapeutic agents and offering a broad spectrum of activity with greater emphasis on preventive action. The present study was aimed at evaluating and comparing the antimicrobial spectrum of various extracts (methanolic, ethanolic, DCM and aqueous) of whole plant and root’s bark of Delonix regia against different pathogenic strains of bacteria and fungi, by adopting the disc diffusion method. Among all studied extracts, methanolic extract of whole plant (seeds, leaves and stem) showed the significant inhibitory response against S. aureus (91.84%), P. aeruginosa (91.05%), B. pumilus (85.75%) and E. coli (78.71 %). The descending sequences of antimicrobial activity of various extracts of whole plant of Delonix regia (seeds, leaves and stem) against studied microorganisms were as follows: methanolic extract, ethanolic extract, DCM extract, and aqueous extract. The results of the present study indicate that methanolic extract of the studied plant is a potentially good candidate for the therapy of antibacterial-resistant bacteria and would therefore require further study.

Keywords: Delonix regia, methanol extract, ethanol extract, dichloromethane extract, antimicrobial activity, disc diffusion method.

INTRODUCTION
Bacterial infections are one of the emerging problem especially in developing countries [1]. Gram +ve bacteria, such as Streptococcus pneumoniae cause bronchopneumonia, sinusitis, conjunctivitis, endocarditic and labor pneumoniae [2] while, Staphylococcus aureus are responsible for the toxic shock syndrome, septicemia, endocarditic, postoperative wound infection and food poisoning [3]. Whereas, Gram –ve bacteria, such as Klebsiella pneumoniae cause chest infections, wound infection and urinary tract infection whereas, E. coli cause lower urinary tract infection, septicemia and another strain [4, 5]. Well reputed antibiotics are available in markets which show their antibacterial action by various mechanisms such as protein synthesis inhibition, cell wall synthesis inhibition, and DNA synthesis inhibition. Almost all the microbes have developed resistance against all introduced allopathic antibiotics, in addition to resistance; antibiotics are associated with serious adverse effects on host including hypersensitivity, geno-toxicity, reproductive disorders, allergic response, nephro-toxicity, immune-suppression and depletion of the gut normal flora [6]. Antimicrobials of plants origin have enormous therapeutic potential; recently much attention has been paid to biologically active compounds isolated from plant species used in herbal medicine. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [7].

Delonix regia (Bojer Ex. Hook) (Syn. Poinciana regia Bojer Ex. Hook) belonging to Caesalpiniaeaceae (family) is a medium-sized, attractive tropical tree found in greater parts of Asia (Pakistan and India) [8]. It is flowering plant, grown as an ornamental tree and given the name, flame tree or flamboyant, peacock, gulmohar,
royal poinciana [9]. Its flowers used as natural color and as an acid-base indicator [10]. Chemical constituents of different classes such as; flavonoid, tannins, and its glycosides, phenolics, sterol (phytosterol) and terpenoids (triterpenoids) [11-13], were reported from its flowers and leaves. Many of distinct steroids are found in plants, animals and fungi [14]. All steroids are made in cells either from the sterols, lanosterol (animals and fungi) or from cycloartenol (plants). Both lanosterol and cycloartenol are derived from the cyclization of the triterpenesqualene [15]. Ethanolic extracts of flower and bark were investigated to anti-inflammatory activity in rats [16]. Leaves and flowers are reported to possess antimalarial [17], anti-bacterial [18], anthelmintic [19], hepato-protective [20], diuretic [21], termiticidal [22], anti-arthritis [23], cytotoxic [24], hypoglycemic [25], and anti-oxidant [26, 27]. However, in the previous papers antimicrobial activity of the leaves of the Delonix regia have been studied [28, 29], but aim of the present study was to investigate and compare the antimicrobial activity of the different fractions (methanol, ethanol, DCM and aqueous) of the whole plant (seeds, leaves and stem) and root’s bark of the Delonix regia (Bojer Ex. Hook).

MATERIALS AND METHODS

Extraction preparation

Adulterants free whole plant (seeds, leaves and stem) and root bark were powdered in the electrical blenders. Extraction of coarse powdered material (# 40) was performed by the triple maceration [31], by using the different organic solvents depending upon their polarity. Extract was prepared by soaking 400g of the whole plant and root barks in a measured volume of dichloromethane, in two separate macerating bottles and agitated at 120 rpm/min for 72 hrs in rotary orbital shaker, at room temperature and this procedure was adopted three times with dichloromethane. After maceration, the soaked coarse powdered material was passed through muslin cloth (double layered), in order to remove vegetative debris and the obtained filtrate was subsequently filtered through a Whatman-1 filter paper. The filtrates were stored in amber glass air-tight bottles. The extraction of marc was carried out with ethanol, methanol and then with distilled water by adopting same procedure. Rotary evaporator (Rotavapor, BUCHI labrotechnik AG, Model 9230, Switzerland) attached with a vacuum pump and a recirculation chiller was used to concentrate the dichloromethane, ethanol and methanol extracts, under reduced pressure at 37 °C. While aqueous extract was concentrated by the lyophilization process. All extracts were stored at −4 °C in a refrigerator.

Preparation of extract solution

In vitro, experiments were performed by dissolving 0.3 gram of the crude extract in 0.1ml (100 µl) of 100% dimethylsulfoxide (DMSO) and volume was made up to 1 ml (1000 µl) with distill water to prepare 0.3 g /ml, w/v stock solution (300 mg/ml), due to its insolubility in distilled water and stored in refrigerator [32]. The dimethylsulfoxide alone did not show any biological and physiological activity. Thereafter serial dilution of stock solution (containing 300 mg/ml) was made, to obtain 30 mg/ml concentration which was used for the antimicrobial sensitivity test.

Micro-organisms and standard drugs (discs) used

All standard drug discs i.e., gentamicin, fluocloxacinil, vancomycin, ciprofloxaclin, ceftriaxone, levofloxaclin and amphotericin-B, having drug concentration of 20 µg/disc (Oxoid Ltd. Basingstoke, Hampshire, England) were purchased from G.M, Scientific shop, Multan, Pakistan. While, all the microorganisms i.e., Bacillus cereus, Bacillus pumilus, Staphylococcus aureus, Streptococcus pneumonia, Pseudomonas aeruginosa, Escherichia coli, Citrobacter freundii, Klebsiella pneumoniae Candida albicans and Aspergillus niger, were collected from the University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. All microbes were cultured overnight in a nutrient agar (pH 5) containing peptone (0.5%), agar (1.2%), yeast (0.3%), and NaCl (0.8%) [33]. Inoculums were prepared by transferring microbial colonies from fresh culture plates to tube containing 10 ml of nutrient broth media. The tubes were shaken occasionally for aeration to promote the microbial growth and were incubated overnight at 37 °C.

Determination of antimicrobial activity

For the determination of the antimicrobial activity, standard disc diffusion method was adopted [34, 35] and three types of discs were used, i.e., discs containing standard antibiotics were used as positive control, discs containing plant crude extract or latex were used as sample discs, and discs containing the DMSO were used as negative control. Punch machine was used to prepare the discs having the diameter of 6 mm from the whatman-1 filter paper. All glassware were sterilized by the dry heat of sterilization. Nutrient agar media and sabouraud dextrose agar media prepared in distilled water and sterilized in autoclave at 121°C for 30 minutes. Pour the media into separate petri dishes and allowed to set as a firm gel on cooling. The thickness of gels layer should range between 2-3 mm. The test petri-dishes were incubated overnight at 37 °C and those showing no growth were selected for further work. The bacterial culture and fungal culture were transferred from inoculums to petri-dishes by using the sterilized aluminum wire loops, which were subsequently spread by streaking method. All the procedure was carried out in the strict aseptic condition using horizontal laminar flow cabinet. Bacterial cultures were incubated at 37 °C in incubator for 24 hours while fungal cultures at room temperature for 48 hours. At the end of the incubation period, zone of inhibition (mm) of the each extract was measured in comparison with the positive and negative control [36].

Relative percentage inhibition

The relative percentage inhibition of the crude extract with respect to positive control was calculated by using the following formula [37, 38]
Relative percentage inhibition of crude extract = \( \frac{100 \times (a - b)}{c - b} \)

Where, 
a: total area of inhibition of the test extract,  
b: total area of inhibition of the solvent  
c: total area of inhibition of the standard drug 

The total area of the inhibition was calculated by using  
\[ \text{Area of inhibitory zone} = \pi r^2 \]

Where \( r \) is radius of zone of inhibition

RESULTS AND DISCUSSION

In Vitro antimicrobial activity

Diameter of the zone of inhibition and relative percentage of inhibition of the methanolic, ethanolic dichloromethane (DCM), and aqueous extract of Delonix regia against different pathogenic bacteria and fungi species, are shown in tables 1 and 2. Methanolic extract of whole plant showed the zone of inhibition (including diameter of disc 6 mm) of 23.00 mm against Staphylococcus aureus, 21.75 mm against Bacillus pumilus, 21.00 mm against Bacillus cereus, 21.50 mm against Pseudomonas aeruginosa, 22.50 mm against Escherichia coli, 21.00 mm against Citrobacter freundii and 18.00 mm against Klebsiella pneumoniae, as compared with standard drugs fluocoxacin (24.00), vancomycin (23.50), ciprofloxacin (24.75), ceftriaxone (25.00), gentamicin (22.00), ceftriaxone (24.50 mm), ciprofloxacin (25.00) and levofloxacin (23.00) with relative percentages of inhibition 91.84, 85.75, 71.95, 73.95, 91.05, 78.71, 70.60 and 61.25 respectively, whereas methanolic extract of root's bark showed the zone of inhibition of 21.00 mm against Staphylococcus aureus, 20 mm against Bacillus pumilus, 20.50 mm against Bacillus cereus, 18.75 mm against Pseudomonas aeruginosa, 19.50 mm against Pseudomonas aeruginosa, 20.00 mm against Escherichia coli, 17.50 mm against Citrobacter freundii and 14.25 mm against Klebsiella pneumoniae, with relative percentages of inhibition 76.60, 72.42, 65.77, 56.32, 77.75, 66.65, 49.00 and 38.44% respectively.

Relative percentage of inhibition (RPI) showed by the ethanolic extract of whole plant of Delonix regia was 84.79, 75.80, 63.69, 64.00, 83.16, 73.47, 57.75 and 42.54 mm, whereas RPI showed by ethanolic extract of root's bark was 62.50, 60.10, 47.80, 43.85, 60.55, 37.50, 38.44 and 39.82% against S. aureus, B. pumilus, B. cereus, S. pneumoniae, P. aeruginosa, E.coli, C. freundii and K. pneumoniae as compare with standard. Whereas Delonix regia showed less response against fungal species i.e., C. albicans and A. niger as compared to pathogenic bacteria species.

It can be inferred that among all studied extracts, methanolic extract of Delonix regia (whole plant and root's bark) showed the maximum zone of inhibition against all studied microbes as compared to ethanolic, DCM and Aqueous extract. After statistical analysis, P value was determined which was found to be significant for the methanolic and ethanolic extract, i.e., less than 0.05 (P < 0.05).

Table 1. Zone of inhibition of the methanolic, ethanolic, DCM and aqueous extract of the whole plant and root's bark of Delonix regia against different strains of bacteria and fungi (values are expressed as mean ± SEM, n = 3).

<table>
<thead>
<tr>
<th>Microbes used</th>
<th>Whole Plant</th>
<th>Root barks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Metha</td>
<td>Etha</td>
</tr>
<tr>
<td>S. aureus</td>
<td>23.00</td>
<td>22.10</td>
</tr>
<tr>
<td>B. pumilus</td>
<td>21.75</td>
<td>20.45</td>
</tr>
<tr>
<td>B. cereus</td>
<td>21.00</td>
<td>19.75</td>
</tr>
<tr>
<td>S. pneumonia</td>
<td>21.50</td>
<td>20.00</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>21.00</td>
<td>20.15</td>
</tr>
<tr>
<td>E. coli</td>
<td>22.50</td>
<td>21.00</td>
</tr>
<tr>
<td>C. freundii</td>
<td>21.00</td>
<td>19.00</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>18.00</td>
<td>15.00</td>
</tr>
<tr>
<td>C. albicans</td>
<td>19.55</td>
<td>17.00</td>
</tr>
<tr>
<td>A. niger</td>
<td>20.00</td>
<td>17.55</td>
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</tbody>
</table>

* Diameter of the zone of inhibition including diameter of disc 6mm. Meth = methanolic extract, Eth = ethanolic extract, DCM = DCM extract, Ag = aqueous extract, -ve = negative control, i.e., Dimethylsulfoxide, +ve control = standard drug discs, - no response.

Table 2. Relative percentage inhibition of methanolic, ethanolic, DCM and aqueous extract of the whole plant and root’s bark of Delonix regia against different strains of bacteria and fungi (values are expressed as mean ± SEM, n = 3).

<table>
<thead>
<tr>
<th>Microbes used</th>
<th>Whole Plant</th>
<th>Root barks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Meth</td>
<td>Eth</td>
</tr>
<tr>
<td>S. aureus</td>
<td>89.14</td>
<td>84.79</td>
</tr>
<tr>
<td>B. pumilus</td>
<td>85.75</td>
<td>75.80</td>
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<td>B. cereus</td>
<td>71.95</td>
<td>63.69</td>
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<tr>
<td>S. pneumonia</td>
<td>73.95</td>
<td>64.00</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>91.05</td>
<td>85.16</td>
</tr>
<tr>
<td>E. coli</td>
<td>78.71</td>
<td>73.47</td>
</tr>
<tr>
<td>C. freundii</td>
<td>70.60</td>
<td>57.75</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>61.25</td>
<td>42.54</td>
</tr>
<tr>
<td>C. albicans</td>
<td>75.00</td>
<td>57.87</td>
</tr>
<tr>
<td>A. niger</td>
<td>74.45</td>
<td>64.70</td>
</tr>
</tbody>
</table>

Meth = methanolic extract, Eth= ethanolic extract, DCM = DCM extract, Ag = aqueous extract
Figure 1. Zone of inhibition of methanolic, ethanolic, DCM and aqueous extract of (A) whole plant (B) root’s bark of Delonix regia in diameter (mm) against different strains of bacteria and fungi (values are expressed as mean ± SEM, n = 3).
Drug resistance in the pathogenic microbes has developed due to the excessive use of the commercial antibiotics for the treatment of the infectious diseases, whereas drug resistance is the major hurdle of this era which is leading towards mortality and morbidity [39]. This condition has forced researcher to search for new antibacterial substances though various sources. In vitro evaluation of the plants for the antimicrobial property is the first step toward achieving the goal for developing eco-friendly management of the infectious diseases [40].

Considering these, Delonix regia was screened for its antibacterial activity against ten (10) human pathogenic microbes. On the basis of the results of the present study it may be revealed that extract of Delonix regia possess activity against Gram +ve and Gram –ve bacteria. In general Gram +ve bacteria are considered more sensitive than Gram –ve bacteria towards different antimicrobial compounds because of the difference of cell wall structure of both [41, 42] but methanolic crude extract of the whole plant of the Delonix regia showed the significant response against *S. aureus*(91.84%), *P. aeruginos* (91.05 %), *B. pumilus*(85.75 %) and *E. coli*(78.71 %), as compared to the ethanolic, DCM and aqueous extract of whole plant, in the same case, methanolic extract of the root bark show the less satisfactory response against *S. aureus*(76.60 %), *P. aeruginos* (77.75 %), *B. pumilus* (72.42 %) and *E. coli* (66.65 %).

The descending sequences of antimicrobial activity of various extracts of whole plant of Delonix regia (seeds, leaves and stem) against studied microorganisms were as follows: methanolic extract, ethanolic extract, DCM extract, and aqueous extract, whereas same descending sequence was also followed by the various extracts of root’s bark of Delonix regia against studied microorganisms but whole plant of Delonix regia (seeds, leaves and stem) showed the more potent inhibitory response as supporting the view, that medicinal plants might be useful in the development of novel antimicrobial agents [43]. Hence it can be inferred that In-vitro results of this plant appear as interesting and promising and may be effective as potential source of novel antimicrobial drug.

**CONCLUSION**

*Delonix regia* is believed to possess the antibacterial activity due to the presence of tannin and flavonoids [11-13]. Tannin and flavonoids are the potent antioxidant and free radical scavenger which prevent oxidative cell damage and also have strong antimicrobial activities [44, 45]. Hence these compounds may be responsible for the antimicrobial activity of the plant. Further research is necessary to determine the identity of the therapeutic compound within this plant and also to determine their full spectrum of efficacy. However, the present study may serve as the primary platform for the further in-vivo studies.

**REFERENCES**


