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Antibacterial and pharmacognostical evaluation of *Delonix regia* root bark

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Abstract

Medicinal plants have been of age long remedies for human diseases since they contain valuable components. In India, indigenous herbal remedies such as Ayurveda and other Indian traditional medicine have since ancient times used plants in treatment of various diseases. *Delonix regia* belonging to the family *Fabaceae* was carried out to assess its Pharmacognostical and Antibacterial activity, using gram positive and gram negative strains, which are harmful to human beings. The results showed significant antibacterial activity at the concentration of 200mg/ml. The pharamcognistical studies like total ash, acid insoluble ash, water soluble ash was found 8.5%, 1.6%, 2.8% respectively.

Key-Words: *Delonix regia*, Antibacterial activity, Pharamacognistical study

Introduction

In developing countries, the frequency of life-threatening infections were caused by pathogenic microorganisms has led to increased worldwide and is becoming an important cause of morbidity and mortality in immune compromised patients. The increasing failure of chemotherapeutics coupled with antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity .Herbal-based and plant-derived products can be exploited with sustainable comparative and competitive advantage. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials.

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 (Stuffness and Douros, 1982) and natural products play an important role in drug development programs of the pharmaceutical industry (Baker et al., 1995; Cordell, 1995).

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Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents. Systematic screening of them may result in the discovery of novel active compounds.

Plant extract has been reported to possess anti-bacterial, anti-malarial and anti-fungal properties (Aqil and Ahmad, 2007; Ankrah *et al*., 2003; Dutta *et al*., 1998). In the present study antibacterial potentiality of root bark of the plant *Delonix regia* has been carried out.

Material and methods

Collection and extraction of plant material

The root bark of *Delonix regia* was collected from Coimbatore in the month of May 2011 and identified by Botanical Survey of India, Tamilnadu. Plant materials were shade dried for 15 days and powdered. The air died powder was subjected to successive solvent extraction with methanol.

Pharmacognostical studies

Physicochemical parameters

Ash Values

The determination of various physicochemical parameters such total ash, water-soluble ash, alkalinity of water soluble ash and acid-insoluble ash values of the leaf and fruit powder was determined as per the Indian Pharmacopoeia (Anonymous 1996).

Fluorescence Analysis

A small quantity of dried and finely powdered leaf and fruit of *Delonix regia* was placed on grease free clean microscopic slide and added 1-2 drops of the freshly prepared reagent solution, mixed gently by tilting the slide and waited for 1-2 minutes. Then the slide was viewed in day light and (365 nm) ultraviolet radiations. The colors observed by application of different reagents in different radiations were recorded. (Pratt *et al*., 1949).

Microorganisms

Gram negative *(Escherichia coli, Pseudomonas aeruginosa, Klebseilla pneumonia*) and Gram positive bacteria cultures *(Staphylococcus aureus, Streptococci pneumonia* and *Bacillus subtilis*) were used in throughout investigation. All the slants were kept at 40ºC in the refrigerator for further studies.

Antimicrobial Activity

Antimicrobial assay of different concentrations of extract were performed by Disc diffusion method (Bauer *et al* 1966). All the bacterial cultures were developed on Muller Hinton using sterile cotton swabs. Streptomycin pre soaked and dried discs of 6 mm diameter of whattman No. 1 filter paper were used as positive control. After the incubation period the inhibition zones around the discs were measured and recorded. 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 18 hrs.After the incubation the size of the inhibition zone were measured.

Antibacterial activity was determined by measuring the diameter of the zone of inhibition surrounding microbial growth. For each strain, controls were included that comprised pure solvents instead of the extract (Parekh and Chanda 2007). The standard antibiotic ampicillin (50µg) was used. The experiment was repeated and the mean values were tabulated.

Results and Discussion

Fluorescence Analysis

The plant powder were subject to fluorescence analysis as per the standard procedure and various shades from light brown to black brown were observed (Table 1).

Ash value

The powdered drug was evaluated for its physico-chemical parameters like Ash values, Acid Insoluble ash, Water soluble ash and all the results are tabulated in Table 2.

Antibacterial activity

The plant samples with various concentrations were tested against 6 different bacterial strains which are pathogenic to humans. The agar disc diffusion method was used to evaluate the antimicrobial activity by measuring the inhibition zone against the test microorganisms. Preliminary phytochemical analysis revealed the presence of secondary metabolites like tannins, Phenols, alkaloids, terpenoids, sterols, cardiac glycosides. Since multidrug resistance of microorganisms is a major medical concern, screening of natural products in a search for new antimicrobial agents that would be active against these microorganisms is the need of the hour. (Zgoda JR).

Based on the investigations, methanol extract of root bark of *Delonix regia* showed effective activity against all bacteria. At the concentration of 100mg/ml and 200mg/ml, the extract showed maximum antimicrobial activity (Table 3).

Further research is necessary to determine the identity of the antibacterial compounds from within this plant and also to determine their full spectrum of efficacy.

As the powders of the test plant were treated with various reagents like NaOH, dil.HCL, HNO_3, acetic acid, methanol, picric acid, H2SO4 the colour changes were observed in the treated powders and the colour varied from brown to black brown shades. Methanol extract of root bark of *Delonix regia* showed effective activity against all bacteria.

Further research is necessary to determine the identity of the antibacterial compounds from within this plant and also to determine their full spectrum of efficacy.

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Table 1: Fluorescence analysis of *Delonix regia* root bark

Powder + reagents	Day light	Uv light (365nm)
Powder as such	Light brown	Brown
Powder + NaOH	Brown	Pale brown
Powder + dil. HCL	Brown	Brown
Powder + HNO_3	Blackish green	Thick brown
Powder + acetic acid	Brown	Light brown
Powder + methanol	Dark brown	Brown
Powder + picric acid	Light brown	Brown
Powder + H_2SO_4	Blackish green	Blackish brown

Table 2: Physico-Chemical Studies of Powdered *Delonix regia* root bark

Ash type	Percentage of ash
Total ash	8.5% w/w
Acid insoluble ash	1.6% w/w
Water soluble ash	2.85 w/w

Table 3: Antibacterial activity of methanol extract of *Delonix regia* root bark

Bacterial strains	Std. ampicillin 50µg/ml	Concentration of extract (25mg/ml)	50mg/ml	100mg/ml	200mg/ml
Escherichia coli	21	NT	NT	8	11
Psuedomonas aeruginosa	19	7	8	11.5	16
Klebseilla pneumoniae	18.5	NT	7	10	14
Staphylococcus aureus	22.5	7	11	16	21
Streptococci pneumoniae	21	NT	NT	NT	NT
Bacillus subtilis	19	7	9	13	19

Zone of inhibition in mm (include 6mm disc) (mean value).
NT - not formed zone.