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Study of antibacterial activity of leaf, stem, root methanolic extract from *Delonix regia*

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Abstract
Medicinal plants are also potent source of biologically active compound and offering broad spectrum of Activity. The present study was aimed evaluating and comparing antimicrobial spectrum of Methanolic Extract of leaves, roots, stem of Delonix regia which is also known as Gulmohar. Its antibacterial activity was performed by using gram negative bacteria Escherichia coli and gram positive bacteria Staphylococcus aureus by agar well diffusion method with Dimethylsulfoxide (DMSO) as standard. The Antibacterial activity was measured by different concentrations of *D. regia* with its minimum dose (20ug/ml) and maximum dose (100ug/ml) and DMSO (100ug/ml). It was evaluated by measuring the zone of inhibition in millimetres. It can be concluded that methanolic extract of *Delonix regia*was found to have antibacterial activity and it is effective against E. coli and Staphylococcus aureus and it also indicate that Methanolic Extract of plant is potentially good for therapy of Antibacterial-resistant bacteria.

Keywords: antibacterial activity, soxhlet extraction, ultrasonic extraction, well diffusion method

Introduction
Bacterial infections are one of emerging problem in developing countries Gram positive especially Streptococcus aureus causes Boils. The most common type of staphylococcus infection is the boil, a pocket of pus that develops in a hair follicle or oil gland, Impetigo. This contagious, often painful rash can be caused by Staphylococcus aureus, Cellulitis, Staphylococcal scalded skin syndrome. Whereas Gram negative Bacteria, such as E. coli, short for Escherichia coli, is a type of bacteria commonly found in the intestines of humans, livestock and other animals that is excreted in faeces. The strain called O157:H7, a top cause of food-borne illness, is particularly dangerous. Some of antibiotics available in market and show their Antibacterial activity by various mechanisms like DNA synthesis, protein synthesis etc. And some of them are having adverse effects like hypersensitivity, gene toxicity, and depletion of Normal gut flora. *D. regia* is a tall tree reaching a height of more than 15 m and a girth of 2 m under favourable conditions. The trunk is buttressed and the stem form above the buttress is generally normal in taper (Webb *et al*. 1984). The trees are almost evergreen, with broad-spreading, open, umbrella-shaped crowns (Randhawa, 1965). It is deciduous in localities which experience long pronounced dry seasons (Streets, 1962; Yusuf and Sheikh, 1986). The bark is grey or brown, smooth or slightly rough, and exfoliating (Gamble, 1902; Sheikh, 1993). The compound leaves of *D. regia* are bipinnate and feathery, up to 60 cm long, pinnae 11-18 pairs, petiole stout. The leaflets are in 20-30 pairs on each pinna, oblong, 7.5-10 mm long, 3.4-5 mm wide (Gamble, 1902; Randhawa, 1965). At the base of the leaf, two stipules occur which have long, narrow comb-like teeth (Luna, 1996). The inflorescence of *D. regia* is a lax terminal or axillary raceme. The flowers appear in corymbs along or at the end of branches and are large, 10 cm across and bright red. They vary considerably in intensity of colouring, ranging from orange-vermillion to deep scarlet. Most of the common names for *D. regia* are derived from the colour of its flowers. The pods are 5 cm broad and 30-60 cm long, ending in a beak when mature (Luna, 1996). They are green and flaccid when young and are compressed, firm and rather thick when mature. Seeds are large, yellowish, oblong, arranged at right angles to the length of pod and transversely mottled (Parker, 1956).

Plant Profile: ***Delonix regia***

- **Botanical Classification (Plant Taxonomy)**

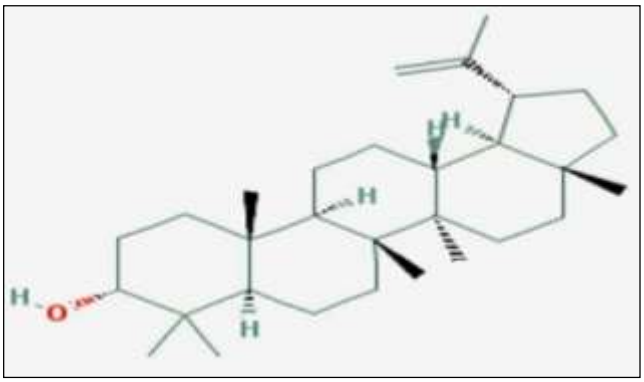
Kingdom	Plantae-Plants
Subkingdom	Tracheobionta-Vascular plants
Division	Magnoliophyta-Flowering plants
Class	Magnoliopsida-Dicotyledons
Subclass	Rosidae
Order	Fabales
Family	Fabaceae/Leguminosae-Pea family
Genus	*Delonix* Raf. - delonix
Species	*Delonix regia* (Bojer ex Hook.) Raf. - royal poinciana

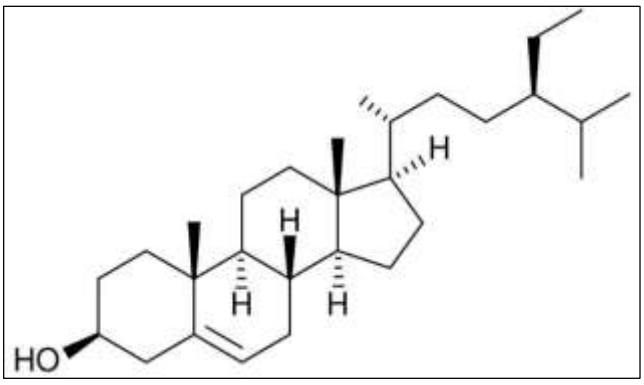
- **Name of plant**
  Delonix regia (Gulmohar).

- **Biological Source:** It consists of dried root, stem, leaves of Delonixregia and species of flowering plant belonging to family Fabaceae.
- **Geographical Source**-Bangladesh, Vietaman, India.
- **Chemical Constituents-**

1. Leaves-Lupeol and β sitosterol.
2. Stem-Bark yield 4 triterpenes- Lupeol, epilupeol, ß sitosterol, Stigma sterol, p-methoxybenzaldehyde.
3. Root-secondary metabolite tannins, phenols, alkaloids, sterols, cardiac glycosides, terpenoids.

- **Structure of β sitosterol, epilupeol-Epilupeol Betasitosterol**





- **Macroscopy-**

Organoleptic evaluation of *D. regia* Leaves, Stem, and Root were done to identity the nature of the plant. The parameters such as colour, odour, taste, were measured.

Sr. No.	Macroscopy	Leaves	Root	Stem
1	Colour	Dark Green	Dark Brown	Brown
2	Odour	Characteristic	Characteristic	Characteristic
3	Taste	Bitter	Bitter	Bitter

Fig 1.1: Leaves

Fig 1.2: Root

Fig 1.3: Stem

Need of Present Investigation

To find out the different concentration from of extracts of *Delonix* regia which should have minimum dose and maximum dose anti-bacterial activity, To develop a possible source for new potent antibiotics to which pathogens strains are not resistant, To develop better drugs against microbial infection.

Aim and Objectives

To study comparative Antimicrobial activity of methanolic extract of leaves, Stem, Root of *Delonix regia* by using

techniques of Ultrasonic extraction and Soxhlet extraction method.

The objectives of my present investigation were to authenticate the *Delonix regia* plant, to extract out the leaves and root of *Delonix regia*by ultrasonic and soxhlet extraction, to check the Phytochemical Constituent of the extract, to check the Antibacterial activity of extract by using Cup-plate method, to know the Antibacterial potency in plants, to avoid premature deaths due to infectious disease, to prepare newer broad spectrum Antibacterial agent.

Plan of Work

Selection of plant then Collection of plant then Authentication plant then Extraction of plant by using Soxhlet and ultrasonic extraction method with methanol solvent then Phytochemical test of extract then Preparation of test microorganisms then Preparation of agar medium then Preparation of wells then Evaluation of antibacterial activity of plant extract.

Method and Material

A. Selection of plant

The fresh leaves, root and stem of the plant will be collected from the Lonand city, from Satara district of Maharashtra in month of September 2017.

B. Authentication of Plant

Plant is authenticate from Yashwantro Chavan Institute of Sciences, Satara.

C. Preparation of Delonix regia plant extract

Plant materials were air dried for 15-20 days and powered. The air dried powder was subjected to solvent extraction with methanol.

D. Micro-organisms used

Stock cultures of gram positive staphylococcus aureus and gram negative organism E.coli were collected from Yashwantrao Chavan department of Microbiology of Y.C.I. Satara.

E. Preparation of culture media and inoculation

Nutrient agar was used as Bacteriological medium. The medium was sterilised by autoclaving at 121 ^{0}C for 30 min. under aseptic conditions, 25ml of culture medium containing microbial culture was dispensed into pre sterilised Petri dish to yield uniform depth.

F. Antibacterial Activity

The antibacterial activity studies were carried out by using cup plate method. The nutrient agar media was sterilized at 121 °C under 15 psi pressures for 30 minutes. After cooling to about 65 ^{0}C, 25 ml of the medium was poured in Petri-dish. The plates were kept at room temperature for solidification and stored at 4 ^{0}C until using. The same process was applied with dextrose agar plates which were used for the growth of *Staphylococcus*. Bacterial culture was spread over the nutrient agar plates by using separate sterile spreader. Holes were made in the medium by using 7 mm corn borer. The dried plant extract was dissolved in methanol to final extract of 100ug/mL. Each hole in plate was filled with 1 ml of plant extract. Methanol was used as a negative control in one of the plates. The plates were incubated for 24-48 hours at 37 °C along with negative controls. Also Dimethylsulfoxide (DMSO) was used as standard. The antibacterial activity of each extract was recorded based on the inhibition of bacterial growth by the extract at the end of incubation period. At the end of the incubation period the zones of inhibitions were measured to the nearest millimetre. The inhibition zone is the area surrounded the hole and there is no growth of inoculated microorganism. For confirmation of the results each test was performed in duplicate.

Observation

- **Standard (DMSO)**

Fig 1.4: Gram positive

Fig 1.5: Gram negative

- **Control (Methanol)**

Fig 1.6: Gram positive

Fig 1.7: Gram negative

- **Antibacterial activity by using Soxhlet method**
- **Leaves**

Fig 1.8: Gram positive

Fig 1.9: Gram negative

- **Stem**

Fig 1.10: Gram positive

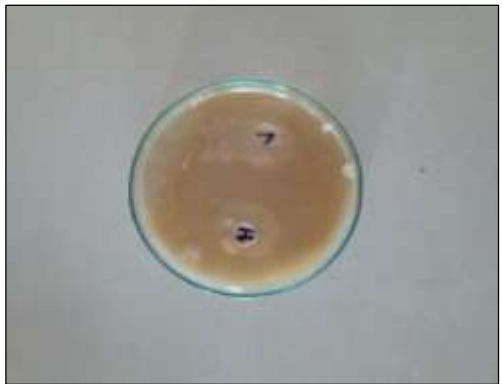


Fig 1.11: Gram negative

- **Root**

Fig 1.12: Gram positive

Fig 1.13: Gram negative

- **Antibacterial activity by using Ultrasonic extract of 45 minutes**
- **Leaves**

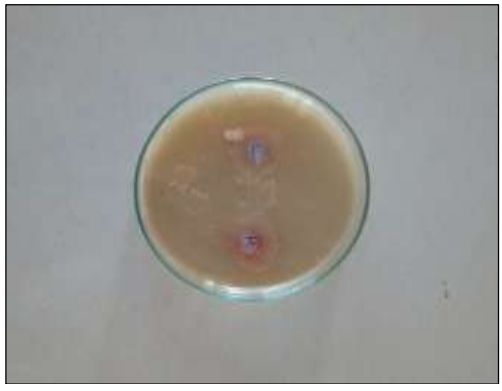


Fig 1.14: Gram positive

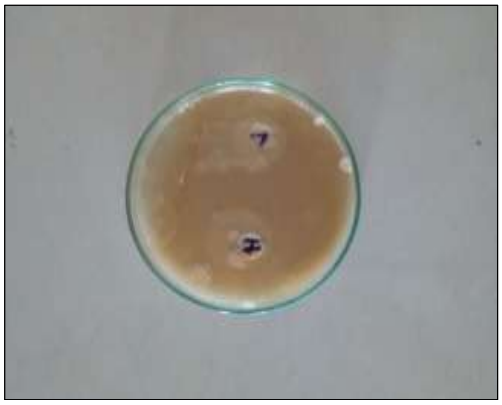


Fig 1.15: Gram negative

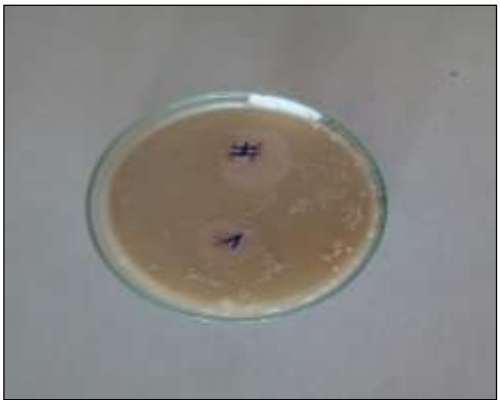


Fig 1.17: Gram negative

- **Stem**

Fig 1.16: Gram positive

- **Root**

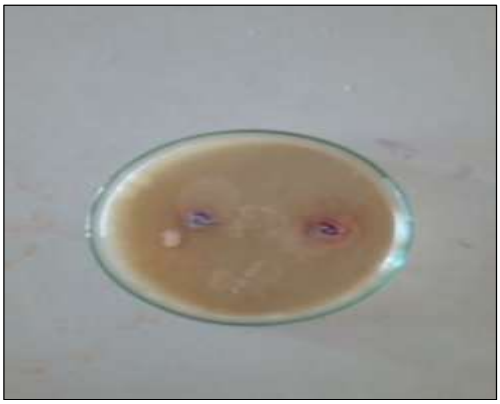


Fig 1.18: Gram positive

Fig 1.19: Gram negative

Result

- **Gram positive:** Staphylococcus aureus.
- **Gram negative:** Escherichia Coli.

Antibacterial Activity by Soxhlet Extraction Technique

Zone of Inhibition in mm										
	Std		Control		Leaves		Stem		Root	
Pathogens	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
Staphylococcus	25mm	30mm	23mm	20mm	24mm	22mm	28mm	25mm	21mm	20mm
E. coli	23mm	27mm	27mm	24mm	28mm	26mm	30mm	27mm	24mm	22mm

Antibacterial Activity by Ultrasonic Extraction Technique

Ultrasonic Extraction										
	Std		Control		Leaves		Stem		Root	
Pathogens	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
Staphylococcus	25mm	30mm	23mm	20mm	22mm	25mm	25mm	27mm	24mm	26mm
E. coli	23mm	27mm	27mm	24mm	27mm	30mm	28mm	31mm	25mm	28mm

Discussion

The crude extracts obtained from leaves, stem, roots of *Delonix regia* exhibited activity against *staphylococcus aureus* which is gram positive and *E. coli* which is gram negative. The finding of current study demonstrates the methanolic extract of *Delonix regia* showed better activity gram positive and gram negative organism.

Conclusion

Therefore, *Delonix regia* may be considered as plant for various health benefits. These plants shows Antibacterial activity with less toxic side effects The Method of extraction was easy and therefore their extract shows Antibacterial activity against *Staphylococcus aureus* and *E. coli*. The extracts possessing high Antibacterial activity should be used for further therapeutic use. As these plants are easily available and economically affordable and thus have many medicinal values. Hence these plants can be used to minimise health problems and for achieving healthy life.

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