ANTIBACTERIAL ACTIVITY OF *DELONIX REGIA* WOOD

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Summary

Objective of present work is to evaluate traditonal antibacterial property of *Delonix regia* Linn. (Caesalpinaceae) wood by using suitable methods. Wood of the plant was collected, authenticated, powdered and extracted with various solvents by Soxhlet apparatus to obtain petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts. These vacuum dried extracts (400 and 600 µg/ml) were screened for antibacterial action by disc diffusion method, cup-plate method, and turbidimetric method against *Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis,* and *Staphylococcus aureus.* Aqueous extract was subjected to column chromatography and was fractionated into five fractions which were again screened for antibacterial action at the concentration of 40 µg/ml by disc diffusion method. Results showed that methanol and aqueous extracts significantly inhibited all the bacterial strain as compared to other extracts in all the tests. Fraction II obtained after column chromatography of aqueous extract showed best antibacterial activity. Hence we can conclude that compound responsible for antibacterial action is present in fraction II of aqueous extract of *D. regia* wood.

Key words: *Delonix regia*, wood, antibacterial, column chromatography, tannins, flavonoids.

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Introduction

Delonix regia is stirringly ornamental medium sized tree, planted in garden in all the warmer and damper parts of India. It has spreading crown and feather foliage and bears flower early in hot season. The flowers with panicles varying in color from deep crimson to scarlet orange to delicate salmon appears in profusion in broad ereil cluster along the branches presenting gorgeous appearance. The wood is white, soft and light. Traditionally plant is used as anthelmintic, antimicrobial, anticancer, emetic, CNS depressant and in the treatment of anemia, fever, malaria, and dysmenorrhoea [1, 2]. As the half of world is suffering from bacterial infection and the sources of infection are very common due to poor sanitation, poor family hygiene, malnutrition, and crowded living conditions. So there is need to develop antibacterial drugs from herbal source. As *D. regia* wood is having antibacterial action traditionally, present work was undertaken to prove it scientifically and to find out constituents responsible for antibacterial activity.

Materials and methods

Plant material

Fresh samples of wood of *D. regia* Linn was collected from wild sources in October 2006 from Ahmednagar district (Maharashtra), shade dried at room temperature and authenticated by Mr. S.C. Mujumdar, Botanical Survey of India, Pune (Voucher Specimen No. MKP1).

Extraction

The powdered wood (400 g) was subjected for successive extraction in Soxhlet extractor using various solvents viz. petroleum ether (60-80 ^{o}C), chloroform, ethyl acetate and methanol [3]. Marc left was extracted using water in reflux condenser. All the extracts were concentrated and vacuum dried for further screening. Percentage yield of all five extracts was found to be 3.05 %w/w, 3.75 %w/w, 2.05 %w/w, 3.65 %w/w and 6.20 %w/w respectively.

Microorganisms

For antibacterial screening *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853) and *Bacillus subtilis* (ATCC6633) strains were procured from National Chemical Laboratory, Pune and maintained in standard condition.

Column chromatography

Aqueous extract (5 g) was subjected for fractionation by using column chromatography (30 cm X 2.5 cm) having silica gel (mesh size 60–120) as stationary phase. Column was eluted by gradient elution using methanol and ethanol (flow rate: 10 drops/min).

Table 1. Fractionation of aqueous extract by column chromatography

Designation	Solvent	Yield (% W/W)
AQ1	Methanol	22.03
AQ2	Methanol : Ethanol (8 : 2)	34.42
AQ3	Methanol : Ethanol (6 : 4)	17.32
AQ4	Methanol : Ethanol (4 : 6)	15.06
AQ5	Ethanol	9.50

Antimicrobial activity

Disk diffusion method

Above mentioned bacterial strains and HIMEDIA M173-Muller Hinton agar media was used for screening antibacterial activity of all extracts and fractions isolated from aqueous extract. In this method suspension of bacterial strains was inoculated in nutrient agar media and then poured in petridish. Whatman filter paper discs soaked in all extracts (400 and 600 µg/disc) and all fractions (40 µg/disc) were placed on the media. A standard disc of Gentamycine (10 µg/disc) was also kept. These plates were kept at 4^0C for 1 hour before keeping them for incubation and then incubated for 24 hrs at 37^0C. Antibacterial activity of various extracts, fractions and standard drug was measured in terms of zone of inhibition [4].

Table 2. Antibacterial activity of various extracts of *D. regia* wood by disc diffusion method

Extracts	Concentration (µg/disc)	Zone of inhibition (mm)			
		B. subtilis	***E. coli***	***S. aureus***	***P. aeruginosa***
Petroleum ether extract	400	-	-	-	-
	600	-	-	-	-
Chloroform extract	400	-	-	-	-
	600	-	-	-	-
Ethyl acetate extract	400	-	-	-	-
	600	-	-	-	-
Methanol extract	400	24	25	22	24
	600	25	30	31	31
Aqueous extract	400	26	25	29	34
	600	27	32	33	35
Gentamycin	10	29	26	27	21

Table 3. Antibacterial activity of various fractions of aqueous extract by disc diffusion method

Fraction	Zone of inhibition (mm)			
	B. subtilis	***E. coli***	***S. aureus***	***P. aeruginosa***
AQ1	17	15	14	16
AQ2	28	24	27	22
AQ3	15	16	16	21
AQ4	14	19	14	20
AQ5	17	16	25	17

Cup plate method

As like Disk-diffusion method, here above mentioned bacterial strains and HIMEDIA M173-Muller Hinton agar media was used for screening antibacterial activity of all extracts. Here in each plate, wells of 6 mm diameter were made using a sterile borer and these microwells were filled with different extracts (400 and 600 µg/ml). A well was filled with standard Gentamycine solution (10 µg/ml). These plates were then incubated at 37^0C for 24 hours and zone of inhibition of microbial growth were measured [5].

Table 4. Antibacterial activity of various extracts of *D. regia* wood by cup-plate method

Extracts	Concentration (µg/ml)	Zone of inhibition (mm)			
		B. subtilis	***E. coli***	***S. aureus***	***P. aeruginosa***
Petroleum ether extract	400	-	-	-	-
	600	-	-	-	-
Chloroform extract	400	-	-	-	-
	600	-	-	-	-
Ethyl acetate extract	400	-	-	-	-
	600	-	-	-	-
Methanol extract	400	34	27	21	27
	600	36	29	22	29
Aqueous extract	400	38	39	24	30
	600	40	40	26	31
Gentamycin	10	39	40	26	32

Turbidimetric method

Nutrient broth having composition, Peptone 1 g, Yeast extract 0.3 g, Sodium chloride 0.5 g and distilled water 50 ml was used in turbidimetric method. Bacterial suspension was prepared in sterile normal saline solution. Different concentrations of all extracts as 333µg/ml, 111µg/ml, 37µg/ml, 12.03µg/ml, 4µg/ml and 1.2µg/ml were prepared in test tubes. Bacterial suspension 0.1 ml was added to all test tubes and tubes were incubated at 37^0C for 24 hours. The growth in tubes was observed by turbidity. MIC was determined by lowest concentration of extract that prevented the development of turbidity [6].

Phytochemical screening of active extract

In order to find out the active constituents responsible for the antibacterial activity phytochemical tests were performed on the methanol and aqueous extracts (Table 6).

Table 5. MIC values of various extracts of *D. regia* wood against bacterial strains by turbidimetric method

Extracts	*B. subtilis*	*E.coli*	*S. aureus*	*P. aeruginosa*
Petroleum ether extract	-	-	-	-
Chloroform extract	-	-	-	-
Ethyl acetate extract	-	-	-	-
Methanol extract	111µg/ml	333µg/ml	333µg/ml	333µg/ml
Aqueous extract	111µg/ml	333µg/ml	333µg/ml	333µg/ml

Table 6. Phytochemical screening of methanol and aqueous extract of D. regia wood

Test performed for	Methanol extract	Aqueous extract
Carbohydrate	+	+
Gums	+	+
Mucilage	+	+
Protein	+	+
Amino acids	+	+
Fats and oils	-	-
Steroids	-	-
Volatile oils	-	-
Glycosides	-	-
Saponin glycoside	+	+
Flavonoids	+	+
Alkaloids	+	+
Tannins and phenolic compounds	+	+

'+' Indicates presence and '-' indicates absence.

Results and conclusion

Results revealed that aqueous extract showed prominent antibacterial activity against all strains followed by methanol extracts in disc diffusion method, while other extracts failed to show antimicrobial activity. The activity was compared with standard drug gentamycin (Table 2). Similar types of results were obtained in case of cup-plate method (Table 4). Further the results were confirmed by turbidimetric method. MIC of methanol and aqueous extracts against all strains are comparable. Inhibition of *B. subtilis* was achieved at lowest concentration by methanol and aqueous extracts, while other extracts showed no inhibition of any strains (Table 5). From this we can conclude that only polar constituents of *D. regia* wood are responsible for antibacterial action. Preliminary phytochemical tests were carried out for methanol and aqueous extracts in order to find out type of secondary metabolites present. It showed that, both extracts contains saponins, flavonoids, alkaloids and tannins. Previous study reported that these types of secondary metabolites are responsible for antibacterial action [7, 8, 9, 10] which supports our findings.

In order to find out bioactive compound, aqueous extract was fractionated using column chromatography. Column was eluted using various concentrations of solvents to offer 5 fractions of different yield (Table 1). These 5 fractions were screened for antibacterial activity using same bacterial strains by disc diffusion method. Results showed that fraction AQ 2 is most potent antibacterial (Table 3). Further purification and spectroscopic study of the fraction is necessary in order to identify antibacterial compound.

References

1. Anonymous. The Wealth of India. Vol. III, Council of Scientific and Industrial Research; New Delhi, 2003, p. 30.
2. K. R. Kirtikar and B. D. Basu. Indian Medicinal Plant. 2nd edition, Vol. III; International Book Distributors; Deharadun, 1987. p.1193.
3. P. K. Mukharjee. Quantity Control of Herbal Drugs. 1st edition, Business Horizons Pharmaceutical Publishers; New Delhi, 2002. p. 117-492.
4. S. M. Pawaskar and K. U. Kale. Antibacterial activity of successive extracts of *Mimosa pudica*. Indian Drugs 2006; **43:** 476–479.
5. P. Selvamani and S. Latha. Studies on the antimicrobial activity of *Cadaba indica* Lam. Ind. J. Pharm. Sci. 2005; **67:** 637-638.
6. Anonymous. The Indian Pharmacopoeia. 4th ed., Vol. II, New Delhi: Government of India, Ministry of Health and Family Welfare; 1996. p. Appendix 105-8.
7. C. Cioacá, C. Margineanu and V. Cucu. The saponins of *Hedera helix* with antibacterial activity. Pharmazie. 1978; **33:** 609-610.
8. A. Hisanori, F. Kazuyasu, Y. Osamu, O. Takashi and I. Keiji. Antibacterial activity of several tannins against *Staphylococcus aureus.* J. Antimicro. Chemother. 2001; **48:** 487–491.
9. F. Scazzocchio, M. F. Cometa, L. Tomassini and M. Palmery. Antibacterial activity of *Hydrastis canadensis* extract and its major isolated alkaloids. Planta Med. 2001; **67:** 561-564.
10. P. Stjpan, K. Zdenka and Z. Marijana. Antimicrobial activity of flavonoids from *Pelargonium radula* (Cav.) L' Herit. Acta Pharm. 2005; **55:** 431–435.