



EVALUATION OF ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT FRACTIONS OF *DELONIX ELATA* BARK

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ABSTRACT

This study was carried out with an objective to investigate the antibacterial and antifungal potential of bark of *Delonix elata*. Antibacterial activity of various fractions obtained from methanolic extract (PE, DM, EA, MR) of bark were carried out against three Gram positive bacteria – *Staphylococcus aureus*, *Staphylococcus albus*, *Enterococcus faecialis* and three Gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella*. The antifungal activity of the fractions was evaluated on two common pathogenic fungi *Candida albicans* and *Cryptococcus neoformans*. The testing was done by the disc diffusion method. Zones of inhibition of fractions were compared with that of standard Amikacin for antibacterial activity and Ketoconazole for antifungal activity. The EA and MR fractions showed significant antibacterial activity but did not exhibit anti-fungal activities comparable with that of standard against the organisms tested.

Keywords: Antibacterial, Antifungal, *Delonix elata*, Amikacin sulphate, Ketoconazole.

INTRODUCTION

Antibiotic resistance has become a global concern in recent years. This problem is of great significance especially in developing countries because infectious diseases are one of the major causes of mortality in these countries¹. Higher plants, as a source for new potential drugs are still largely unexplored and only a small percentage of them have been subjected to phytochemical investigation and the fractions submitted to pharmacological screening are very low. Such screening of various natural organic compounds and identifying active agents is the need of the hour as due to successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development. Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases². The plant extract and fractions have been developed and proposed for use as antimicrobial substances³. Many plants used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine⁴. Due to rapid increase in the rate of infections, antibiotic resistance against microorganisms and side effects of synthetic antibiotics, medicinal plants with antimicrobial potentials are gaining popularity⁵. Thus it is important to screen traditional medicinal plants for their antimicrobial potentials⁶⁻⁸. Antimicrobial activities of many plants have been reported by the researchers^{9, 10}. The antimicrobial activities of medicinal plants can be attributed to the secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids that are present in these plants¹¹.

Delonix elata is a flowering plant belonging to the family Fabaceae. This plant was reported to have anti-oxidant¹², anti-arthritic and anti-inflammatory activities¹³. Hence an attempt was made to fractionate the methanol extract and study them for anti-microbial activity.

MATERIALS AND METHODS

Plant material

Delonix elata barks were collected from the outskirts of Tiruchengode, Tamilnadu, India, in the month of August 2011. It was authenticated by Prof. Dr. M.D.Rajanna, GKVK, Bangalore, Karnataka, India. The barks were dried under shade after they were made into small pieces. It was then powdered and passed through sieve 40 before subjecting to extraction.

Extraction and fractionation

400 grams of shade dried powder bark material was extracted continuously by methanol until the plant material became colourless. The crude methanol extract obtained was concentrated by rotary flash evaporator and fractionated with petroleum ether (PE), Dichloromethane (DM), ethyl acetate (EA) successively. The remaining portion was considered as mother residue (MR). All the fractions were subjected for phytochemical tests to analyse the chemical constituents present in them.

Microorganisms

The test microorganisms used for the antimicrobial activity screening were gram positive bacteria *Staphylococcus aureus*, *Staphylococcus albus* and *Enterococcus faecialis* and gram negative bacteria's *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The antifungal activity of the fractions was evaluated on two common pathogenic fungi *Candida albicans* and *Cryptococcus neoformans*. The strains were maintained in sterile conditions and grown on Nutrient Agar (NA) for bacteria and Sabouraud dextrose agar (SDA) for fungi in the Dept. of Microbiology, PES Institute of Medical Sciences and Research, Kuppam-517425, India.

Antimicrobial Activity

Antibacterial assay

Paper disc diffusion method was employed for screening antibacterial activity. Samples of each PE, DM, EA and

MR fractions (1mg/ml and 2mg/ml) were dissolved in methanol and sonicated and filtered. Sterile 5mm diameter of whatman no4 paper discs were impregnated with these fractions of different concentrations. Adequate amount of Muller Hinton Agar (HIMEDIA -M173) were dispensed into sterile plates and allowed to solidify under aseptic conditions. The test organisms were inoculated on nutrient broth and incubated for 2 hours at 37 ± 0.1°C. The test fractions were placed with a sterile spreader on the surface of solid medium in plates and incubated for 24 hours at 37 ± 0.1°C.

All the plates were observed for zones of inhibition and the diameters of these zones were measured in millimetres. All the procedures were performed under sterile conditions. Amikacin standard disc 30µg/disc (HIMEDIA-SD035) was used for the antibacterial assay. The experiments were performed in duplicate.^{14, 15}

Antifungal assay

The antifungal activity was tested by disc diffusion method. The sabouraud dextrose agar (HIGHMEDIA-M063) plates were inoculated with *Candida albicans* and *Cryptococcus neoformans* cultures by point inoculation. The filter paper disc (5mm in diameter) impregnated with 1mg/ml and 2mg/ml concentration of the fractions were placed on test organism-seeded plates. Ketoconazole standard disc 30µg/disc (HIGHMEDIA-SD275) was used for the antifungal assay. The activity was determined after 72hours of incubation at 28°C. The inhibition zones were measured in mm. All tests were performed under sterile

conditions. The experiments were performed in duplicate¹⁶.

RESULTS AND DISCUSSION

Delonix elata when subjected for extraction with methanol yield 15% w/w of extractive value. Upon fractionation of methanol extract it yield 7.5% of PE, 19% of DM, 29.5% EA fractions and 44% was left out as mother residue.

In the qualitative chemical tests PE fraction gave positive tests for fixed oils, phytosterols, and steroidal compounds. DM fraction showed positive for steroidal compounds and trace of alkaloids while EA and MR gave positive answers for the presence of flavonoids, saponins, Tannins. The current study revealed that among the tested methanolic extract fractions EA possess significance antibacterial activity against most organism except *P.aeruginosa*. MR showed better protection of growth against *E.faceialis*, *P.aeruginosa*, *K.Pneumonia* While PE and DM were not significantly protected the growth (Table 1, Figure 1). In antifungal screening also EA fraction exhibited 18mm zone of inhibition against *Candida albicans* and 20mm against *Cryptococcus neoformans* (Table 2). This may be due to the presence of phenolics and flavonoids in EA and MR fractions. All other fractions were not shown significant activity. Further research is necessary to determine the identity of the antibacterial compounds from EA and MR fractions.

Table 1: Antibacterial activity of different fractions of *Delonix elata* barks on bacterial strains.

Average diameter of zone of inhibition of different bacteria(mm)								
S.no	Fraction/Standard	(mg/ml)	<i>S. aureus</i>	<i>S. albus</i>	<i>E. faceialis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae.</i>
1	PE	1	7	NA	8	8	8	12
		2	7	NA	12	10	11	13
2	DM	1	8	NA	NA	10	10	11
		2	8	NA	NA	11	12	11
3	EA	1	11	NA	11	11	7	11
		2	13	NA	14	14	9	14
4	MR	1	6	NA	15	8	14	13
		2	10	NA	15	9	16	13
5	Amikacin	30µg	16	38	14	19	21	8

Zone including 5mm of paper diameter

NA- No activity

Table 2: Antifungal activity of different fractions of *Delonix elata* barks on fungal strains.

Average diameter of zone of inhibition of fungi (mm)				
S.no	Fraction/ Standard	(mg/ml)	<i>Candida albicans</i>	<i>Cryptococcus neoformans</i>
1	PE	1	10	11
		2	10	13
2	DM	1	9	8
		2	10	11
3	EA	1	16	17
		2	18	20
4	MR	1	10	12
		2	10	15
5	Ketoconazole	30µg	39	36

Zone including 5mm of paper diameter

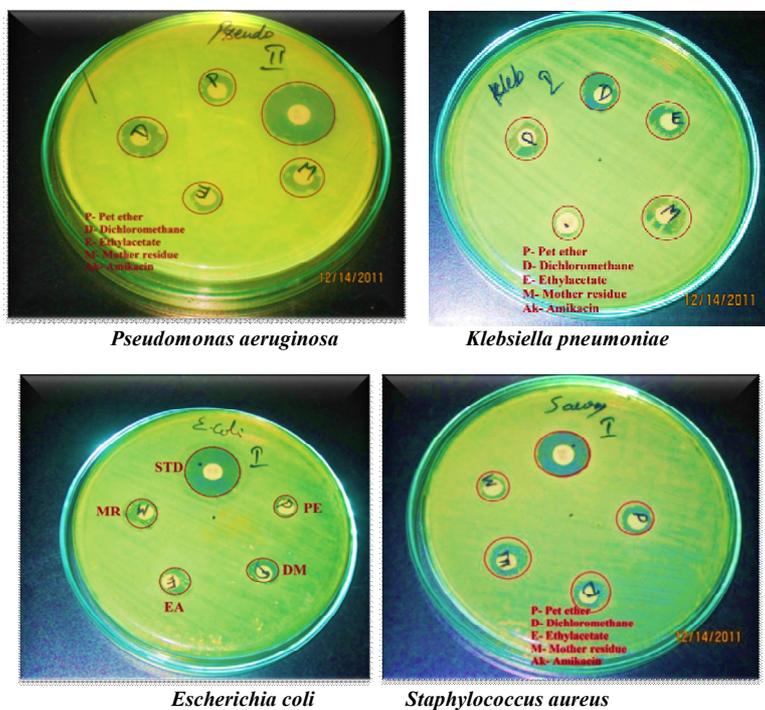


Figure 1: Antimicrobial activity of the fractions against few Bacteria's

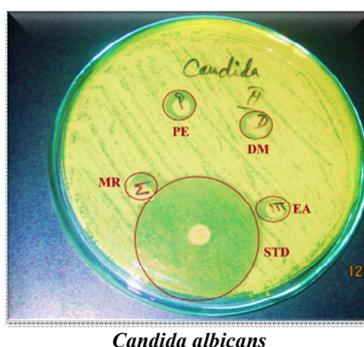


Figure 2: Antimicrobial activity of the fractions against fungi

CONCLUSION

Among *Delonix elata* fractions EA and MR fractions showed significant protection against bacteria and fungus. These can be further subjected for isolation of active principles which may lead to potential molecules from natural source. Thus this plant could be utilized as an alternative antimicrobial source.

REFERENCES

- Kroschwitz JI, Howe-Grant, Kirk-Othmer. Encyclopaedia of Chemical Technology. 1992; 2: 893.
- Starry F, Hans S. The National guides to medical herbs and plants. Tiger Books. Int. Plc. UK 1998.
- Del Campo J, Amiot MJ, Nguyen C. Antimicrobial effect of Rosemary extract. Journal of Food Protection 2000; 63(10):1359-1368.
- Mann A, Bansa A, Clifford LC. An antifungal property of crude plant extracts from *Anogeissus leiocarpus* and *Terminalia avicennioides*. Tanzania Journal of Health Research. 2008; 10 (1):34-38.
- Reddy PS, Jamil K, Madhusudhan P. Antibacterial activity of isolates from *Piper longum* and *Taxus baccata*. Pharmaceutical Biology. 2001; 39(3):236-238.
- Bajpai M, Pande A, Tewari SK, Prakash D. Phenolic contents and antioxidant activity of some food and medicinal plants. International Journal of Food Sciences and Nutrition, 2005;56(4): 287-291.
- Wojdylo A, Oszmianski J, Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chemistry.2007; 105(3):940-949.
- Mothana RAA, Lindequist U. Antimicrobial activity of some medicinal plants of the island Soqatra. Journal of Ethno pharmacology, 2005;96(1-2):177-181.
- Murganathan G, Sathyachethan P. Antimicrobial constituents from Plants. International Research Journal of Pharmacy. 2012; 3(1):5-9.
- Sher A. Antimicrobial activity of natural products from medicinal plants. Journal of Medicinal Sciences. 2009; 7(1):72-78.
- Sini KR, Sinha BN, Karpagavalli M. Determining the antioxidant activity of certain medicinal plants of Attapady, (Palakkad), India using DPPH assay. Current Botany.2010; 1(1):13-16.
- Murganathan G, Mohan S. Anti-inflammatory and Anti-arthritis activities of *Delonix elata* bark extracts. International Journal of Research in Ayurveda and Pharmac. 2011; 2(6):1819-1821.
- Sivanarayan V, Suriyavathana. Preliminary studies on phytochemicals and antimicrobial activity of *Delonix elata* and *Prosopis cineraria*. International Journal of Current Research, 2010 Sep; 8:66-69.
- Rahul R, Asha A, Anjali D, Rukiar B, Priya S, Ankur AK, Nirmala R, Jyoti P. Antimicrobial activity of different extracts of *Juglans regia* L. against oral microflora. International Journal of Pharmacy and Pharmaceutical Sciences.2011; 3(2):200- 201.
- Jigna P, Sumitra VC. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal Plants. Turkish Journal of Biology.2007; 31(1):53-58.
- Gayathri G, Saraswathy A, Vijayalakshmi K. Antimicrobial activity of medicinal plant *Bauhinia variegata* Linn. International Journal of Pharmacy and Biological Sciences.2011; 1(4):400-408

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