

ANTI BACTERIAL ACTIVITY OF FLOWER EXTRACT OF *PONGAMIA PINNATA* Linn. - AN ELITE MEDICINAL PLANT

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ABSTRACT

Antibacterial potential of flower extracts of *Pongamia pinnata* Linn was traced in the present investigation using agar well diffusion method. The flower pigments were extracted by using Liquid-Liquid extraction method with 80% acetone in water and petroleum ether as solvents, which inhibited the growth of six potential pathogenic bacteria *Escherichia coli*, *Bacillus cereus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Enterobacter aerogenes*. Maximum zone of inhibition was recorded in *S. aureus* (24 mm), and *B. cereus* (23 mm) followed by *E. coli*, *B. subtilis*, *E. aerogenes* and *K. pneumoniae* with 22, 19, 19 and 17 mm respectively at the concentration of 1600 µg. This study confirms the efficacy of *Pongamia* flower pigment extracts as natural and broad spectrum antibacterial activity and suggests the possibility of their use in the treatment of various bacterial infections and to discover the new bioactive compounds which can also be used for prophylactic treatment.

Keywords: Antibacterial potential; *Pongamia pinnata*; agar well diffusion method; Liquid - Liquid extraction method; bioactive compounds.

INTRODUCTION

In view of the increasing incidences of infections with emerging multidrug resistant and treatment failures hitherto caused by them there is very little choice left for the physicians to treat such infections. There have been only fewer drugs introduced in the market in the past two decades compared to the increased number of bacterial population including both the gram-positive and gram-negative bacteria developing resistance. The occurrence of gram positive cocci like, *Staphylococcus aureus* (MRSA) resistant to most of the drugs like vancomycin and others including *Enterococcus* spp, coagulase negative *Staphylococci* which are multidrug resistant is a cause of concern¹. *S. aureus* is a normal flora of 20-40% of healthy individuals in the anterior nares and has also been the most notorious agent in causing serious Hospital Acquired Infections (HAI). Resistance to Methicillin (MRSA) has been reported both in the hospital and the community. The spread of such drug resistant bacteria can be responsible for future treatment failures².

Multidrug resistant gram-negative bacteria due to production of β -lactamases, metallo- β -lactamases and carbapenemases are difficult to treat. Considering the above facts it's imperative that as the microbial population develops resistance, we have to keep up the pace to discover newer, effective and easily manufactured chemotherapeutic agents.

Plant-derived chemicals are valuable sources for a variety of pharmaceuticals, flavors, dyes, oils, and resins^{3,4}. Bioactive compounds currently extracted from plants are used as food additives, pigments, dyes, insecticides, cosmetics, perfumes and fine chemicals⁵. Many of the plant species that provide medicinal herbs have been scientifically evaluated for their possible medical applications⁶. Medicinal and aromatic plants are the most exclusive source of life saving drugs for the majority of the world's population. The World Health Organization estimated that over 65% of the world's population relies on traditional medicine for their primary health needs^{7,8}.

Phytomedicine represents one of the most imperative fields of traditional medicine all over the world. To uphold the proper use of phytomedicines and to find out their potential use as a source of new drugs, it is essential to study medicinal plants, which have traditional reputation. As a part of this, for the first time the present experiment was conducted to investigate the antibacterial activity of *P. pinnata* flower extracts.

Pongamia pinnata Linn. (Fabaceae) is one of the important medicinal plants and commonly known as *Karanja* or *Kanuga*

originated in tropical and temperate Asian countries. This plant contains several medicinally important phytochemicals and hence traditionally plant parts are used in treatment of different diseases. In unani and ayurveda systems of medicine, it is used as anti-inflammatory⁹, antinociceptive, anti-plasmodial, anti-lipidoxidative, anti-hyperglycemic, anti-ulcer, anti-hyperammonic, CNS depressant¹⁰. The fruits and sprouts of *P. pinnata* were used in folk remedies for tumors¹¹. Seed extracts are hypotensive effects and produce uterine contractions. Seed powder is used in chronic fever and chronic skin diseases and painful rheumatic joints. Seed oil is used in scabies, leprosy, piles, ulcers, chronic fever, liver pain and lumbago. Its oil is a source of biodiesel and also used as fuel for cooking and lamps¹². Traditionally, its bark is used in treatment of piles; leaves are effective as medicated bath and rheumatic pains; and the seeds are used in hypertension, bronchitis, whooping cough, skin diseases and rheumatic arthritis^{13, 14, 15}. In Malaysian and Indian folk medicinal system, root extracts are applied to abscesses; other plant parts, especially crushed seeds and leaves are regarded as having antiseptic properties¹⁶. The literature survey showed that *P. pinnata* is a potential medicinal plant. Since, there is no report on antibacterial activity of flower extract of *P. pinnata*; the present investigation was conducted to find the antibacterial properties.

MATERIALS AND METHODS

Plant Material

P. pinnata flowers were collected from Kakatiya University medicinal garden and authenticated by Prof. N. Rama Swamy, Department of Biotechnology, Kakatiya University, Warangal. The collected fresh flowers were washed with sterilized distilled water followed by blot drying and used for the current investigation.

Preparation of extract

The floral pigments were isolated from petals of fresh flowers by using liquid-liquid extraction method. Hundred grams of petals were blended by using sterile pestil and mortar and extracted with 100 ml of 80% acetone in water. Then extraction was filtered through four layered cheese cloth. This filtrate was extracted with 30 ml of petroleum ether by using separating funnel. This step was repeated for three times to collect the pigments present in acetone extract and the collected organic solution was washed with distilled water to remove the impurities. Later the crude pigment extract present in organic layer was concentrated using a rotary evaporator and the solvents were completely removed *in vacuo* and stored at 4°C for further use.

Bacterial strains used

The standard pathogenic bacterial strains were procured from the Department of Microbiology, Kakatiya University, Warangal and used in the present study. The bacterial strains such as *Escherichia coli*, *Bacillus cereus*, *B. subtilis*, *Klebsiella pneumoniae*, *Styphalococcus aureus* and *Enterobacter aerogenes* were used for antimicrobial assay. The bacterial cultures were revived in Mueller- Hinton broth (Hi-media laboratories, Mumbai, India) at 37°C for 16 - 18 h and then preserved at 4°C. Subcultures were prepared from the stock for bioassay. A loopful of culture was inoculated in 10 ml of sterile nutrient broth and incubated at 37°C for 3-4 h. Turbidity of the culture was standardized to 10⁵ CFU with the help of Standard Plate Count and turbidometer.

Screening of antibacterial activity

Antibacterial activity of flower pigments extract of *P. pinnata* was carried out by agar well diffusion method¹⁷. Petri plate containing 20 ml nutrient agar medium was inoculated with 0.1 ml of 18 hour old bacterial suspension culture by spread plate method to form lawn cultures. The floral pigment extract was dissolved in Dimethyl sulfoxide (DMSO) and sterilized by using Sortorius syringe filter of pore size 0.22 µm. Various concentrations of the extracts 400 µg, 800 µg, 1200 µg and 1600 µg were added into the sterile 6 mm diameter well (stock solution 0.04 g/1ml) and incubated for 24hrs at 37°C. Streptomycin sulphate (Hi-media laboratories, Mumbai, India) (30 µg) and DMSO (15 µl) were used as positive and negative control. Incubation was made at 37°C for 24hrs. Antibacterial

activity was assayed by measuring the diameter of the inhibition zone formed around the well. The diameter of the clearing zones was measured in mm to obtain a semi quantitative determination of the concentration of the antibacterial pigment.

RESULTS AND DISCUSSION

In the present study, crude floral pigment extract of *P. pinnata* showed significant inhibitory effect against *E. coli*, *B. cereus*, *B. subtilis*, *K. pneumoniae*, *S. aureus* and *E. aerogenes* and the results are presented in Table 1. The crude extract, showed maximum inhibition for the species *S. aureus* (24 mm), and *B. cereus* (23 mm) which is almost equal to antibiotic streptomycin inhibition followed by *E. coli* (22 mm), *B. subtilis* (19 mm), *E. aerogenes* (19 mm) and *K. pneumoniae* (17 mm) at the concentration of 1600 µg (Fig 1). It was reported that the chloroform extract of *P. pinnata* bark showed an inhibition zone of 14.51 mm in *S. aureus*, 14.18 mm in *B. subtilis* and 9.38 mm in *E. coli* at a concentration of 1000 µg/ml¹⁸. At the same time the petroleum ether extract of *P. pinnata* leaves showed an inhibition of 11 mm in *E. coli*, 14 mm in *E. aerogenes* and no inhibition activity on *B. subtilis* and *K. pneumoniae* at a concentration of 2000 µg/ml¹⁹. Crude *P. pinnata* floral extract showed a minimum inhibition at 400 µg in *B. subtilis* (6mm) followed by *E. aerogenes* (7 mm), *K. pneumoniae* (8 mm), *B. cereus* (9 mm), *S. aureus* (10 mm) and *E. coli* (13 mm), showed similar results to that of crude ethanol flower extract of *Rumex vesicarius*²⁰. The findings of this study reveal that the floral extracts showed better inhibition activity than bark extracts (chloroform) of *P. pinnata*.

Table 1: Anti bacterial activity of crude flower pigment extract of *P. pinnata*.

Test organisms	Zone of inhibition (mm)				Positive control (30µg)	Negative control (15µl DMSO)
	400µg	800µg	1200µg	1600µg		
<i>Escherichia coli</i>	13	15	18	22	26	0
<i>Bacillus cereus</i>	9	14	18	23	23	0
<i>Bacillus subtilis</i>	6	10	15	19	24	0
<i>Klebsiella pneumoniae</i>	8	10	13	17	18	0
<i>Staphylococcus aureus</i>	10	15	20	24	25	0
<i>Enterobacter aerogenes</i>	7	11	14	19	22	0

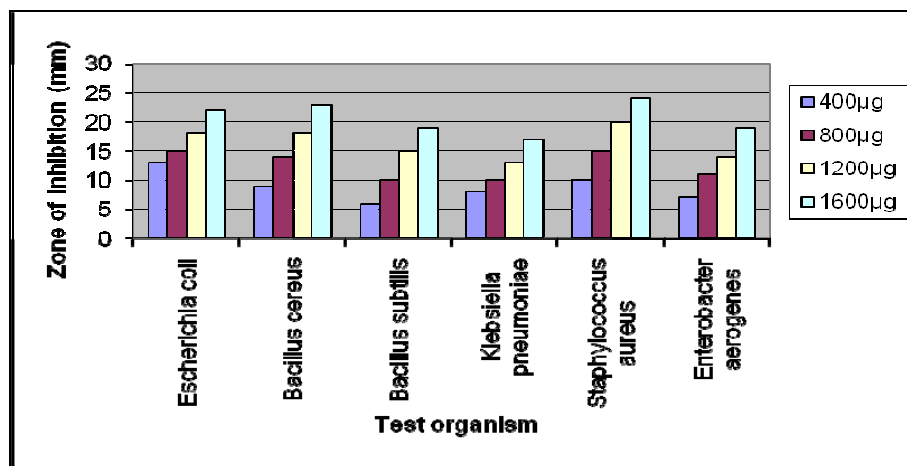


Fig. 1: Antibacterial activity of flower extract of *P. pinnata*

From these results it is clear that extracts obtained from the floral pigments of *P. pinnata* showed significant broad spectrum antibacterial activity on pathogenic bacteria and the inhibitory property was found to be increased with rise in the minimum inhibitory concentration of floral extract.

CONCLUSION

The above results show promising evidence for the antibacterial properties of flower extracts of *P. pinnata*. The flower extracts showed antibacterial activity against enteric pathogens like *E. coli*, *B.*

cereus, *B. subtilis*, *K. pneumoniae*, *E. aerogenes* and *S. aureus* which are the most significant bacterial agents causing serious infections. Therefore we suggest further studies on analyzing the chemical entity that is present in the floral extract of *P. pinnata* and evaluate its usefulness in the treatment of various bacterial infections and to discover the newer bioactive compounds.

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