

Antibacterial Effect of *Pongamia pinnata* Leaf Extract against Some Human Pathogenic Bacteria

Aviral Asaiya*, Diwyansh Raj, Chokhelal Prajapati

Forest Protection Division, Tropical Forest Research Institute, Jabalpur, (M.P.) India

ABSTRACT

Medicinal plants play an important role in the discovery of novel drugs used in modern medicine. The medicinal plant *Pongamia pinnata* leaf possesses the wide range of medicinal properties which were confirmed through literature reviews. The leaves of the *Pongamia pinnata* have been used for the medicinal purposes since ancient time research has suggested that *Pongamia pinnata* leaf extracts have antimicrobial properties. The present study was to determine its leaves have any antibacterial activity leaf aqueous extract were screened for against different species of human pathogens, *Chromobacterium violaceum*, *Citrobacter freundii*, *Staphylococcus aureus* and *Micrococcus luteus*. The activity of leaf aqueous extracts was measured by agar well diffusion method. Nutrient Agar Media (NAM) was prepared for growth of bacteria strains; leaf aqueous extract was obtained by mixing the pulverized leaf materials with ethanol, filtered with Whatman No. 1 filter paper and concentrated to dryness. The collected extracts were tested for antibacterial activities, after that plates placed in incubator for incubation at $37 \pm 2^\circ\text{C}$ for 24 hours. Measure the zones of inhibition obtained. The obtained data were statistically analyzed by using standard deviation and standard error. The highest zone of inhibition of *P. pinnata* ethanolic leaf aqueous extract was measured against gram-positive bacteria *Micrococcus luteus* (38 mm) and smallest was measured against *Citrobacter freundii* (17.6 mm). In this present study we have evaluated the efficacy of *P. pinnata* leaf extract against four different human pathogenic bacteria.

Keywords: Antimicrobial activity; *Pongamia pinnata*; Human pathogen; Medicinal plant; Agar well diffusion; Facultative anaerobes; Obligate aerobes

INTRODUCTION

Pongamia name has been coined from the Tamil name of plant Pongam, in Western India the tree goes under the name of karanj and it is probably due to the abundance of such tree in the area that the Island of Karanja, near Bombay was so named. It's a mangrove plant belonging to the family, Fabaceae (Papilionaceae) [1]. It is also called as *Derris indica* and *Pongamia glabra* [2]. The tree is occasionally seen along roads or streets in peninsular India it is indigenous throughout India from the foothills of the Himalayas down to the south of the Peninsula especially not far from the sea-coasts, it is found in deep forests. It is one of the few nitrogen-fixing trees producing seeds/kernels containing 18-27% oil [3]. Leaf and oil obtained from *Pongamia pinnata* shows antimicrobial activity against *Aspergillus niger*, *Staphylococcus aureus*, *Chromobacterium violaceum*, *Citrobacter freundii*, *Chromobacterium* and *Pseudomonas aeruginosa*, which was carried out by employing Minimum Inhibitory Concentration (MIC) [4]. This plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hitherto

unmet therapeutic needs [5]. It is hoped that study would lead to the establishment of some compound that used to formulate new and more potent antimicrobial drug of natural origin. Many herbal remedies individually or in combination have been recommended in various medical treatises for the cure of different diseases [6].

Different parts of the plant have been used in traditional medicine for bronchitis, whooping cough, rheumatic joints and to quench thirst in diabetes. Leaves are hot, digestive, laxative, anthelmintic and cure piles, wounds and other inflammations [7]. A hot infusion of leaves is used as a medicated bath for relieving rheumatic pain and for cleaning ulcers in gonorrhoea and scrofulous enlargement. Different extracts of leaves, roots and seeds are used to treat infectious diseases such as leucoderma, leprosy, lumbago, muscular and articular rheumatism [8]. Fruits and sprouts of *P. pinnata* were used in folk remedies for abdominal tumours in India and seed extract has hypotensive effects and produces uterine contractions. Flowers are prescribed for glycosuria and diabetes [9]. The bark is used internally for bleeding piles, beriberi and as an antimicrobial. Karanja seed is used as a medicinal plant, particularly with the

Correspondence to: Aviral Asaiya, Forest Protection Division, Tropical Forest Research Institute, Jabalpur (M.P.) India, Tel: +919424371609; E-mail: aaviral143@gmail.com

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Ayurvedic and siddha medicine system of India [10]. Leaves are active against microcococcus, their juice are use for cold, cough, diarrhoea, dyspepsia, gonorrhoea and leprosy and seed oil is used in itches, abscess, and other skin diseases [11]. Roots are used for cleaning gums, teeth and ulcers. Powdered seeds are valued as febrifuge, tonic and in bronchitis [12] and examined *in vitro* for antiplasmodial properties against *Plasmodium falciparum*. Ethanol extract of *P. pinnata* shows significant anti plasmodial activity [13] and leaf extract of *P. pinnata* shows circulatory lipid per oxidation and antioxidant activity [14].

Chromobacterium violaceum is a gram-negative, facultative anaerobic that can grow without need of oxygen, non-sporing coccobacillus [15]. It is motile with the help of single flagellum. It is part of the normal flora water and soil of tropical and sub-tropical regions of the world. *C. violaceum* rarely infected humans, but when it does it cause skin lesions, sepsis and liver abscesses that be fatal [16]. To date cause have been reported from Argentina, Australia, Brazil, Canada, Cuba, India, Japan, Nigeria, Singapore, Shri Lanka, Taiwan, United states and Vietnam [17]. The disease usually started as a limited infection of the skin of the point of entry of the bacteria which progress to necrotizing metastatic lesions then multiple abscesses of the liver, lungs, spleen, skin, lymph nodes or brain, leading to severe septicemia, culminating in multi organ failure which may be fatal [18].

Citobacter freundii is a gram-negative, facultative anaerobes, belongs to Entrebacteriaceae family. The bacteria have a long rod shape. *C. freundii* have several flagella used for the locomotion, but some do not and non-motile. *C. freundii* is found in soil but can also be found in water, sewage, food and in the intestinal tracts of animal and human [19], *C. freundii* caused urinary tract infection, healthcare associated infection, especially pediatric and immunocompromised patients, muco hemorrhagic diarrhea in animals being bacteremic and septicemia many organs and tissues are affected besides the gut [20].

Staphylococcus aureus is a gram-positive, round shaped, non-motile and does not form spores, facultative anaerobes, belongs to the family Staphylococcaceae. It is a member of the micro-biota of the body [21]. *S. aureus* usually acts as a commensally bacterium asymptotically colonizing about 30% of the human population, it can sometime causes infection like respiratory infection such as sinusitis, food poisoning [22]. Bacteremia and infective endocarditic additionally it can various skin and soft tissue infection including abscesses.

Micrococcus luteus is a gram-positive to gram-variable non-motile, coccus tetrad-arranging, pigmented, saprotrophic [23]. It is catalase positive and obligate aerobe [24]. That belongs to Micrococcales family. *M. luteus* found in soil, dust, water and air and as part of the normal micro-biota of the mammalian skin. *M. luteus* causes diseases are brain abscesses, Central nervous system infections [25].

MATERIALS AND METHODS

Sample collection and sterilization

Pongamia pinnata healthy leaves were collected from the field of Tropical Forest Research Institute, Jabalpur (M.P.) Surface sterilization of leaves with HgCl₂ (0.1%) for a duration of 6 minutes significantly reduced microbial contamination (7.78%) but also reduced the survival percentage (51.77%) and caused drying

of explants within 10 days of inoculation which may be due to phytotoxicity caused by long exposure of leaves with HgCl₂. All the treatment was statistically significantly different to each other [26]. leaves washed with household detergent to remove the impurities and then washed thoroughly under tap water and then sterilization was done with 70% alcohol for 2 minutes followed by 1% HgCl₂ for 2~3 minutes [27].

Leaf extract preparation

The extractions were done by following the methods mentioned elsewhere with slight modification [28]. For ethanol extraction, sample was mixed with ethanol at a ratio of 2:10 into sterile flask and was placed in orbital shaker water bath at 130r/min at 37°C overnight. The liquid samples were then filtered with Whatman No.1 filter paper. The extracted samples were stored in universal bottles and refrigerated at 4°C prior to use.

Culture media

The media use for the study was Nutrient Agar Medium (NAM) used for the bacteria growth.

Agar well diffusion assay

The well diffusion assay was used for testing antimicrobial activity. Each sterilized Petri plate was pre-seeded with 15 ml of respective growth agar medium and 80µl of bacterial culture and lawn was prepared by spread plate method to dry for 30 minutes, and then make wells with the help of sterile cork borer, prepared wells numbers was (A) Leaf aqueous extract (50 µl), (B) Blank, (C) Negative control (ethanol 70%) (50 µl) and last one was (D) Positive control (Antibiotic) so as to allow the diffusion of the substances and then incubated at 37±2°C for 18-24 hours in case of bacteria [29]. Measure the zones of inhibition obtained; the obtained data were statistically analysed by using standard deviation and standard error.

Statistical analysis

This Experiment was performed in triplicate in an independent manner. The data were expressed as mean and standard error of three replicates and values were analysed statistically (Figure 1).

RESULTS AND DISCUSSION

Antibacterial activity of leaf extract of *Pongamia pinnata* was evaluated against following pathogenic bacterial strains *Micrococcus luteus*, *Citrobacter freundii*, *Staphylococcus aureus*, and *Chromobacterium violaceum* both gram positive and gram negative bacteria has produced after 24 hours of incubation. It has found that leaf extract has showed minimum inhibitory concentration (MIC) against *Micrococcus luteus* was 38 mm, *Citrobacter frendii* 17.6 mm, *Staphylococcus aureus* 37.8 mm and *Chromobacterium violaceum* 33.6 mm (Table 1).

The traditional use of plants provides the basis for indicating the type of plant extracts useful for controlling various pathogenic microorganisms. Historically, many plant extracts have antimicrobial properties [12]. Also the renewal of interest in the food industry and increasing consumer demand for effective, safe, natural products means the qualitative data on plant extracts are well documented [5]. Therefore, plant extracts are known to be the

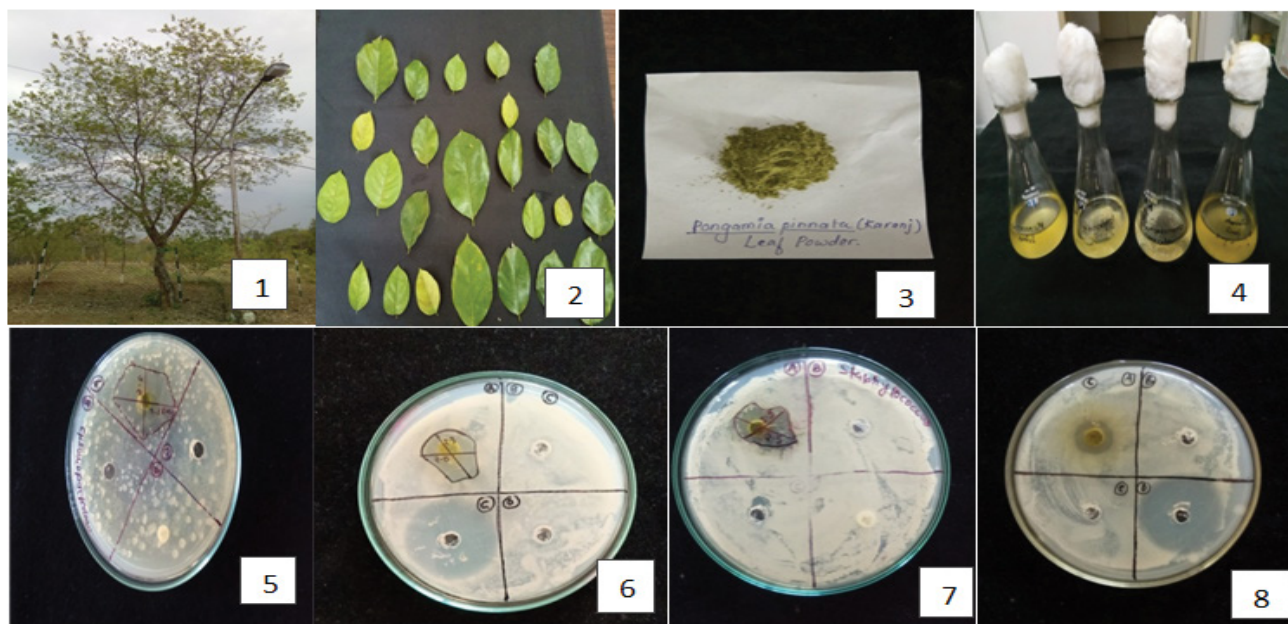


Figure 1: 1) Plant of *Pongamia pinnata*, 2) Fresh leaves of *Pongamia pinnata*, 3) Leaf powder, 4) Pathogenic bacterial cultures in NAM broth, 5), 6), 7) and 8) These four plate showing antimicrobial activity against *Chromobacterim violaceum*, *Citrobacter freundii*, *Staphylococcus aureus*, *Micrococcus luteus*.

Table 1: Zone of inhibition of *Pongamia pinnata* leaves extract in millimeters (mm).

Human pathogenic bacteria	Zone of inhibition* (in mm.)		
	Negative control (Ethanol)	Leaf extract	Positive control (Streptomycin)
<i>Micrococcus luteus</i>	-	38	85
<i>Citrobacter freundii</i>	-	17.6	79
<i>Staphylococcus aureus</i>	-	37.8	85
<i>Chromobacterium violaceum</i>	-	33.6	90

promising antimicrobial agents to inhibit the bacterial pathogens. The leaves and stem of *Pongamia pinnata* consist of several flavones, chalcone derivatives [30]. Reported the antimicrobial activity of flavonoids from leaf extract of *Pongamia pinnata* but it did not show activity against *S. epidermis*, *M. smegmatis*, *S. typhimurium*, and *C. candida*. That study to the lead to the establishment of some compound that used to formulated new and more potent antimicrobial drug against antibiotic resistant human and plant pathogens [31].

CONCLUSION

The leaves of *Pongamia pinnata* are Antioxidant and Anti-hypermmmonemic and have also Anti-diarrheal activity. The result of the antibacterial screening showed that leaf extract of ethanol have potential antibacterial effects against some pathogens: *M. luteus*, *C. freundii*, *S. aureus* and *C. violaceum*. *Pongamia pinnata* mediated organic extracts could be a source of natural antimicrobial agents for use in food or pharmaceutical industries to control food-borne pathogenic bacteria. Further study is in preparation to evaluate the bioactive compounds present in various organic extracts *Pongamia pinnata* are to be used for drug or food preservation, issues of safety and toxicity will always need to be addressed.

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