Original Article

Evaluation of Antibacterial Activity of *Pongamia pinnata linn* on Pathogens of Clinical Isolates

Mary Shobha Rani[1], C D Dayanand*[2], Jeevan Shetty[3] Pradeep Kumar Vegi[4], and A V Moideen Kutty[5]

[1]Central research Laboratory Department of Allied Health Sciences Sri Devaraj Urs Academy of Higher Education and Research Kolar, Karnataka, India.
[2]Department of Biochemistry: Head, Department of Allied Health Sciences Sri Devaraj Urs Academy of Higher Education and Research Kolar, Karnataka, India.
[3]Department of Microbiology Sri Devaraj Urs Medical College Kolar, Karnataka, India.
[4]Department of Biochemistry Central research Laboratory Department of Allied Health Sciences Sri Devaraj Urs Academy of Higher Education and Research Kolar, Karnataka, India.
[5]Department of Biochemistry & Dean, Department of Allied Health Sciences Sri Devaraj Urs Academy of Higher Education and Research Kolar, Karnataka, India.

Address for Correspondence

Department of Biochemistry: Head, Department of Allied Health Sciences Sri Devaraj Urs Academy of Higher Education and Research Kolar, Karnataka, India.
E-mail: cd8905@yahoo.co.in

ABSTRACT

Introduction: Plants are well known for the presence of antimicrobial compounds. Our study was to screen the antibacterial activity of the seed extracts of *Pongamia pinnata Linn*.
Material and Methods: *Pseudomonas aeruginosa*, *Staphylococcus aureus, Serratia marcescens, Micrococcus luteus*, *Proteus vulgaris* and *Klebsiella pneumonia* were isolated and cultured from clinical samples obtained from Department of Microbiology, R.L.Jalappa Hospital and Research Center, Tamaka Kolar. Good quality seeds were collected from local region of Kolar and authenticated by College of Horticulture science. Extracts were made with methanol (M) and ethanol (E) solvents. A fixed inhibitory concentration of 100µg/ml of seed extract was tested by using Agar well Diffusion method and the same compared with the antibiotic Ceftazidime at equal concentration.
Results: Methanol extracts of *Pongamia pinnata L*(PPM) showed higher antibacterial activity than ethanol extracts of *Pongamia pinnata L*(PPE).
Conclusion: *Pongamia pinnata L* has good bactericidal activity against the selected Hospitalized pathogens and the maximum activity evinced on *Pseudomonas aeruginosa* with zone of inhibition 20mm by methanol extract and 18.5mm on *Pseudomonas aeruginosa* in ethanol extract in comparison to Ceftazidime.

Keywords: Antibacterial activity, Ethanol extract, Methanol extract,

Pongamina pinnata linn.

INTRODUCTION

In Ayurveda the medicinal values of plants are well documented and revealed in the literature since ancient times. The expeditions for such medicinal plants are increasing day by day on account of man's quest for finding out newer compounds to health benefit[1]. The compounds obtained from plants were rich in phytochemicals such as phenolic acids, flavonoids, tannins, lignin and other small compounds [2].Such plants signify rich source of active principle exhibits numerous health related effects such as antimicrobial, antimutagenic, anticarcinogenic and vasodilatory activity[3]. The potential source of vascular plants is still not completely explored for the community utility. The screening of such plants for phytochemical compounds in order to evaluate pharmacological effect has become a random tool, very few vascular plants group with respect to antibacterial activity were studied[4,5]. Apart from medical uses, some of the plants are used as ingredients in composting in the manufacture of organic manure. These plants contain phyto-chemicals which inhibits the growth of pathogenic microbes causing disease in plants. The present study is aimed to evaluate the antibacterial activity of *Pongamia pinnata L.* on hospitalized pathogens, since this plant has been documented for several beneficial uses to mankind.

Pongamia pinnata L commonly known as Karanj or Indian beach tree belongs to family of Fabaceae is a flower and fruit generating angiosperm. The activities such as anti-plasmodium characteristics[6,] Anti-inflammatory activity[7], antidiarrheal[8], antiulceric[9], hypoglycemic property[10], wound healing property[11], like Jatropa for oil yielding or bio-fuel source[12], anticonvulsant activity[13] are reported. The antibacterial activity by *Pongamia* seed oil through tube dilution method was also reported[14]. Although, few reports are available on *Pongamia pinnata L* in terms of leaf, root and stem with respect to bactericidal activity against gram positive and negative bacteria in vivo and invitro[15]. However, there is a need to evaluate the antibacterial activity of *pongamia* seed on pathogens.

MATERIALS AND METHODS

Collection of Plant Material

The seeds of *Pongamia pinnata L.*were obtained from the local area of kolar and authenticated by College of Horticulture Science- Kolar, Karnataka, India.

Seed Description

Pods of *Pongamia pinnata L* measures generally 3-6 cm long and 2-3 cm wide, thick walled and usually contain a single seed. Seeds are 1-2 cm long, elliptical and reniform, fig, oblong and light brown colour as shown in figure 1.

Solvent Extraction

The seeds were selected according to their conditions; seeds were cleaned and deshelled, naturally, dried and made into fine powder in mixer. 15gms of Seed powder is weighed separately, added separately to 50 ml of ethanol and 50ml of methanol solvent in beakers. The mixture was placed in orbital gel shaker for 3 days. The extracts were concentrated to dryness by evaporating the solvent under reduced pressure using rotary evaporator and final powder was preserved in air tight container to maintain its viability and stored at 4ºC till usage.

Preparation of Inoculum

The organisms like *Pseudomonas aeruginosa, Serratia marcescens, Proteus vulgaris, Micrococcusluteus, Klebsiella pneumonia, Staphylococcus aureus* were isolated from the clinical samples collected from Department of Microbiology, R.L. Jalappa Hospital and Research Center,Tamaka, Kolar. The confirmed pathogenic cultures were grown in nutrient broth at 37°C , maintained in nutrient agar slants and stored at 4ºC for determining the antimicrobial activity of this selected medicinal plant.

Media preparation

To find out inhibitory effect/ antimicrobial susceptibility on agar well diffusion method, agar-agar media plates were prepared using nutrient agar 4gm%, this was considered as minimum inhibitory concentration after several trails with serial dilution technique. Prior to carrying out the preparation of media plates, Prepared agar was allowed for attaining sterilization, the sterilized media cooled to 50°C in a water-bath. Pouring of about 25 ml agar media into pre-labeled sterile petri plates, allowed to set at room temperature and dried in order to avoid moisture on the surface of the agar. For bacterial cell growth the suspension culture prepared using 2% Luria Broth (w/v), the media prepared was subjected for sterilization at 121°C for 20 min in autoclave technique at 15 lbs pressure.

Agar well Diffusion method

Antibacterial activity measured as per the method described by E. Christy Jeyaseelan *et al*[18]. Agar plates were swabbed with 0.1ml of 24hours cultured pathogens such as *Pseudomonas aeruginosa, Serratia marcescens, Klebsiella pneumoniae, Proteus vulgaris, Micrococcus luteus and Staphylococcus aureus* in separate set of experiments to be tested for antibacterial activity. Wells were made on agar surface with 6mm in diameter spacing 3cm using sterile cork borers and marked. Each of the well was filled with 100µg/ml of plant extract prepared with methanol and ethanol. Well filled with only ethanol and only methanol served as negative control. However, well filled with antibiotic ceftazidime serves as positive control. A separate set of experiments carried out with 100g/ml plant seed extract in each solvent. These were incubated at 37°C for 24 h. The complete absence of growth at applied concentration was considered as the bactericidal concentration measured in millimeters (mm) is the zone of inhibition that was calculated by measuring of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in duplicates and its average values were documented and tabulated in table 1 and figure 2, 3.

RESULTS AND DISCUSSION

The experimental results obtained from the present study illustrates that methanolic extracts found to be more effective to control the pathogens growth compared to less effective inhibition by ethanol extract as shown in table 1. Infectious diseases have become the major cause and serious concern in public health issues. The occurrence of drug resistant strains with less susceptibility to antibiotics due to mutation is challenges amongst the researcher to invent newer drugs are in progress. At this scenario, evaluation of antimicrobial substances from various sources of medicinal plants is considered to be a pivotal role. Few studies states that *Pongamia pinnata L* seeds have antimicrobial properties and thus being used in bronchitis, leprosy and chronic skin disease[16,17]. In field of agriculture, *Pongamia pinnata L* seeds are used as fertilizer to enhance the soil fertility[18,19].

The phytochemical investigation of *Pongamia pinnata L* also indicated the

presence of abundant prenylated flavonoids such as furanoflavoids, chromenoflavones [20,21]. The seeds contain a flavones derivative called pongal. The structures of Karangin and pongal of *Pongamia pinnata L* were elucidated[21,22] which have antimicrobial activity. However, in the present study results also exhibited the confirmation of the antimicrobial property that showed bactericidal action on the pathogens commonly encountered in hospitalized patients. Even though, further studies are required to exploring the mechanism of biochemical active principle in the seed extract for the inhibitory action on various pathogens selected in the study.

CONCLUSION

The seed extract of *Pongamia pinnata Linn* with methanol and ethanol solvent at 100µg/ml concentration showed significant antibacterial activity on selected pathogens in clinical isolates.

ACKNOWLEDGEMENT

We express our thanks to authorities of the Sri Devaraj Urs Academy of Higher Education and Research for their support. Appreciation is also expressed to College of Horticulture NH-4, Kolar, Karnataka for authentication of Pongamia seeds.

REFERENCES

1. Abascal, K. and E. Yarnell. Herbs and drug resistance. Alternative and Complementary Therapies 2002; 8:237-241.
2. Cowan, M.M. Plant products as antimicrobial agents. *Clin. Microbiol. Rev* 1999; 12:564– 82.
3. Bidlack, W.R., S.T. Omaye, M.S. Meskin, and D.K.W. Topham, 2000. Phytochemicals as Bioactive Agents. CRC press, Boca Raton, FL.
4. Kroschwitz J.I. and M. Howe-Grant Kirk–Othmer encyclopedia of chemical Technology 1992; 2: 893.
5. Srivatsava J.J. Lambert and N Vietmayer. Medicinal plants An expanding role in development world bank Technical paper No 320.1996
6. Simonsen HT, Nordskjold JB, Smitt UW. In vitro screening of Indian Medicinal Plants for antiplasmodial activity. *J Ethno-pharmacol* 2001; 74:195-04.
7. Srinivasan K, Muruganandan S, Lal J. Evaluation of anti-inflammatory activity Of *Pongamia pinnata* leaves in rats. *J Ethnopharmacol* 2001; 78:151-57.
8. Brijesh S, Daswani PG, Tetali P. Studies on Pongamiapinnat (L.) Pierre leaves: Understanding the mechanism(s) of action in infectious diarrhea. *J Zhejiang UnivSci B* 2006; 7:665-74.
9. Prabha T, Dora M, Priyambada S. Evaluation of *Pongamia pinnata* root Extract on gastric ulcers and mucosal offensive and defensive factors in rats. *Indian J ExpBiol* 2003;41:304-10
10. Punitha R. and Manoharan S. Antihyperglycemic and antilipidperoxidative effects of *Pongamia pinnata* (Linn.) Pierre flowers in alloxan induced diabetic rats. *J Ethnopharmacol* 2006; 105: 39–46.
11. Ayyanar M, Ignacimuthu, S. Herbal medicines for wound healing among tribal people in Southern India: Ethnobotanical and Scientific evidences *International Journal of Applied Research in Natural Products* 2009;2(3): 29-42.
12. Naik M., Meher L.C., Naik S.N. and DasaL.M., Production of biodieselfrom high free fatty acidKaranja (*Pongamia pinnata*) oil. Biomass and Bioenergy 2008; 32: 354–57.
13. Ashish Manigauha *et al*. Evaluation of anticonvulsant activity of *Pongamia pinnata* Linn in experimental animals. *International Journal of PharmTech Research* 2009; 1(4):1119-21.
14. Baswa M, Rath CC, Dash SK, Mishra R K. Antibacterial activity of Karanj (*Pongamia pinnata*) and Neem (*Azadirachtaindica*) seed oil: a preliminary report. Method Microbios 2001; 105 (412):183-9.

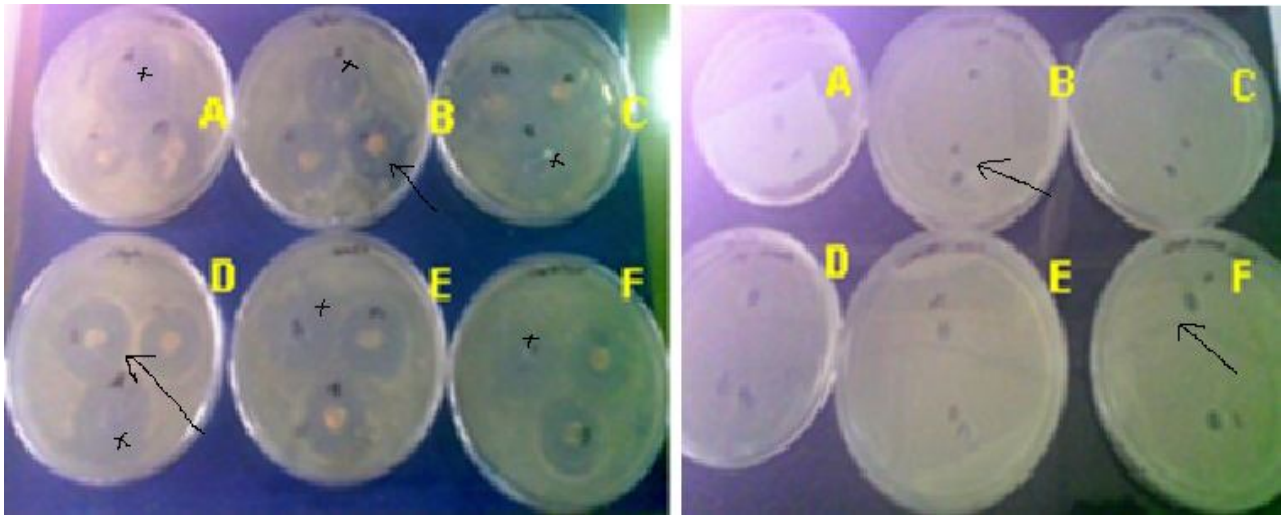
15. SavitaSagwan *et al*, in vivo and in vitro proportional antimicrobial activity in karanj [*Pongamia pinnata*]: an imperative leguminous tree. *International Journal of Research and Reviews in Pharmacy and Applied science IJRRPAS* 2012; 2(6): 981-95.
16. WaghP, M. Rai, S. K. Deshmuk, M.L. Durate. Bioactivity of oils of Trigonella-foenumgraecum and *Pongamia pinnata*. Afric, *J. Biotech* 2007; 6:1592-1596.
17. Simin, K, Z.Ali, S.M. Khaliq, V.U. Ahmad. Structure and biological activity of a new retinoid from *Pongamia pinnata*. Nat. Prod. Letters, 16: 351-357 (2002).
18. E. Christy Jeyaseelan, S. Tharmila, V. Sathiyaseelan, K Niranjan. Antibacterial Activity of Various Solvent Extracts of Some Selected Medicinal Plants Present in Jaffna Peninsula. *International Journal of Pharmaceutical & Biological Archives* 2012; 3(4):792-96.
19. Shivani Chaturved, Vishal Kumar, Santosh Satya. Composting effects of *Pongamia pinnata*on tomato fertilization. Archives of Agronomy and Soil Science 2009; 55(5):535-46.
20. Yadav PP, Ahmad G, Maurya R. Furanoflavonoids from *Pongamia pinnata* fruits. Phytochemistry 2004; 65: 439-43.
21. Hao Yin, Si Zhang, Jun Wu. Prenylated Flavonoids from *Pongamia pinnata* Z. Naturforsch 2005; 60b: 356-58.
22. Shameel S, Usmanghani K, Ali MS. Chemical constituents from seeds of *Pongamia pinnata* (L.) Pierre. *Pak. J. Pharm.Sci*-1996;9:11-20.

Table 1. Showing the Antibacterial activity of Pongamia pinnata L seed extarct with concentartion of 100µg/ml methanol and ethanol extracts compared to Ceftazidime

	Zone of inhibition in mm		
Name of the Microorganism	Methanol Extract 100µg/ml	Ethanol Extract 100µg/ml	Ceftazidime 100µg/ml
K pneumonia (A)	16	9	21
P vulgaris (B)	14	13	17
P aeruginosa (C)	20	18	23
S aureus (D)	15	16	21
S marcescens (E)	16	14	19
M luteus (F)	14	12	20

 ISSN 2321 – 2748

Figure 1. Showing Pods of *Pongamia pinnata Linn*



(a) (b)

Figure 2. (a) Showing Zone of inhibition with seed extract of *pongamia pinnata L* with methanol and ethanol solvents along with Ceftazidime indicated with (X) in the figure.
(b) Showing no zone of inhibition in Controls with Ethanol and Methanol solvents.

 ISSN 2321 – 2748

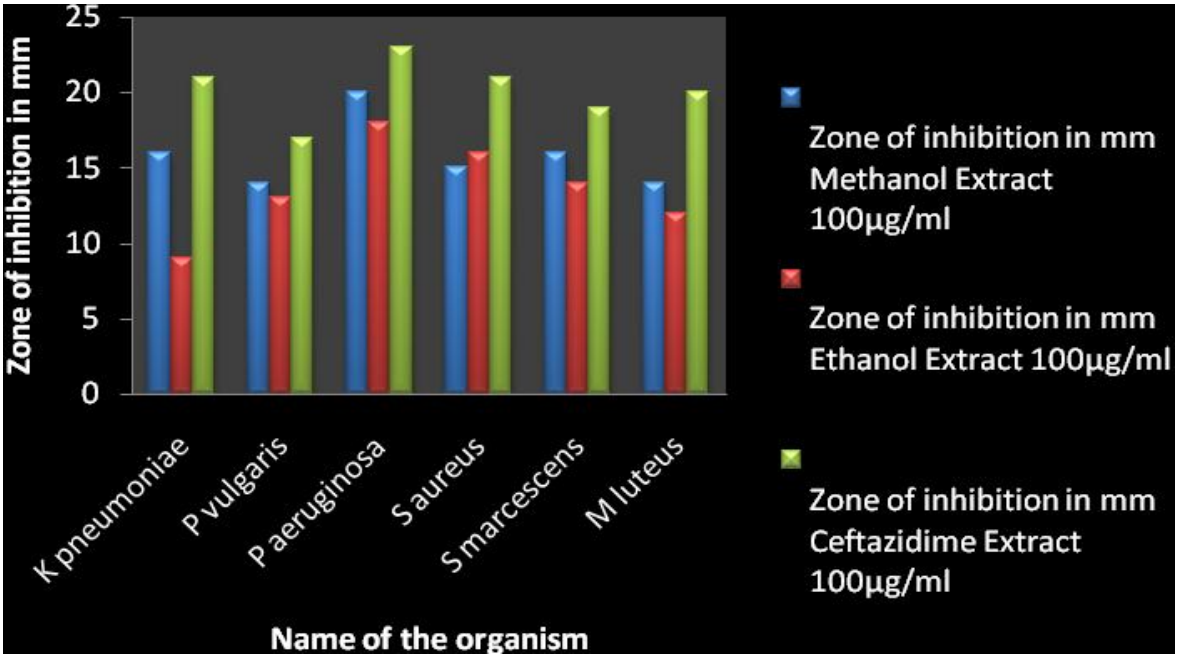


Figure 3. Showing the comparision of Antibacterial activity of *pongamia pinnata L* seed extarct with concentartion of 100µg/ml methanol and ethanol extracts compared to Ceftazidime