CODEN (USA): IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com *Research Article*

ANTIMICROBIAL EFFICACY OF *PONGAMIA PINNATA* (L) PIERRE AGAINST DENTAL CARIES PATHOGENS OF CLINICAL ORIGIN

Sowjanya Pulipati*, P. Srinivasa Babu, R. Sampath, N. Bindu Sree,
Vignan Pharmacy College, Vadlamudi- 522 213, Guntur (Dt), Andhra Pradesh, INDIA.

Abstract:
Dental caries is one of the most prevalent diseases in humans. Endogenous oral bacterial species play a major role in the initiation and progression of the dental caries. Effective prevention of dental caries can be achieved by mechanical plaque removal or chemical agents. All parts of plant have been used as a crude drug for the treatment of tumors, piles, skin diseases, itches, rheumatoid arthritis, wounds, ulcers, diarrhea etc. Traditionally young stems are used as tooth brush for oral hygiene. The fresh young stems were extracted by cold maceration with Ethanol. The preliminary phytochemical screening was carried out and the presence of steroids, cardiac glycosides, alkaloids, flavonoids, tannins and phenolic compounds were observed.

The antimicrobial efficacy of stems of Pongamia pinnata was carried by agar well diffusion method at concentrations of 250 µg, 500 µg, 750 µg and 1000 µg against dental caries pathogens like Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Lactobacillus acidophilus, Pseudomonas aeruginosa and Candida albicans. The diameters of zone of inhibition range from 15.33±0.57 to 28.0±1.0 mm at different concentrations. The antimicrobial activity was compared with the standards tetracycline and fluconazole. The PPEE exhibited maximum activity against S. aureus (26.0±1.0 mm) and moderate activity against E. faecalis (25.66±1.15 mm) and minimum activity against P. aeruginosa (21.66±1.52 mm).The plant also effectively inhibited C. albicans (28.0±1.0 mm). The results of the current study demonstrate the antimicrobial activity of stems of Pongamia pinnata to prevent dental caries. MIC was performed by agar dilution method and the range was found to be 31.2 mg/ml to 62.5 mg/ml.

Key words: *Pongamia pinnata, antimicrobial activity, agar well diffusion.*

Corresponding Author:
Sowjanya Pulipati,
Vignan Pharmacy College,
Vadlamudi, Guntur (Dt),
Andhra Pradesh, INDIA.
Email: *sowjypulipati@gmail.com*

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Please cite this article in press as Sowjanya Pulipati et al, **Antimicrobial Efficacy of *Pongamia Pinnata* (L) Pierre against Dental Caries Pathogens of Clinical Origin**, *Indo Am. J. Pharm. Sci, 2016; 3(5).*

INTRODUCTION:

Dental caries is a common disease worldwide [1,2,3]. Dental caries also known as tooth decay or cavities is a bacterial infection. This causes demineralization and destruction of hard tissues such as enamel, dentin and cementum [4,5]. This leads to pain, tooth loss and infection. Tooth decay results from production of acid by bacteria on fermentation of food debris accumulated in between or on the surface of teeth. The mouth contains a wide variety of oral bacteria, but few specific bacteria cause dental caries. The bacteria responsible for dental caries are *Streptococcus mutans, Streptococcus sobrinus* and *Lactobacillus acidophilus*. *Actinomyces viscosus, Nocardia* spp., are also most closely associated with dental caries. Several agents are commercially available for dental caries treatment; these chemicals can alter oral microbiota and have undesirable side-effects such as vomiting, diarrhea and tooth staining. The adverse effects of some antibacterial agents, increased resistance by bacteria to antibiotics used in dentistry, there is a need for alternative prevention and treatment options that are safe, effective and economical. Hence, the search for alternative products continues and natural phytochemicals isolated from plants used as traditional medicines are considered as good alternatives. In this study, *Pongamia pinnata* stem extract or phytochemicals are used to inhibit the growth of oral pathogens. Traditionally the stems are used as tooth brush to maintain oral hygeine, it reduce the development of biofilms and dental plaque and also reduce the symptoms of oral diseases.

Pongamia pinnata (Linn.) Pierre belongs to Fabaceae family is popularly known as Karanja in Hindi, Indian Beech tree in English and Kanuga in Telugu [6]. *Pongamia pinnata* exhibits various pharmacological activities. *Pongamia pinnata* is used to treat various kinds of diseases in the Indian system of traditional medicine Ayurveda and Siddha[7]. It is also used traditionally as folklore medicinal plant [8]. The entire plant have been used as a crude drug for the treatment of tumours, piles, skin diseases, itches, abscess, painful rheumatic joints, wounds, ulcers, diarrhea etc [9]. The effectiveness of phytoconstituents from *P. pinnata* has been reported specifically as antimicrobial and therapeutic agents[10]. The seed oil can be converted to biodiesel by trans-esterification method [11]. The anti-plasmodium [12], anti-inflammatory[13], antiulceric[14], wound healing [15] properties were reported. The literature survey on *P. pinnata* plant showed that it is a potential medicinal plant. Since, there is no report on antimicrobial efficacy of stem extract of *P. pinnata* against pathogens associated with dental caries; the present investigation was conducted to find the antimicrobial properties.

MATERIALS AND METHODS:

Plant Material

The fresh stems of *Pongamia pinnata* (L) were collected at our college premises and authenticated by P. Satyanaraya Raju, Plant Taxonomy Consultant, Department of Botany & Microbiology, Acharya Nagarjuna University, Guntur. The collected stems were washed and blotted to dry. The healthy fresh stems were used for extraction.

Preparation of the Extract

The stems were extracted by cold maceration using ethanol. The *Pongamia pinnata* ethanolic extract (PPEE) was prepared by crushing 100g of fresh stems with 500mL of ethanol kept in a shaker for 24 h. The extract was collected by filtration through 5 layers of muslin cloth. The extraction was repeated twice. The collected filtrates were pooled, concentrated and dried at mild temperature. The extract was preserved in refrigerator at 4°C and used for further study.

Phytochemical Screening

The phytochemical screening for the extract was carried out by standard protocol. The presence of alkaloids, glycosides, tannins, flavonoids, phenolic compounds, steroids was investigated [16,17].

Microbial strains used

Six dental caries causing bacteria *Staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis, Escherichia coli, Lactobacillus acidophilus, Pseudomonas aeruginosa* and one yeast *Candida albicans.* The pathogenic bacterial and fungal strains procured from Microbiology lab. The organisms were subcultured in specific media, bacteria in nutrient agar and yeast in malt yeast agar and incubated aerobically at 37°C. The strains were identified and confirmed by standard biochemical and staining methods [18,19,20].

Antimicrobial susceptibility test

Antimicrobial activity was measured by agar well diffusion method [21,22]. Nutrient agar plates were inoculated with bacteria and potato dextrose agar plates were inoculated with fungi in separate set of experiments to be tested for antimicrobial activity. The wells of 6 mm diameter were made equidistantly in the agar plate with sterile borer. The extract was dissolved in dimethyl sulphoxide to get a final concentration of 10mg/ml. The wells were filled with 250, 500, 750 and 1000 µg/ml of drug respectively. The activity was compared with standard antibiotics tetracycline (30 µg) for bacteria and fluconazole (100 µg) for yeast. The plates were incubated for 24 h and zones of inhibition were measured with ruler.

RESULTS:

The results of antimicrobial activity cold ethanolic extract of *Pongamia pinnata* stems by agar well diffusion method proved that the plant possess antimicrobial constituents that inhibited all tested dental pathogens. The preliminary phytochemical screening revealed the presence of carbohydrates, steroids, glycosides, alkaloids, flavonoids, tannins and phenolic compounds. The PPEE exhibited highest antimicrobial activity against *S. aureus* (26.0±1.0 mm) and followed by *E. faecalis* (25.66±1.15 mm), *S. mutans* (24.33±1.52), *E. coli* (23.33±1.29), *L. acidophilus* (23.33±0.57) and then against *P. aeruginosa* (21.66±1.52 mm).The plant also effectively inhibited *C. albicans* (28.0±1.0 mm). The MIC of 31.2 mg/ml was observed against *S. aureus, E. faecalis, C. albicans* and 62.5 mg/ml against *S. mutans, L. acidophilus, E. coli* and *P. aeruginosa.*

The positive control for bacteria tetracycline showed antimicrobial inhibitory zone of 28.33±0.57 against *S. aureus*, 26.33±1.15 against *S. mutans*, 20.0±1.0 against *E. faecalis*, 21.33±1.52 against *L.acidophilus*, 27.66±1.15 against *E. coli*, 18.66±1.15 against *P. aeruginosa*. The positive control for fungi fluconazole showed inhibitory zone of 19.33±0.57 against *C. albicans.* The negative control Dimethyl sulphoxide (DMSO) produced no observable zone against any of the tested microorganism.

Table 1: Preliminary Phytochemical Screening of PPEE

S. No	Name of the test	PPEE
1.	Carbohydrates	+
2.	Aminoacids	-
3.	Proteins	-
4.	Steroids	+
5.	Glycosides	+
6.	Flavonoids	+
7.	Alkaloids	+
8.	Phenolic compounds & Tannins	+
9.	Terpenoids	-

Note: "+" indicates positive and "–" indicates negative.

Table 2: Antimicrobial Efficacy of PPEE against Dental Pathogens of Clinical Origin

S. No.	Name of the Microorganism	Zone of inhibition in mm						
		Concentration of PPEE & standards µg/well						
		250	500	750	1000	TET (30)	FLU(100)	DMSO
1.	*S. aureus*	16.66±0.57	18.33±0.57	20.33±1.52	26.0±1.0	28.33±0.57	NA	-
2.	*S. mutans*	17.66±0.57	20.66±1.52	22.0±1.0	24.33±1.52	26.33±1.15	NA	-
3.	*E. faecalis*	15.33±0.57	18.66±0.57	20.66±1.52	25.66±1.15	20.0±1.0	NA	-
4.	*L. acidophilus*	16.33±1.15	19.33±1.52	21.33±1.52	23.33±0.57	21.33±1.52	NA	-
5.	*E. coli*	15.66±0.57	20.66±1.15	22.0±1.0	23.33±1.29	27.66±1.15	NA	-
6.	*P. aeruginosa*	16.33±0.57	18.66±1.15	19.33±0.57	21.66±1.52	18.66±1.15	NA	-
7.	*C. albicans*	16.66±1.15	19.33±0.57	23.66±1.15	28.0±1.0	NA	19.33±0.57	-

Note: Values are mean ± SD of triplicates

Table 3: Minimum Inhibitory Concentration (MIC) of PPEE

Name of the organism	Extract concentration mg/ml								
	0.97	1.95	3.90	7.81	15.6	31.2	62.5	125	250
S. aureus	+	+	+	+	+	β	-	-	-
S. mutans	+	+	+	+	+	+	β	-	-
E. faecalis	+	+	+	+	+	β	-	-	-
L. acidophilus	+	+	+	+	+	+	β	-	-
E. coli	+	+	+	+	+	+	β	-	-
P. aeruginosa	+	+	+	+	+	+	β	-	-
C. albicans	+	+	+	+	+	β	-	-	-

Note: += Turbidity observed, - = No turbidity observed, β= MIC value

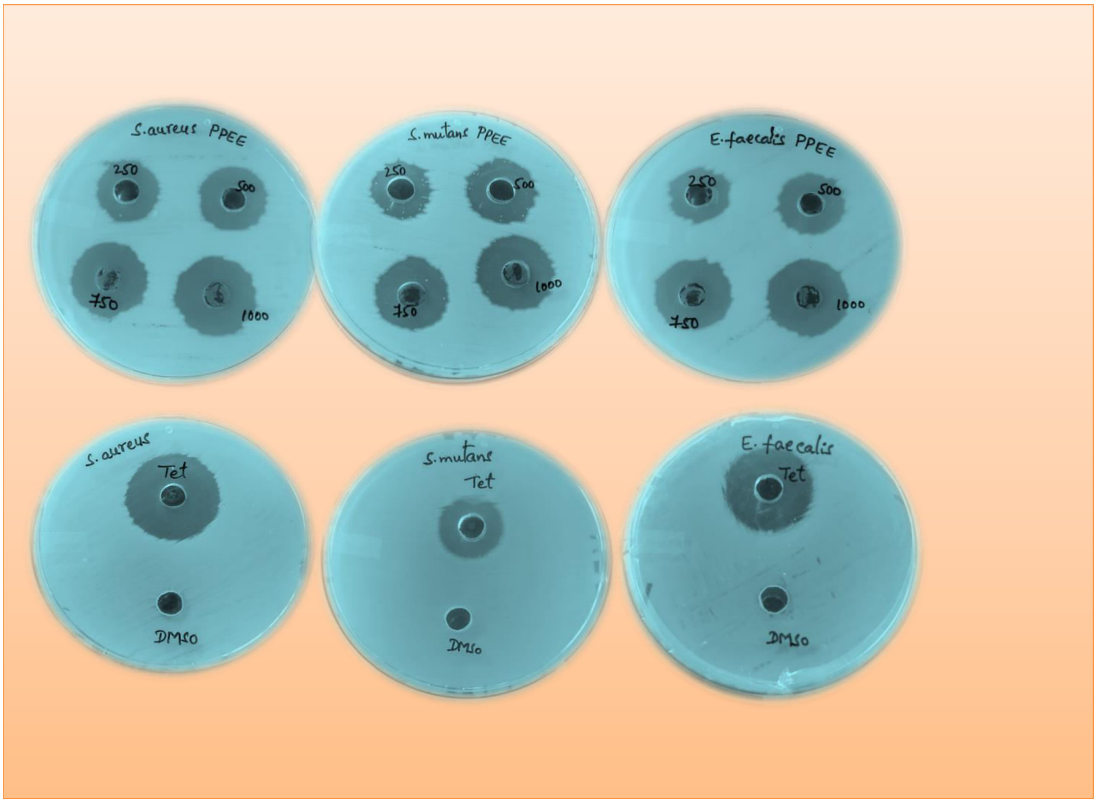

Fig 1: Antimicrobial Activity of PPEE and Tetracycline against Gram Positive Bacteria

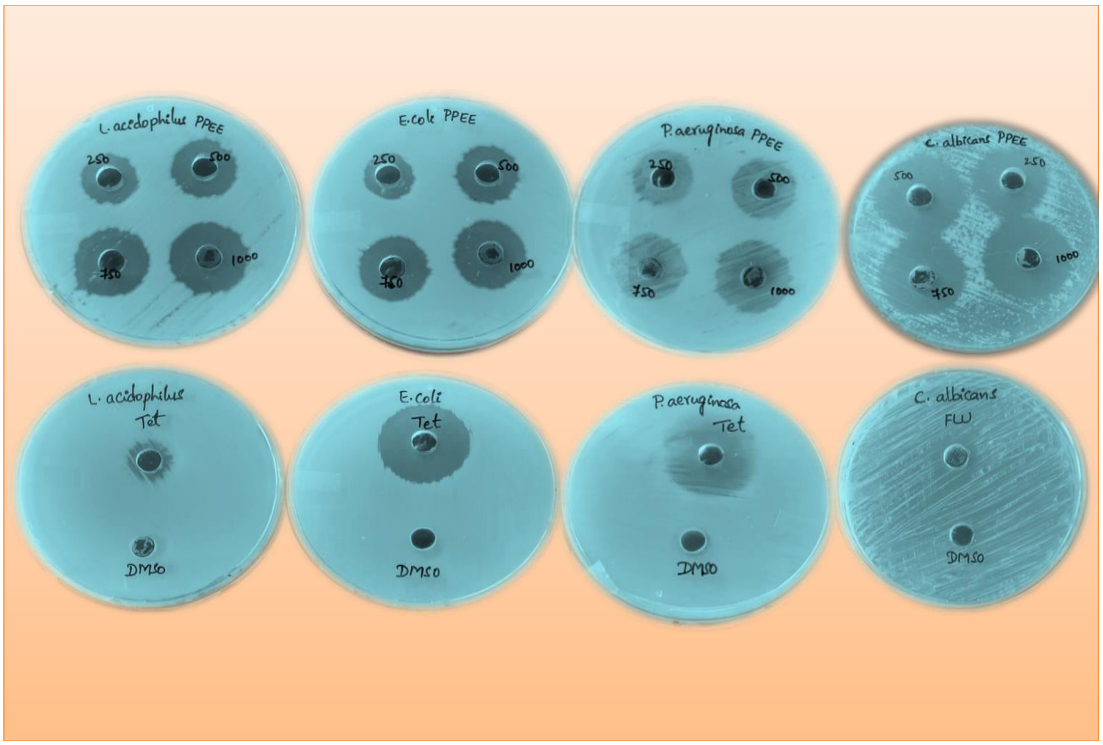

Fig 2: Antimicrobial Activity of PPEE, Tetracycline & Fluconazole against Gram Negative and Fungi Organisms

DISCUSSION:

Nowadays dental caries remain one of the most common diseases throughout the World. *Azadirachta indica* commonly known as Neem was reported for inhibition of *Streptococcus sanguis* to saliva-conditioned hydroxyapatite, a composite of bone and enamel. Neem extract inhibited insoluble glucan synthesis, which indicates the ability of neem to reduce the adherence of *Streptococci* to tooth surface[23]. It is also reported that neem extract produced highest zone of inhibition against *Streptococcus mutans* at 50 % concentration [24].

The present study was carried choosing the test organisms *S. aureus*, *S. mutans, E. faecalis, L.acidophilus, E. coli* and *C. albicans* because they have been implicated in dental caries [25,26]. *S.aureus,* a major human pathogen, is responsible for a number of hospital acquired infections and propagates mainly in mouth and hands acquired in the hospital environment [27,28,29]. *Candida albicans* is also the most common yeast and is associated with fungal oral infections, endocarditis and septicemia [30]. *S. mutans, L. acidophilus, E. faecalis, E. coli* and *P. aeruginosa* considered to be an opportunistic pathogen in the oral cavity, may induce significant oral risks by acting as a tertiary colonizer in dental caries and causing both superficial and invasive infections.

CONCLUSION:

The present study has established that *P. pinnata* is a potential plant, which could be incorporated into orodentrifice. The antimicrobial activities can be enhanced if the phytoactive components are purified and adequate dosage determined for proper administration. As the global scenario is now changing towards the use of traditional medicinal plants, development of modern drugs from *P. pinnata* should be emphasized for the treatment and management of dental caries.

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