

**Study of Antimicrobial Activity of Stem Extracts of *Pongamia pinnata* L**

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**Abstract:** In the present study, the stem extracts of the plant *Pongamia pinnata*, L. has been selected to investigate upon its antimicrobial activity against tooth bacteria and its antibacterial activities were compared with market products. The antimicrobial tests were conducted by using two different solvent based plant extracts i.e. aqueous and ethanol stem extracts. Least bacterial colonies were found in nutrient agar plate streaked with tooth bacteria, after brushing with stem of *Pongamia pinnata*, L. As compared to bacterial colonies obtained on nutrient agar plate, after brushing with sodium monofluorophosphate. From the MIC test of aqueous and ethanol stem extracts against gram positive tooth bacteria, it was found that both aqueous and ethanol extracts were effective against gram positive tooth bacteria. It is also seen that ethanol stem extract is more effective than aqueous stem extract. In disc diffusion method, it was found that both aqueous and ethanol stem extracts showed inhibition zone at different concentrations against gram positive tooth bacteria and inhibition zone indicated that ethanol extract was more effective than aqueous extract of *Pongamia pinnata*, L dry stems.

**Keywords:** *Pongamia*, Anti-bacterial, Tooth, Antimicrobial activity.

**INTRODUCTION**

The presence and growth of microorganisms in food may cause spoilage and result in a reduction in quality and quantity [1]. Further, microbial contamination of food still possesses important public health and economic concerns for human society.

Plant secondary metabolites such as plant extracts are studied for their antimicrobial activities and most of the plant-derived extracts are known to possess antibacterial, insecticidal, and antifungal activities [3]. With the increase of bacterial resistance to antibiotics, interest has been generated to investigate the antimicrobial effects of different extracts against a range of bacteria, to develop other classes of natural antimicrobials useful for infection control [3].

*Pongamia pinnata*(L.) Pierre (Fabaceae), a medium-sized glabrous tree, found throughout India and further distributed eastwards, mainly in the littoral regions of south eastern Asia and Australia [4]. In the literature of India, different parts of this plant have been recommended as a remedy for various ailments [5]. The seed and seed oil of this plant have been used for treating various inflammatory and infectious diseases such as leucoderma, leprosy, lumbago, muscular and articular rheumatism [6]. The leaves are spicy, digestive, laxative, anthelmintic and cure piles, wounds and other inflammations [7]. A liquid solution of the leaves are used as a medicated bath for relieving rheumatic pains and for cleaning ulcers in gonorrhea and scrofulous enlargement [8, 4]. Root and seed

extracts of *P. pinnata* L. have been reported to have anti-inflammatory activity [9]. Owing to its various ethno pharmacological properties, there is no report available in the literature on the screening of different solvent extracts of *P. pinnata* stem for their antibacterial activity.

A lot of work has already been done for the extraction of biodiesel from the seeds of a plant called *Pongamia pinnata* (L) apart from *Jatropha* sp. Another long history of plant science is that, it can keep our tooth clean in a natural way. There are different ways of taking care of teeth i.e. using toothpaste while brushing & sometimes using powders of plant origin. But, it is a matter of deep concern that the chemical ingredients present in tooth paste are very harmful and even carcinogenic. So, one should be careful about all these things otherwise a lot of damage may occur to our teeth as mouth is the most absorbent place in our entire body. So, optimum care should be taken while using any sort of chemical product. That is because mouth and gums are the direct gateway to every system in our body. Therefore, one should aware about the toothpaste chemistry minutely.

It has been [10] reported that, fruits of *Pongamia pinnata* (L) afforded three furanoflavonoid compounds such as glucoside, pongamoside, and flavonol glucosides. Studies showed [25] that antibacterial compounds in some medicinal plants. These compounds possess anti-inflammatory activities. Phytochemistry[11] of *Vitex negundo* L. roots and found compounds like fatty acid, steroids and their derivatives with the help of physicochemical techniques. The medicinal value of different parts of pongamia pinnata, L. and two important flavinoids which could be immense source for biofuel industry has been reported [12,13]. It has been reported [14] that the secondary metabolites are necessary for medicinal properties.

It has been [15] reported that, the phytoextract or bioactive compounds are of great pharmacognostic significance. It is reported that no single method is sufficient to study the activity of phytochemicals from a given plant. An appropriate assay is required to first screen for the presence of the source material, to purify and subsequently identify the compounds therein. It has been reported [16] that the phytochemical evaluation of *Pongamia pinnata* (L) seed oil, which resulted in the isolation of furanoflavonoid compound called methyloleate. Presence of furanoflavonoids in pongamia fruits [22] and in vitro anti microbial activity against staphylococcus has been reported [23,24].

It has been reported that pongamia is an important medicinal plant [17,19] and the bark has abounding prenylated flavinoids which are the potent source of crude drug [18]. Kumar *et al.* [19] reported presence of antibacterial and antifungal agents from some Indian medicinal plants.

Keeping a view on the available literature, the present study aims to find out role of different types of solvents on the extractability, investigating the antimicrobial activity of secondary metabolites and identifying the antipyretic activity of *Pongamia pinnata* (L) extract.

## MATERIALS AND METHODS

The stem of *Pongamia pinnata* Linn (Family: Fabaceae) were collected from Khandagiri area of Bhubaneswar in the month of July 2017 and authenticated by Dr. Mamata Mohapatra, Head of the Department and Asst. prof. in Botany, Khallikote Autonomous college, Odisha, with the help of Hain's flora book. After collecting the stem it was washed thoroughly with tap water. The plant material was dried in shade, pulverized to powder by using a mechanical grinder and stored in air tight plastic container for further studies.

### Preparation of plant extract

For this study maceration extraction process was selected. 20 gm of shade dried, powdered stems

were soaked separately in 100 ml of distilled water and in 100 ml of ethanol for 72 hours. Each mixture was stirred periodically using sterile glass rod. At the end of 72 hours, each extract was filtered through Whatman filter paper (No: 1). Filtered extracts were evaporated on a rotary evaporator and reduced to 30 ml of each extract. Collected stem extracts were stored at -4°C in an air tight bottle for further use.

At the time of use, dried plant extracts were dissolved in their same corresponding solvents. For this experiment, concentration of 200mg/ml of aqueous and ethanolic stem extracts was required. So the extracts were diluted as per their requirement.

### Collection of Tooth Bacterial Samples

Before brushing, bacteria from the tooth were isolated with the help of a sterile ear bud and one nutrient agar plate was streaked with it by streak plate technique. This procedure is repeated after brushing with sodium monofluorophosphate. Then after 8-10 hours this procedure is again repeated after brushing the tooth with stem of *Pongamia pinnata*. All the 3 inoculated nutrient agar plates were incubated for 24-48 hours at 37°C in an inverted position.

### Antibacterial activity assay

The antibacterial activity was evaluated by paper disc-diffusion method. Minimum inhibitory concentration (MIC) values were also studied for microorganisms. The MIC was identified as the lowest concentration of the chemical agent, which resulted in confirmed inhibition of the growth of the tested microorganism, after 24 hr of optimal incubation conditions [20, 21]. Antibiotics activity assay method was the zone diameter of inhibition, measured to determine the inhibition capacity of plant extract [20].

### Paper disc diffusion method

The antibacterial activity of *Pongamia pinnata* L. stem with ethanol and aqueous extracts were analyzed separately by using disc diffusion assay. Commercial Ampicilin (10 mcg/disc) and Gentamycin (10 mcg/disc) antibiotic disc were used as standard drugs. Sterile antibiotic discs were used for the present investigation. The extract of *Pongamia pinnata* L. was incorporated to the sterile paper discs, individually with 0.62 to 20 µg./ml respectively using a micropipette. Activity of the above mentioned extracts were tested separately, using disc diffusion method.

### MIC determination of Plant drugs against bacteria

The ethanol and aqueous extracts of *Pongamia pinnata* L. were incorporated to the sterile test tubes containing broth for serial dilution individually using micropipette. This can be done in a series of 12 test tubes, which, were incubated at appropriate culture conditions and examined by turbidity. Each extract was assayed in triplicate

## RESULTS AND DISCUSSIONS

**Table-1: Minimum inhibitory concentration for aqueous extract of *Pongamia pinnata***

SL NO.	CONCENTRATION ( $\mu\text{g/ml.}$ )	OPTICAL DENSITY (at 610 nm)
1	20	2.3
2	10	2.3
3	05	3.05
4	2.5	3.5
5	1.25	3.5
6	0.62	3.3

**Table-2: Minimum Inhibitory Concentration for Ethanolic Extract of *Pongamia Pinnata***

SL NO.	CONCENTRATION ( $\mu\text{g/ml.}$ )	OPTICAL DENSITY (at 610 nm)
1	20	1.97
2	10	1.97
3	05	2.58
4	2.5	2.57
5	1.25	2.57
6	0.62	2.42

**Table-3: Comparison of inhibition zone of antibiotic control with e thanolic extract of *Pongamia pinnata***

Sl. No.	Concentration ( $\mu\text{g/ml}$ )	Inhibition zone Observed in (mm)	Antibiotics of 10mcg	Inhibition zone of antibiotic (mm).
1	20	02	Ampicillin	03
2	50	03		
3	100	05	Gentamycin	04
4	200	07		

The ethanol stem extract of *Pongamia pinnata* L. showed maximum inhibition against growth of gm +ve bacteria. It has been seen from the OD value that microbes are susceptible to aqueous extract than the ethanol extract. So, selecting extraction medium (ethanol, methanol, acetone or distilled water) is very crucial to study antimicrobial activity of phyto compounds. Ethanol had almost no side effects than any other type of organic extracts. Therefore, Ethanol had been used in homeopathic medicine since prehistoric time. Which means that, Ethanol has had no cytotoxic hindrance. Antioxidants are the compounds responsible for the protection of living organisms from the damage caused by the abnormal production of reactive oxygen species produced by lipid peroxidation, protein damage, and others including DNA strand breaking etc. The chemistry of ethanol extract is highly significant in this regard.

Many studies on cell structure and animals have suggested that phytochemicals attenuate the inflammatory system of the cell. Basically flavonoids are having antimicrobial and antipyretic activities. Phenolic compounds possess the biological properties like anti- inflammatory, anti- aging and anticancer etc. Studies on different medicinal plants have also shown the antioxidant properties are in phenolic compounds. In this study aqueous extract (in distilled water) showed considerable activity against micro- organisms. The

activity in ethanol extract was higher than aqueous extraction .The main challenge now a day is the search for plants with promising antimicrobial activities and isolation of active principles. There are many difficulties which seriously impede this type of analysis. Firstly, existence of a broad range of structurally diverse compounds and their synergistic effects. Secondly, there is an urgent need for most appropriate pharmacological model for clinical efficiency.

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**REFERENCES**

1. Soliman KM, Badeaa RI. Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food and chemical toxicology. 2002 Nov 1;40(11):1669-75.
2. Tepe B, Donmez E, Unlu M, Candan F, Daferera D, Vardar-Unlu G, Polissiou M, Sokmen A. Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* (Montbret et Aucher ex Benth.) and *Salvia multicaulis* (Vahl). Food chemistry. 2004 Mar 1;84(4):519-25.

3. Bakri IM, Douglas CW. Inhibitory effect of garlic extract on oral bacteria. Archives of oral biology. 2005 Jul 1;50(7):645-51.
4. Satyavati GV, Raina MK, Sharma M. Medicinal plants of India. Indian Council of Medical Research; 1987.
5. Kirtikar KR, Basu BD. Feronia elephantum Corr. Indian Medicinal Plants. Vol-I.1995.
6. Agarwal SK, Singh SS, Verma S, Kumar S. Antifungal activity of anthraquinone derivatives from Rheum emodi. Journal of ethnopharmacology. 2000 Sep 1;72(1-2):43-6.
7. Kirtikar KR, Basu BD. Vol. 3. Deheradun: International Book Distributors. 1933:1823-4.
8. Chopra RN, Chopra IC, Handa KL, Kapoor LD. Indigenous drugs of India Academic publishers. Calcutta-New Delhi. 1982;306.
9. Singh RK, Joshi VK, Goel RK, Gambhir SS, Acharya SB. Pharmacological actions of Pongamia pinnata seeds--a preliminary study. Indian journal of experimental biology. 1996 Dec;34(12):1204-7.
10. Ahmad G, Yadav PP, Maurya R. Furanoflavonoid glycosides from Pongamia pinnata fruits. Phytochemistry. 2004 Apr 1;65(7):921-4.
11. Gangwar AK, Ghosh AK, Saxena V. Antiinflammatory activity of ethanolic extracts of *Vitex negundo* L. roots. International Journal of Herbal Medicine. 2015;2(6):01.
12. Brijesh S, Daswani PG, Tetali P, Rojatkar SR, Antia NH, Birdi TJ. Studies on Pongamia pinnata (L.) Pierre leaves: understanding the mechanism (s) of action in infectious diarrhea. Journal of Zhejiang University Science B. 2006 Aug 1;7(8):665-74.
13. Chauhan D, Chauhan JS. Flavonoid glycosides from Pongamia pinnata. Pharmaceutical biology. 2002 Jan 1;40(3):171-4.
14. Kumar KT, Ranjini CE, Sasidharan VK. Antibacterial and antifungal activity of secondary metabolites from some medicinal and other common plant species. Journal of Life Sciences Royal Dublin Society. 1997;2:14-9.
15. Harborne J. Phytochemical Methods: A grade to Modern techniques of plant analysis, Chapman and Hall, London, UK. 1998.
16. Kalidhar SB, Meera B, Kumar S. A review of the chemistry and biological activity of *Pongamia pinnata* L. Med Aromat Plant Sci. 25, 2003, 441-65.
17. Arote SR, Yeole PG. Pongamia pinnata L: a comprehensive review. Int J Pharm Tech Res. 2010 Oct;2(4):2283-90.
18. Yin H, Zhang S, Wu J. Prenylated flavonoids from Pongamia pinnata. Zeitschrift für Naturforschung B. 2005 Mar 1;60(3):356-8.
19. Kumar VP, Chauhan NS, Padh H, Rajani M. Search for antibacterial and antifungal agents from selected Indian medicinal plants. Journal of ethnopharmacology. 2006 Sep 19;107(2):182-8.
20. Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature protocols. 2008 Feb;3(2):163.
21. Khandelwal KR. Phytochemical Screening. Practical Pharmacognosy techniques and experiments, Nirali Publications, Pune. 2001,149\_156.
22. Yadav PP, Ahmad G, Maurya R. Furanoflavonoids from Pongamia pinnata fruits. Phytochemistry. 2004 Feb 1;65(4):439-43.
23. Parekh J, Chanda S. In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turkish Journal of Biology. 2007 Apr 9;31(1):53-8.
24. Panigrahi S, Mahapatra S. Evaluation of Antibacterial Activity of Pongamia pinnata L., Curcuma longa L. and Mentha arvensis L. Against Staphylococcus aureus. International Journal of ChemTech Research. 2016;9(02):205-12.
25. Samuel LA. Screening, Isolation and Characterisation of Antibacterial compounds from folklore medicinal plants M. Sc. Dissertation submitted to Andhra University. 2006, 9-11.