ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Assessment of Antimicrobial Activity of Different Parts of *Pongamia pinnata* L. extracts obtained by Sequential Extraction against Pathogen Isolates from Raipur region.

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ABSTRACT

Pongamia pinnata belonging to the family Fabaceae (Leguminosae), the third largest family of flowering plant with 730 genera and over 19,400 species, is cosmopolitan in distribution. In the present study an assessment of antimicrobial activity of leaf, seed and root extract of *P. pinnata*, with four solvents namely, petroleum ether, acetone, ethyl alcohol and water, against clinical isolates of *Escherichia coli, Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* was carried out following agar disc diffusion technique. Seed of *P. pinnata* emerged as the most effective part with maximum activity against *Staphylococcus aureus* followed by *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* with acetone extract as compared from ethanol and aqueous extracts. The root was the next in order showing the antimicrobial property. Aqueous extract of seed and root showed inhibitory effect on *Escherichia coli* and *Staphylococcus aureus*. The phytochemical studied revealed that all parts of *P. pinnata* possess high degree of chemicals such as alkaloid, flavonoids, saponins, sterol, tannins and terpenoids. The presence of these secondary metabolites may contribute for their antimicrobial activity. Activity index of seed of *P. pinnata* was found to be highest followed by the root and leaf.

Keywords: Antimicrobial activity, solvent extract, antimicrobial agent, clinical isolates, activity index.

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ISSN: 0975-8585

INTRODUCTION

Antibiotics are generally considered as a wonder drug because of their wide range of action, safe and well tolerance by the host organisms. However, it has been subsequently realized that they are associated with various adverse effects; one of them is antibiotic resistance developed by the microorganism. Antibiotics resistance is a growing problem in current times due to their indiscriminate uses not only in control of several diseases but also as growth promoters in food of animals [1]. Antibiotics are sometime associated with several side effect that can result from interaction with other drugs, such as elevated risk of tendon damage from administration of a quinolone antibiotic with a systemic corticosteroid [2]. Neurotoxic effect also associated with antibiotics such as Cephalosporin, Penicillin, Tetracyclin and Quinolones. Aminoglycosides have also been known to cause toxicity most commonly, through peripheral neuropathy; encephalopathy and neuromuscular blockade have also been reported [3]. This situation has forced to search new antimicrobial substances and develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [4, 5]. Use of medicinal plants may overcome this problem due to their secondary metabolites production abilities. Attention has been given to extracts and isolates of the active compounds from plant species used in herbal medicine to treat infections [6, 7]. Plant extracts have great potential as antimicrobial compounds against microorganisms [8]. They can be used in the treatment of infectious diseases caused by resistant microbes. Synergistic effect from the association of antibiotic with plant extracts against resistant bacteria leads to new choices for the treatment of infectious diseases. This effect enables the use of the respective antibiotic when it is no longer effective by itself during therapeutic treatment. All drugs of the past were substances with a particular therapeutic action extracted from plants. Thus, medicinal plants can be put to culinary or medicinal use [9]. Medicinal plants with emphasis on their antimicrobial properties were also reviewed that clearly showed that products derived from plants may potentially control microbial growth in diverse situations and in the specific case of disease treatment, numerous studies have aimed to describe the chemical composition of these plant antimicrobials and the mechanisms involved in microbial growth inhibition, either separately or associated with conventional antimicrobials [10]. *Pongamia pinnata* is leguminous tree, commonly known as Karanj [11] and has many traditional uses. The phytochemical constitutents of *P. pinnata* contribute for their biological activity [12-17]. The objective of the present study was to assess the antimicrobial activity of different parts of *Pongamia pinnnata* L extracted in four different solvents.

MATERIALS AND METHODS

Collection of Plant material

The plants to be explored for antimicrobial activity were identified using taxonomical principle. Plant materials were collected from the field (Charoda) and brought to the laboratory. Different parts of the plants were washed separately with tap water and shade dried at room temperature for constant weight. The air dried parts were powdered in an electric blender, packed in plastic bags and stored at 4^{o} C till further use.

Extraction

The dried powder material was extracted sequentially in four different solvents *viz*., petroleum ether, acetone, ethanol and aqueous from non polar to polar. 20 g powdered substance was extracted first in 150 ml of petroleum ether (nonpolar), followed by acetone (dipolar), ethanol (polar) and finally in water (polar), sequentially using soxhelet apparatus for 8 hrs each. The crude extract thus obtained was filtered using Whatman No. 1 and concentrated in an incubator at 40°C until the solvent evaporated completely and later stored at 4°C until use.

Preparation of stock solution

The crude extract of the plant material was dissolved in desired amount of mother solvent to prepare a 10 percent stock solution (w/v).

Microorganism used

Four selected bacterial species namely *E. coli*, *S. aureus*, *K. pneumonia* and *P. aeruginosa* used in the present study were obtained from Pt J. N. Medical College, Raipur (C.G). The bacterial species were maintained by sub-culturing in nutrient agar slants.

Preparation of inoculums

24 hrs cultures were optimized by adjusting the optical density of the culture broth with normal saline to required OD of 0.08 at 620 nm using a spectrophotometer which is equivalent to 10^6 colony forming units (CFU) [18].

Antimicrobial Activity

The antibacterial assay of petroleum ether, acetone, ethanol and aqueous extracts was performed using agar disc diffusion method [19]. The 6 mm disc was injected with 20µl of crude test extract. Controls were included that comprised of mother solvents. Reference sets with standard antibiotic chloramphenicol (10µg/disc) were simultaneously maintained. The zone of inhibition was measured in mm and expressed as Mean ± Standard Error (SE). The activity index was calculated using the following formula: Activity Index = Inhibition area of test sample / Inhibition area of standard antibiotic

RESULTS AND DISCUSSION

The leaf, root and seeds of *Pongamia pinnata* exhibited remarkable antimicrobial property. The total yield of phytochemicals in *Pongamia pinnata* is presented in Fig. - 1. The total yield of leaf was found to be 21.07%, whereas for seed it was 21.34% and for root the total yield was 25.51%. The aqueous extraction of leaf showed the yield of 8.40%, followed by acetone (5.31%), ethanol (4.76%) and petroleum ether (2.60%). This might be due to the fact that water is a universal solvent and extracts most of the compounds as reported by [20, 21). The antimicrobial activity of leaf, seed and root of *Pongamia pinnata* is presented in Fig. - 2. Leaf, seed and root extracts were tested on one strain of gram positive (*S. aureus*) and three strain of gram negative bacteria (*E. coli, K. pneumonia* and *P. aeruginosa*). The study revealed higher activity in acetone extracts as compared from ethanol extract. Petroleum ether and aqueous extract did not exhibit antimicrobial activity in all strains except *E. coli* and *S. aureus* which exhibited some activity in seed and root aqueous extracts. The seed emerged as the most effective part of this plant where maximum zone of inhibition was noticed for all the tested organism; the greatest zone of inhibition was recorded on *S. aureus* (9.0 ± 0.57) (Fig-3), followed by *E. coli* (4.50 ± 0.50), *K. pneumonia* (4.00 ± 1.52) and *P. aeruginosa* (2.33 ± 0.88) for acetone extract. The root and leaf acetone extracts, though less effective, exhibited similar pattern like that of seed (Fig. 2). During the study, ethanol extract of seed exhibited higher antimicrobial activity when compared from root and leaf ethanol extract. In seed, effective zone of inhibition was observed for *S. aureus* (4.00 ± 1.52), *E. coli* (4.00 ± 0.57) and *K. pneumonia* (4.00 ± 2.08). *P. aeruginosa* was less effective with 2.00 ± 1.00 mm zone of inhibition. The root was the second important part showing the antimicrobial property, wherein the maximum zone of inhibition was recorded on *S. aureus* (4.00 ± 1.52), followed by *K. pneumonia* (2.33 ± 0.88) and it was same for both *E. coli* (2.00 ± 1.00) and *P. aeruginosa* (2.00 ± 0.57) on ethanol extract. The maximum zone of inhibition was noticed with the ethanol extract of leaf of *Pongamia pinnata*, on *S. aureus* (1.66 ± 0.88), followed by both *E. coli* (1.00 ± 0.57) and *K. pneumonia* (1.00 ± 0.57) and least zone of inhibition was observed on *P. aeruginosa* (0.66 ± 0.33) (Fig. - 2). The activity index of acetone extract of leaf of *Pongamia pinnata* (Table - 1) was more or less similar to that of ethanol extract of leaf extract except in *K. pneumonia*. The ethanol extract of leaf showed maximum activity against *K. pneumonia* followed by *S. aureus*, *P. aeruginosa* and *E*. coli. The acetone extract of leaf showed maximum activity for *K. pneumonia* followed by *P. aeruginosa* & *S. aureus* and minimum activity was noted for *E. coli*. The activity index of ethanol extract of seed of *Pongamia pinnata* (Table - 1) was greater for *K. pneumonia* followed by *E. coli* and was similar for *S. aureus* and *P. aeruginosa*. The acetone extract of seed also showed the maximum activity index for *K. pneumonia* followed by *S. aureus*, *E. coli* and *P. aeruginosa*. The activity index of ethanol extract of root of *Pongamia pinnata* (Table-1) was lesser than that of the acetone extract of root of *P. pinnata.* The ethanol extract of root showed maximum activity index for *K. pneumonia* followed by both *S. aureus* & *P. aeruginosa* and minimum for *E. coli*. In acetone extract, highest activity index was noticed on *K. pneumonia* followed by *S. aureus*, *P. aeruginosa* and *E. coli*. The activity index was calculated to express the relationship between the zone of inhibition of the extracts with the standard

ISSN: 0975-8585

antibiotics [22]. The present study clearly demonstrates the antimicrobial activity of *P. pinnata* and the result indicates that seed was the most effective part as compared from root and leaf. Seed is an important organ of storage in plant. It contains oil which has high content of triglycerides and flavonoids. Antimicrobial activity of pongam oil of *P. pinnata* on *Bacillus anthracis, B. mycoides, B. pulius, E. coli, Pseudomonas mangiferae, Staphylococcus aureus* and *S. albus* has been reported [23]. The antimicrobial activity of Karanj seed oil *in-vitro* against fourteen strains of pathogenic bacteria was also suggested with dose dependent inhibition of the pathogens [24]. In the present study the sensitivity of tested bacteria in all extracts revealed that *S. aureus* was highly affected by the antimicrobial substances present in *P. pinnata*. In *P. pinnata* acetone extract showed maximum activity against all the tested bacteria as compared from ethanol extract. This finding corroborated to some other research work [25, 26]. Aqueous extract of seed showed some activity against *E. coli* and *S. aureus*. Root aqueous extract had inhibitory effect on *S. aureus*. Higher antimicrobial properties of acetone fraction suggest that the bioactive compounds present *in P. pinnata* of dipolar nature. The activity index of leaf, seed and root of *P. pinnata* was also observed. Maximum activity index was observed in the seed of *P. pinnata* against *K. pneumonia*, followed by *S. aureus*, *E. coli* and *P. aeruginosa* in acetone extracts. In ethanol extract the activity index was higher for *K. pneumonia* and comparable for other species. Similar pattern was observed for leaf and root of *P. pinnata*. Higher activity index (greater than 1) could be of economic value in exploring antimicrobial properties of a plant species. Moderate activity index for *Cathranthus roseus* against *Streptococcus pyogens*, *Bacillus cereus, Pseudomonas aeruginosa* and *Proteus mirabilis* with reference to penicillin was also observed [27]. In the current study low to moderate activity index was observed for *P. pinnata* as crude extracts used which may contain low concentration of phytochemical constituents. The activity index (≥0.5) in the crude extract indicates potential antimicrobial activity in the plant [28]. Further purification of the phytochemicals might be useful to assess their potential as antimicrobial compounds.

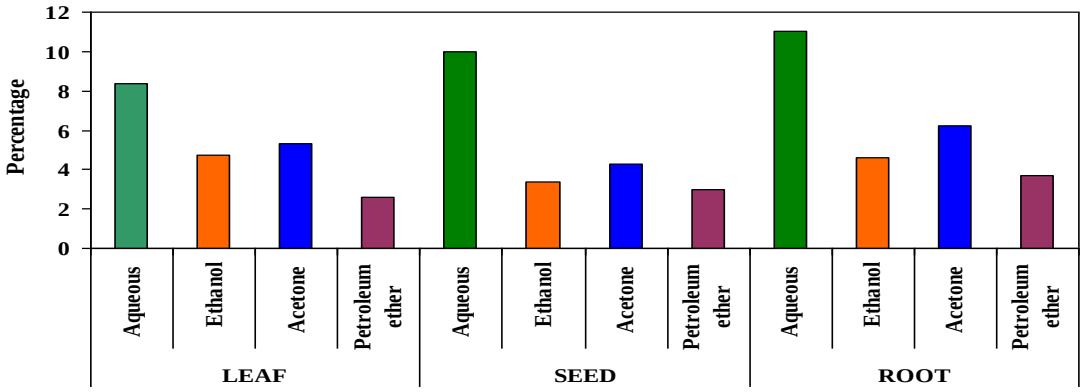

Figure 1: Yield of phyto-compounds from *Pongamia pinnata* in various solvent by hot extraction method.

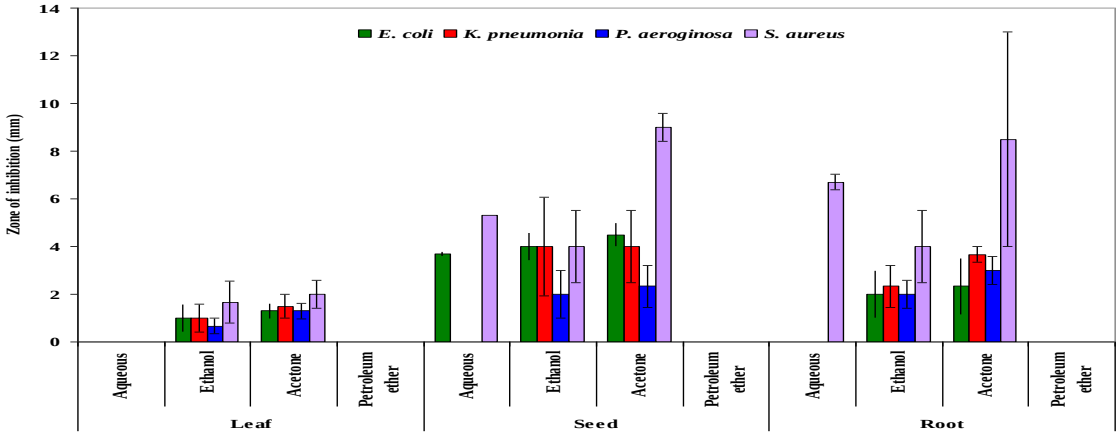

Figure 2: Antimicrobial activity of *Pongamia pinnata* by Agar Disc Diffusion method

ISSN: 0975-8585

Table 1: Activity index of Leaf, seed and root extracts of *Pongamia pinnnata*

Solvent	Activity index											
	Escherichia coli			*Klebsilla pneumonia*			*Pseudomonas aeruginosa*			*Staphylococcus aureus*		
	Leaf	Seed	Root	Leaf	Seed	Root	Leaf	Seed	Root	Leaf	Seed	Root
Ethanol	0.04	0.14	0.07	0.08	0.53	0.31	0.06	0.13	0.13	0.07	0.13	0.13
Acetone	0.05	0.16	0.08	0.20	0.53	0.49	0.08	0.15	0.19	0.07	0.30	0.28
Petroleum ether	-	-	-	-	-	-	-	-	-	-	-	-

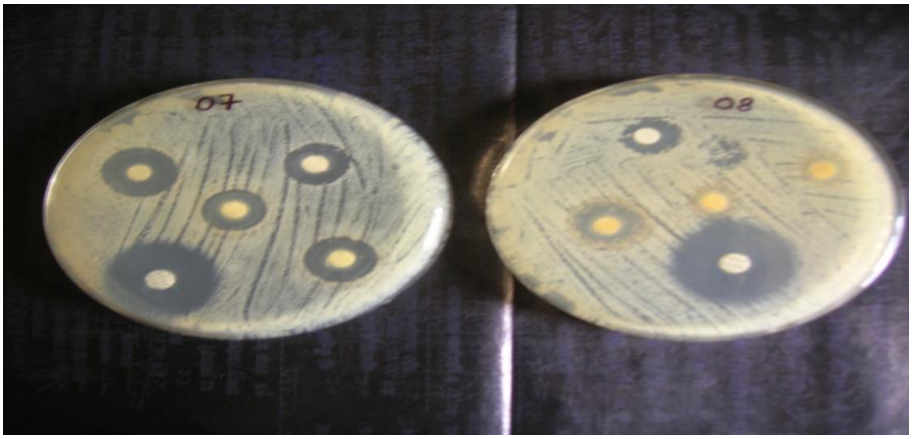

Figure 3: Zone of inhibition with acetone (07) and ethanol (08) extracts of seed of *Pongamia pinnata* against *Staphylococcus aureus*

CONCLUSION

This preliminary screening study revealed that *P. pinnata* posses antimicrobial activity and gave better results with acetone followed by ethanol extract against all the tested bacteria. Seed emerged as the most effective part of this plant. Root and leaf were next in order. The maximum activity was recorded against *Staphylococcus aureus* followed by *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa.* Aqueous extract of seed and root showed some inhibitory effect on *Escherichia coli* and *Staphylococcus aureus*. Activity index of seed of *P. pinnata* was found to be high followed by the root and leaf.

ACKNOWLEDGEMENTS

The authors are thankful to Head, School of Life Sciences, Pt. Ravishankar Shukla University, Raipur for providing the research facility to carry out present work.

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