An assessment of Pongamia pinnata (L) Pierre species extracts as potential antimicrobial agents against Bacterial pathogens of fishes

Vijay Tulshiram Gorgile
Department of Botany, Shahir Annabhau Sathe Mahavidyalaya Mukhed Dist. Nanded M.S., vijay.gorgile@gmail.com

Abstract

In human nutrition, fish is a substantial source of dietary animal protein. Bacteria are one of the principal causal agents of fish infections in both wild and domesticated fish, causing significant economic losses. Few pathogens affect freshwater and marine fish; nevertheless, numerous diseases may manifest as solely skin infections, in particular flexibacteria, aeromonads, and vibrios. Ulcers, bleeding, scale loss, tail and fin rot, and dropsy are all bacterial illnesses found in fish. Aeromonas sp. and Pseudomonas sp. bacteria are ubiquitous facultative parasites whose pathogenicity becomes a concern for fish only under adverse conditions.

Aeromonas sp. and Pseudomonas sp., which are commonly associated with large carps and live fish in freshwater, were described and tested against a plant extract of Pongamia pinnata. This study found that while both pathogenic bacterial isolates from fish are vulnerable to P. pinnata extracts, intermediate to total resistance to various antibiotics develops.

INTRODUCTION

Medicinal plants are plants that are used to prevent or cure disease, illness, or to alter a pathological process. Plant-based drugs and therapies provide a significant contribution to human health and well-being. These natural compounds account for more than half of all drugs utilized in clinical trials worldwide. Natural and traditional medicine are gaining popularity as a source of new commercial products due to their effectiveness. Because of its effectiveness, natural and traditional medicine is gaining popularity as a source of new commercial products.

Most microbial pathogens are quite dangerous, with an increased vulnerability to bacterial infection. As a result, most rural communities lack adequate sanitation, hygiene, and living conditions in comparison to urban areas. The gastro-intestinal tract is a sensitive channel that distributes nutrients throughout the body, and bacterial infection produces vomiting, diarrhea, and systemic disease (Barde, 2021). There are many different types of bacteria that can infect the digestive tract, such as Salmonella spp., Shigella spp., Escherichia coli, Staphylococcus aureus, Helicobacter pylori, and Vibrio spp.

An estimated 25 million Indians rely on traditional medicines for primary health care, raising awareness of herbal medicine as an alternative health care option. India has a diverse range of medicinal plants, which is reflected in the country's formal and informal medicinal systems. People's informal traditional medicinal systems, such as Ayurveda and folk medicine, have yet to be systematized (More and Baig, 2013). This sad lack of accurate records of the usage of traditional medicinal plants in India could be attributed to the effects of urbanization and

cultural changes. Antibiotics are microorganism-produced chemicals with numerous applications, including infection control, prophylaxis, food preservation, and growth promotion (Baig et al., 2002).

The formation of resistance is an extremely complex process, and its environmental significance is poorly understood. Resistance to germs from various environments is increasing at an unprecedented rate and is becoming a major concern globally. The frequency of resistant bacteria in the environment is determined by the bacterium's exposure to various antibiotics, which results in the formation of new strains of bacteria resistant to certain antibiotics.

The goals of this study were to evaluate the extraction of compounds from plant materials using acetone, water, and methanol as well as to assess the antimicrobial activity of crude extracts against the fish pathogens Pseudomonas fluorescens and Aeromonas hydrophila using the minimal inhibition concentration (MIC).

Material and Methods

For extraction, 1 g of powdered plant material from each plant component was extracted in centrifuge tubes for an hour with 10 ml of technical graded acetone, methanol, distilled water, and heated distilled water. For 10 minutes, the samples were centrifuged at 400X rpm. The cold water extract was created by soaking the plant material in water overnight and stirring it occasionally. The sample materials and water were allowed to boil in the boiling water extract. The reactions were allowed to cool to room temperature before being allowed to settle overnight. The supernatant was filtered into pre-weighed vials using Whatman No. 1 filter paper. Extraction on the pellet was performed three times with the same extractant to completely extract the plant (Kothari and Baig, 2013).

Yield determination

The extractants utilized were acetone, methanol, and water. Acetone was chosen because of its low toxicity and capacity to extract both non-polar and polar components (Eloff, 1998), whereas water was chosen since it is the only extractant that has historically been utilized. The water extract of the Leaves produced the highest yield (2.96%), followed by the water extract of the seeds (2.5%). The Acetone extract of the bark yielded the lowest yield (0.14%), followed by the acetone extracts of the roots, which yielded 0.15%, as shown in Table 4.1.

Isolation of Microorganisms

Normalization of fish specimens at room temperature is accomplished by immersing them in distilled water for 1 hour, followed by the addition of 1 gram of the sample to nutrient broth for enrichment of microorganisms. On nutritional agar, microorganisms were isolated using the spread plate and streak plate methods. Physiological and biochemical tests, as well as Bergey's Manual of Systematic Bacteriology, were used to identify the bacteria.

Table 1. Percentage yield of the Pongamia pinnata plant parts: Seeds, leaves, bark and roots, extracted with different solvent

Solvents	Solvents	Yield(g)	Yield %
Seeds	Acetone	0.152	0.51
	Water	0.749	2.50
	Methanol	0.076	0.25
Leaves	Acetone	0.066	0.22
	Water	0.889	2.96
	Methanol	0.329	1.10
Bark	Acetone	0.043	0.14

	Water	0.213	0.71
	Methanol	0.216	0.72
Roots	Acetone	0.044	0.15
	Water	0.113	0.38
	Methanol	0.312	1.04

Antimicrobial bioassays

There are several methods for determining antibacterial activity, including disc diffusion, serial microplate dilution (Barde et al., 2021). Disc diffusion is a straightforward approach for analyzing a wide variety of samples, but it has limits when it comes to analyzing the antibacterial activity of oils and non-polar compounds. A 96-well microtiter plate is used to determine the minimal inhibitory concentration of an active plant extract (Eloff 1998). The method is used to determine the bacteriostatic and bacteriocidal activities of a substance at a specific concentration. Bioautography is a qualitative technique that combines thin-layer chromatography with in vitro bioassay to localize an active chemical in an extract (Gibbons and Gray 1998).

Minimal inhibitory concentration

Eloff (1998) employed the serial microplate dilution method to assess the minimal inhibitory concentration (MIC) for the extracts using iodonitrotetrazolium violet (INT) as a growth indicator.

With 1% sugar and 1% dextrose, a nutritional medium of Tryptone soy agar (38 g in 1 liter) was made. Organism isolates were streaked on agar and incubated at 37 °C for 18–24 hours. Colonies were collected from the prepared plates and sub-cultured on new agar for MIC; the subcultures were incubated at 37 °C for 24 hours. The tryptone broth was made. Acetone extract residues were reconstituted to a concentration of 10 mg/ml in acetone.

In a sterile 96-well microtitre plate, 100 l of the extract examined was serially diluted twice into 100 l of sterile water for each of the enteric bacteria utilized. Similarly, two-fold serial dilutions of 100 l gentamycin at a concentration of 0.1 mg/ml were utilized (positive control). As negative controls, acetone and sterile water were utilized. Each experiment and control were carried out in triplicate. Each well received 100 microliters (l) of each bacterial culture. The plates were covered and incubated overnight at 37°C. To detect bacterial growth, 40 l of 0.02 mg/ml INT was added to the plates and incubated at 37°C for 30-40 minutes. Bacterial growth in each well was shown by a pink-red color, whereas bacterial inhibition was represented by a blue-green color.

Results

Antimicrobial drugs are used in aquaculture as growth promoters to maximize food production and for the clinical treatment of infectious disorders in farm animals to minimize further transmission of infection in animals and people. Inadequate antimicrobial agent selection or indiscriminate use of antimicrobial agents may result in bacterial resistance (Darak & Barde, 2014). As a result of the development of bacterial resistance to most first-line antibiotics (amoxicillin, ciprofloxacin, and levofloxacin), the clinical management of these illnesses has grown more challenging. These resistant bacteria can enter the human body through the food chain (Papadopoulou et al., 1997), contact through industrial exposure, or waste discharge from animal production facilities (Schroeder et al., 2002), and are the source of foodborne illnesses.

Several studies have been published on the antibiotic susceptibility of bacteria, with many resistant bacteria isolated from fish-related samples, including Escherichia spp.,

Enterobacter spp., Salmonella spp., and other Enterobacteriaceae members (Spanggaard et al., 2000). Antimicrobial resistance in enteric bacteria, particularly Enterobacteriaceae, is a symptom of the emergence of resistant bacteria in the food and related industries (Kijima-Tanaka et al., 2003).

Table 2 shows the MIC values of the extracts against the four intestinal pathogens. Acetone and methanol extracts of the leaves, bark, and roots were more effective against the tested bacteria than water. Methanol extracts had MIC values as low as 0.03 mg/ml against both the bacterial isolates. Methanol extracts from the leaf inhibited both the bacterial isolates the least, with a concentration of 0.06 mg/ml and 0.07 mg/ml from the leaf, bark, and root extracts respectively. With a minimum concentration of 0.07 mg/mL, water extracts were efficient against both the bacterial isolates. Because the minimum concentration of water extracts was 0.3 mg/mL, they were ineffective against enteric bacteria. Despite the fact that methanol extracts were also efficient against enteric pathogens, only the acetone extracts were evaluated for the following phase, solvent-solvent separation.

Conclusion

Minimum Inhibitory Concentration (MIC) determination and the dry-weight method were used to assess the antifungal and antibacterial activity of different concentrations of oil derived from Pongamia pinnata against fungal and bacterial isolates (Wagh et al., 2007). Gas chromatography (GC) and gas chromatography/mass spectrometry (GC-MS) studies of oil revealed the presence of fatty acids. They proposed using this plant's fatty oil to generate plant-derived antibacterial medicines (Wagh et al., 2007).

Although water was a decent extract solvent in terms of yield, the extracts were ineffective against the tested bacterial pathogens, with the lowest MIC of 0.3 mg/ml and the highest MIC of >2.5 mg/ml. The Seeds were less potent against all of the bacteria examined, with MICs ranging from 0.3 mg/ml to 1.25 mg/ml in acetone extracts and 1.25 mg/ml to >2.5 mg/ml in water extracts. Acetone has previously been shown to be the best solvent due to its low toxicity to pathogens (Eloff, 1998), which suggests that the activity of the acetone extracts is derived from the extracted components rather than the solvent employed to extract them. The leaf, bark, and root extracts were efficient against the pathogens tested, with the lowest MIC of 0.07 mg/ml.

Table 2. Minimal inhibitory concentrations (mg/ml) of the Seed, leaves, bark and roots extracts against bacterial isolates.

Plant parts	Solvents	*Aeromonas sp.*	*Pseudomonas sp.*
Seeds	Acetone	1.25	1.25
	Methanol	0.03	0.06
	Water	2.5	2.5
Leaves	Acetone	0.15	0.6
	Methanol	0.03	0.07
	Water	1.25	1.2
Bark	Acetone	0.6	0.3
	Methanol	0.3	0.15
	Water	1.25	1.25
Roots	Acetone	0.15	0.3
	Methanol	0.3	0.3
	Water	0.6	0.07

REFERENCES

Baig, M. M. V., Baig, M. I. A., & Muley, S. M. (2002). Enhanced growth of groundnut by plant growth promoting rhizobacteria. International Arachis Newsletter.22; 60-62

Barde, R. D. (2021). Bacterial Diversity Associated With Labeo Rohita Isolated From Cultured And Natural Water Bodies. European Journal of Molecular & Clinical Medicine, 7(11), 2020.

Barde, R. D., Apastambh, A. R., & Baig, M. M. V. (2021). Association of Oomycetes Aphanomyces invadans with Freshwater fishes. Annals of the Romanian Society for Cell Biology, 25(6), 14118-14125.

Eloff, J. N. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta medica, 64(08), 711-713.

Gibbons, S., & Gray, A. I. (1998). Isolation by planar chromatography. Natural products isolation, 209-245.

Kijima-Tanaka, M., Ishihara, K., Morioka, A., Kojima, A., Ohzono, T., Ogikubo, K., ... & Tamura, Y. (2003). A national surveillance of antimicrobial resistance in Escherichia coli isolated from food-producing animals in Japan. Journal of Antimicrobial Chemotherapy, 51(2), 447-451.

Kothari, M.N. and Baig, M.M.V., 2013. Production and characterization of extracellular polygalacturonase by Erwinia carotovora MTCC 1428. Int J Adv Biotechnol Res, 4(1), pp.981-998.

More, D.R. and Baig, M.M.V., 2013. Fungitoxic properties of Pongamia pinnata (L) Pierre extracts against pathogenic fungi. International Journal of Advanced Biotechnology and Research, 4(4), pp.560-567.

Papadopoulou, C., Dimitriou, D., Levidiotou, S., Gessouli, H., Panagiou, A., Golegou, S., & Antoniades, G. (1997). Bacterial strains isolated from eggs and their resistance to currently used antibiotics: Is there a health hazard for consumers?. Comparative immunology, microbiology and infectious diseases, 20(1), 35-40.

Schroeder, C. M., Meng, J., Zhao, S., DebRoy, C., Torcolini, J., Zhao, C., ... & White, D. G. (2002). Antimicrobial resistance of Escherichia coli O26, O103, O111, O128, and O145 from animals and humans. Emerging infectious diseases, 8(12), 1409.

Spanggaard, B., Huber, I., Nielsen, J., Nielsen, T., Appel, K. F., & Gram, L. (2000). The microflora of rainbow trout intestine: a comparison of traditional and molecular identification. Aquaculture, 182(1-2), 1-15.

Wagh, P., Rai, M., Deshmukh, S. K., & Durate, M. C. T. (2007). Bio-activity of oils of Trigonella foenum-graecum and Pongamia pinnata. African journal of Biotechnology, 6(13).