

Original Article

# Phytochemical Analysis and Antimicrobial Activity of Pongamia Pinnata: A Comprehensive Study

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## ABSTRACT

**Background:** Antimicrobial resistance (AMR) poses a significant global health challenge, exacerbated by the misuse of antimicrobial agents. With the rising prevalence of AMR in various plant and animal pathogens, there is an urgent need to explore alternative therapeutic options. Plant extracts, historically utilized for disease treatment, offer a promising avenue. Pongamia pinnata, also known as Indian Karanja, is notable for its rich phytochemical content and has been the subject of recent scientific interest.

**Objective:** The primary aim of this study is to investigate the antimicrobial efficacy of P. pinnata extracts, prepared using the Ultrasonic Assisted Extraction (UAE) method, against a range of bacterial and fungal species known to exhibit AMR.

**Methods:** In this study, an experimental laboratory design was utilized to evaluate the antimicrobial properties of Pongamia pinnata. Aqueous and alcoholic extracts of P. pinnata were prepared and subjected to biochemical analysis to identify the spectrum of phytochemicals present. The antimicrobial activity of these extracts was then assessed using the well-diffusion method against selected bacterial and fungal pathogens.

**Results:** Both aqueous and alcoholic extracts of P. pinnata demonstrated significant antimicrobial effects against the tested microbial species. The study highlights the potent antimicrobial properties of P. pinnata, attributed to its diverse phytochemical composition.

**Conclusion:** The findings of this study underscore P. pinnata as a valuable source of natural antimicrobial agents. Its efficacy against AMR pathogens suggests its potential application in the development of new treatments for infectious diseases. P. pinnata holds promise for medicinal use, particularly in contexts where conventional antibiotics are ineffective.

**Keywords:** Pongamia pinnata, antimicrobial resistance, phytochemicals, plant extracts, ultrasonic assisted extraction, well-diffusion method.

## INTRODUCTION

The emergence of antibiotic resistance among pathogens is a growing global health concern, highlighted by a substantial body of research indicating the alarming increase in multidrug-resistant bacteria. This resistance, often attributed to the misuse and overuse of antibiotics, has been identified as a major cause of escalating mortality rates, particularly in the developing world (1, 2). Notably, the excessive use of antibiotics in both agricultural and medical settings has exacerbated this issue (3-5). For instance, the application of antibiotics in livestock to promote growth has been linked to the rise of antibiotic-resistant infections in humans (6, 7), while the inefficacy of antimicrobial treatments in agricultural crops has also been documented (8). This scenario is not limited to bacterial pathogens; the development of resistance among fungal species to antifungal drugs is also a significant concern, with pathogenic fungi such as Candida, Epidermophyton, and Alternaria posing serious health threats (9, 10).

In response to these challenges, the exploration of alternative antimicrobial sources has become imperative (11-13). Historically, plant extracts have been used for millennia in the treatment of various ailments, reflecting a rich tradition of botanical medicine (11, 14). This tradition includes notable examples such as the use of quinine from the cinchona tree for treating malaria, the derivation of aspirin from willow bark, and the application of aloe vera in wound healing (15). In this context, Pongamia pinnata, commonly known as Indian Karanja, has garnered significant attention due to its diverse range of bioactive compounds found in different plant tissues. Recent studies have highlighted the potent antimicrobial properties of P. pinnata, particularly in its seed extracts which have

demonstrated antibacterial and antifungal effects (16). These extracts are rich in bioactive compounds such as flavonoids, alkaloids, and saponins, and have shown efficacy against a variety of microbial diseases (17). Moreover, leaf extracts of *P. pinnata* containing tannins and quinones have exhibited bactericidal and fungicidal properties, suggesting their potential application in agriculture to protect crops from plant pathogenic bacteria and fungi (12).

Significantly, *P. pinnata* has been a source of isolated chemicals with pronounced antibacterial properties, including karanjin and pongapin. Karanjin, in particular, has shown effectiveness against human bacterial strains and fungal species like *Candida albicans* (18). The extensive investigation into the antimicrobial properties of *P. pinnata* underscores its potential as an alternative source of antimicrobial agents, which could play a crucial role in addressing the challenge of antibiotic and antifungal resistance (7, 10, 19-22). The growing body of evidence supporting the efficacy of *P. pinnata* in combating microbial infections positions it as a promising candidate in the ongoing search for novel and effective antimicrobial compounds (17, 23-26).

## MATERIAL AND METHODS

In this study, an experimental laboratory design was utilized to evaluate the antimicrobial properties of *Pongamia pinnata*. In this study, various bacterial and fungal strains were utilized, including *S. aureus* (ATCC6538), *S. typhimurium* (ATCC2515), *E. coli* (ATCC43888), *P. aeruginosa* (ATCC6432), *K. pneumoniae* (ATCC13883), *B. subtilis* (ATCC6633), *L. monocytogenes* (ATCC19118), *Alternaria solani* (ATCC 58177), *Epidermophyton floccosum* (ATCC 52066), and *Candida albicans* (ATCC 10231). These strains were provided by the microbiology lab of the University of Central Punjab, Pakistan. Stock solutions of all microbial cultures were prepared and refreshed weekly in a broth culture medium. For fungal strains, spore cell suspensions were prepared by adding sterile water to 8-day old slant cultures (1, 14, 27, 28).

The leaves of *Pongamia pinnata* were collected from the countryside of Lahore, Pakistan, in November 2021 and authenticated by the University of Agriculture Faisalabad, Pakistan. These leaves were washed, shade-dried for four weeks, finely ground, and stored in an air-tight glass jar away from light, heat, and moisture. The leaves' powder extract was prepared using the Ultrasonic Assisted Extraction (UAE) Method in a 100 ml flask (29-33). This process involved adding 1 g of fine leaf powder to 50 ml of ethanol as a solvent. The UAE was performed at 400 W, at temperatures of 35°C, 50°C, and 60°C for durations of 10, 25, and 60 minutes (22, 29). Phytochemical screening of both aqueous and alcoholic extracts of *P. pinnata* leaves and seeds was conducted to detect primary and secondary metabolites. Various qualitative tests were performed to identify carbohydrates, proteins, fats, terpenes, sterols, tannins, alkaloids, flavonoids, flavones, saponins, and cardiac glycosides (34-40).

For proteins, the Biuret, Ninhydrin, and Xanthoproteic tests were conducted. The Biuret test involved mixing equal volumes of extract and 4% sodium hydroxide solution in a test tube, followed by the addition of 1% copper sulfate, which resulted in a violet color indicating the presence of peptide linkages. The Ninhydrin test required heating 2ml of extract with 0.25% Ninhydrin reagent, where a blue color appearance indicated proteins. In the Xanthoproteic test, a few drops of nitric acid added to a small volume of extract resulted in a yellow color, also indicating proteins.

Phenol presence was assessed using the Ferric chloride, Lead acetate, Gelatin, and Mayer's reagent tests. The Ferric chloride test involved adding a gelatin solution (1%) and NaCl to a small volume of extract, resulting in a bluish-black color signifying phenols. In the Lead acetate test, the mixture of equal volumes of extract and alcoholic solution was diluted with sulfuric acid (20%) and a few drops of NaOH, leading to a red to blue color change. The Gelatin test used 2 mL of gelatin solution (1%) in the extract, where a white precipitate indicated phenols. The Mayer's reagent test involved mixing 1mL of Mayer's reagent with the extract, with the appearance of white precipitate also indicating phenols.

Flavonoid detection employed Shinoda's, Lead acetate, and Alkaline reagent tests. The Shinoda's test added concentrated HCl and magnesium ribbon fragments to 1mL of extract, where a pink color indicated flavonoids. The Lead acetate test involved adding a few drops of lead acetate to the extract and shaking well, with a yellow precipitate signifying flavonoids. The Alkaline reagent test changed the mixture to colorless after adding dilute acid, indicating flavonoids.

Tannins were detected using the Gold Beater's skin and Gelatin's tests. The Gold Beater's skin test involved soaking the ox skin in HCl (2%) and *P. pinnata* extract, which turned black upon immersion in ferrous sulfate solution (1%), indicating tannins. Gelatin's test added a gelatin solution (1%) containing NaCl to the extract, with the formation of white precipitates signifying tannins.

Tests for Steroids & Triterpenoids involved the Libermann Burchard's and Salkowski's tests. The Libermann Burchard's test formed a violet to blue-color ring at the junction of the solutions upon adding a few drops of acetic anhydride followed by sulfuric acid to the extract.

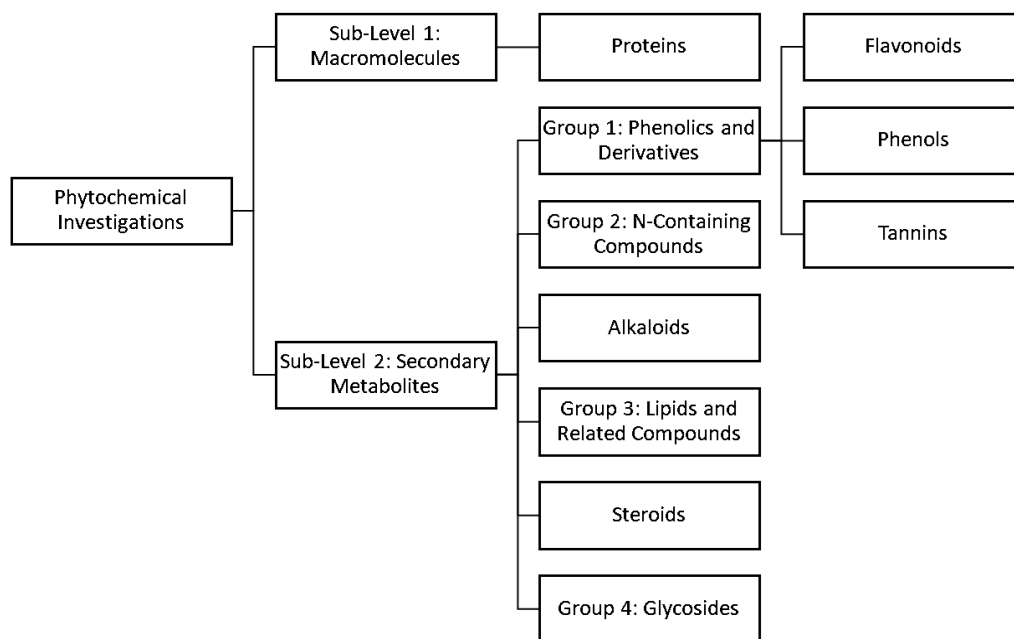


Figure 1 Phytochemical Screening of *P. pinnata* extract

The Salkowski’s test for steroids and triterpenoids involved the addition of a few drops of conc. Sulphuric acid to the *P. pinnata* extract mixed with double the volume of chloroform. The formation of golden yellow precipitates indicated the presence of triterpenes.

For the detection of alkaloids, Dragendorff’s, Wagner’s, and Hager’s tests were utilized. The Dragendorff’s test

involved combining an equal volume of the extract with Dragendorff’s reagent, leading to the formation of an orange-red precipitate, indicative of alkaloids. In Wagner’s test, the addition of Wagner’s reagent to the extract resulted in a reddish-brown precipitate, while in Hager’s test, the formation of a yellow precipitate after mixing with Hager’s reagent confirmed the presence of alkaloids.

Glycosides were identified using Bontrager’s, Legal’s, and Keller–Killiani tests. Bontrager’s test required boiling the extract with diluted HCl, followed by extraction with benzene and the addition of ammonia solution, resulting in a pink color indicative of glycosides. The Legal’s test involved adding sodium nitroprusside to the extract, followed by NaOH, where a pink-to-red precipitate indicated glycosides. In the Keller–Killiani test, diluting the extract with water and adding lead acetate, followed by chloroform extraction and mixing with glacial acetic acid, ferric chloride, and sulphuric acid, resulted in a reddish-brown layer, signifying the presence of digitoxose, a component of glycosides.

The antimicrobial activity of *P. pinnata* extracts was evaluated using the well-diffusion method. The fungicidal effects were tested against *Epidermophyton floccosum*, *Candida albicans*, *Alternaria solani*, and *Helminthosporium turcicum*, forming zones of inhibition in their respective culture media. Amphotericin B was used as a positive control for the aqueous and alcoholic extracts. Similarly, the antibacterial efficacy was assessed against bacterial strains including *S. aureus*, *S. typhimurium*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *B. subtilis*, and *L. monocytogenes*, with zones of inhibition observed in the culture media. Amoxicillin served as a positive control against these bacterial strains. The optimization conditions such as time gap, dosage concentration, temperature range, and environmental exposure were maintained consistently, ensuring the reliability of the results (8, 9, 41, 42).

## RESULTS

Phytochemicals	Tests Performed	Results
Proteins	Biuret Test	+
	Ninhydrin Test	+
	Xanthoproteic Test	+
Steroids	Lieberman's Test	+
	Salkowski’s Test	+
Phenols	Ferric Chloride Test	+
	Lead Acetate Test	+
	Gelatin Test	+
	Mayer’s Test	+
Alkaloids	Dragendorff’s Test	+
	Wagner’s Test	+

Phytochemicals	Tests Performed	Results
	Hager's Test	+
Flavonoids	Shinoda's Test	+
	Lead Acetate Test	+
	Alkaline Reagent Test	+
Glycosides	Bontrager's Test	+
	Legal's Test	+
	Keller-Killiani Test	+
Tannins	Gold Beater's Skin Test	+
	Gelatin Test	+

## DISCUSSION

The growing public health threat posed by antimicrobial resistance (AMR) organisms necessitates an urgent exploration for novel antimicrobial agents (43). While antibiotic resistance results from both judicious and injudicious antibiotic use, the quest for new antimicrobial sources has become imperative (8, 9). The resurgence in the valorization of medicinal plants for their therapeutic potential has shed light on their capacity to yield novel antibacterial compounds (2, 15, 18, 43-45). *Pongamia pinnata*, commonly referred to as *Karanja*, has a storied history of medicinal use and has recently been subjected to scientific scrutiny for its antibacterial efficacy (6, 43, 46-48).

Research by Walia et al. (2017) delved into the antimicrobial activities of phytochemicals derived from *P. pinnata*, revealing that various parts of the plant, including leaves, seeds, and roots, exhibit significant bioactivity (49). This positions the plant as a rich repository of natural antimicrobial agents, a fact that was further corroborated by the comprehensive studies of Radice et al. (2022) (42, 49-51). The assortment of phytochemicals present in *P. pinnata*—such as proteins, phenols, flavonoids, tannins, steroids, alkaloids, and glycosides—has been shown to harbor potent antimicrobial properties against a wide spectrum of microbial diseases, encompassing both bacterial and fungal pathogens (30, 31) further bolster the argument for harnessing these phytochemicals in developing new antimicrobials to combat microbial infections (17, 24).

The antifungal potential of *P. pinnata*'s phytochemicals has been a subject of interest, with Tripathi and Bhattacharyya (2004) discussing its capacity to yield compounds effective against fungal pathogens (23, 49). Investigations into the inhibitory effects of *P. pinnata* extracts against fungi such as *Candida albicans*, *Epidermophyton floccosum*, *Alternaria solani*, and *Helminthosporium turcicum* revealed that flavonoids and tannins, in particular, displayed considerable antifungal activity. Such findings open avenues for application in the development of natural antifungal treatments and agricultural crop protection strategies.

Dye (2014) places a spotlight on the antibacterial potential of phytochemicals from *P. pinnata*, especially given the plant's abundance of bioactive constituents (20). The study's aim was to assess the inhibitory effects of *P. pinnata* extracts on a variety of bacterial strains, including both Gram-positive and Gram-negative bacteria. The outcomes indicated that the plant's phytochemicals possess an expansive array of antibacterial properties, with alcoholic extracts showing greater potency. These results reinforce the potential of *P. pinnata* as a source of innovative antimicrobial substances capable of tackling bacterial infections, particularly those resistant to existing antibiotics.

While the antimicrobial activities of *P. pinnata* extracts are promising for both medicinal and agricultural applications, there are limitations to be considered. For instance, the broad-spectrum action against pathogens is a strength, but the specific mechanisms of action and the isolation of individual active components remain underexplored. The potential of *P. pinnata* extracts in suppressing plant pathogenic microorganisms suggests their suitability for eco-friendly agricultural practices, yet the translation of these findings to field applications requires careful scaling. Future research should prioritize the identification and isolation of the active phytochemicals to develop more targeted antimicrobials. Furthermore, rigorous toxicological evaluations are essential to ensure the safety and efficacy of these plant-derived compounds for human and animal health.

*Pongamia pinnata* harbors a wealth of phytochemicals with potent antimicrobial activities, presenting a viable alternative to combat infectious diseases caused by pathogens, including those resistant to conventional antibiotics (6, 47-51). The plant's broad-spectrum antimicrobial action, alongside potential applications in both medical and agricultural spheres, underscores its value as a substitute for synthetic antimicrobials. Nonetheless, a comprehensive understanding of its bioactive components and their safety profile is critical for the advancement of *P. pinnata* as a therapeutic agent.

## CONCLUSION

In conclusion, *Pongamia pinnata* emerges as a formidable ally in the global fight against infectious diseases, particularly those caused by antibiotic-resistant pathogens. With a diverse array of phytochemicals exhibiting potent antimicrobial properties, *P. pinnata* holds substantial promise for the development of new therapeutic agents. Its efficacy against a broad spectrum of bacteria and fungi not only positions it as a valuable resource for advancing human healthcare but also suggests its utility in enhancing agricultural disease management practices. The integration of *P. pinnata*-derived treatments could mark a significant stride in mitigating the public health threat of antimicrobial resistance, thereby reinforcing the imperative to further investigate and harness its medicinal potential while ensuring safety and efficacy for human use.

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