© IJCIRAS | ISSN (O) - 2581-5334
December 2024 | Vol. 7 Issue. 7

PHYTOCHEMICAL EVALUATION ANTIBACTERIAL ACTIVITY OF PROSOPIS SPECIES PODS.

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Abstract

This study investigates the phytochemical composition and antibacterial activity of aqueous pod extracts from Prosopis juliflora and Prosopis cineraria, two underutilized legumes with significant ethnomedicinal value. Extracts were prepared using the decoction method, and qualitative phytochemical screening revealed the presence of phenolics, flavonoids, tannins, saponins, alkaloids, glycosides, terpenoids, and steroids in both species. Antibacterial activity was evaluated using the turbidity method against E. coli, S. pneumonia, S. aureus, and K. pneumonia.. The results demonstrated dose-dependent antibacterial activity, with P. juliflora showing higher efficacy than P. cineraria, particularly against K. pneumonia. The findings validate the traditional use of Prosopis species in treating bacterial infections and highlight their potential as natural sources of bioactive compounds. Future research should focus on isolating and characterizing active constituents to enhance their pharmacological applications.

Keyword: Phytochemistry, Antibacterial activity, Prosopis juliflora, Prosopis cineraria.

1.INTRODUCTION

Prosopis species is an underutilized leguminous plant belonging to the Leguminosae family and the Mimosaceae subfamily1. This genus comprises 44 species, including notable ones like Prosopis juliflora and Prosopis cineraria 2. Geographically, 40 species originate from North and South America, while three species are native to Asia, and one is found in Africa 3. Prosopis thrives in arid, semi-arid, tropical, and subtropical regions, including countries like the United States, India, Argentina, Chile, Kenya, and Pakistan . In South America, Argentina is particularly rich in Prosopis biodiversity, harboring 29 species, including 14 endemic taxa. The genus grows extensively from the southwestern United States to Patagonia in Argentina, with significant presence in the Monte and Chaco desert regions 4. The ecological resilience of Prosopis is remarkable, as it withstands extreme heat, drought, salinity, and alkalinity, and contributes to soil improvement through nitrogen fixation 5. Additionally, Prosopis is a perennial plant that does not require annual sowing and can coexist with other crops, such as millet in India 6. The leaves of Prosopis cineraria are either smooth or slightly hairy, deciduous, and range in length from 2 to 7 cm. Its fruits are slender pods, measuring 10–21 cm in length, with a brittle and thin outer peel 7. The pods of P. cineraria consist of approximately 70% pericarp and 30% seeds, with the seeds being ovate and brown in color 8. Prosopis has versatile applications in food, being used to produce beverages, flour, sweets, jams, bread, cakes, cookies, and syrups 9. The flour derived from Prosopis pods is brown, sweet, and has an aroma reminiscent of coffee, cocoa, coconut, or caramel 10. In addition, the gum exuded from Prosopis bark serves as an emulsifier, film-forming agent, foaming agent, tablet binder, and stabilizer. Beyond its culinary uses, Prosopis provides firewood, timber, livestock feed, construction materials, fencing, medicine, and shade 11. Its applications in traditional medicine are extensive, with decoctions of its twigs and flowers exhibiting antidiabetic properties. Moreover, extracts from Prosopis leaves have demonstrated antibacterial, antihyperglycemic, antihyperlipidemic, and antioxidant activities 12. Due to the multiple benefits derived from all parts of the tree, Prosopis is often referred to as “kalpataru” in India, meaning “wonder tree” or “king of the desert”. This epithet

reflects its status as a valuable resource for both ecological sustainability and human livelihood 11.
This study focuses on the evaluation of Prosopis species pods highlighting their robust nutritional and phytochemical profiles, making them valuable for food security and health. Rich in macronutrients, essential minerals, and bioactive compounds, these pods exhibit significant antioxidant activity, suggesting potential use in functional food development. Additionally, their adaptability to harsh climates positions them as a sustainable resource for nutraceutical exploration. Further research is warranted to explore bioavailability and application in various food systems, ensuring optimal utilization of this underutilized resource.

2. MATERIALS AND METHODS

2.1. Sample Collection Species: Pods from selected Prosopis species (*Prosopis juliflora*, and *Prosopis cineraria* were collected from arid and semi-arid regions. Mature pods were harvested during peak fruiting season (late summer).

2.2. Preparation: Pods were cleaned, dried under shade, and pulverized into fine powder for analysis.

2.3. Preparation of Aqueous Pod Extract of Prosopis juliflora and Prosopis cineraria Using Decoction Method

2.3.1. Pod Preparation: Collect mature and dried pods of Prosopis juliflora and Prosopis cineraria. Wash thoroughly with distilled water to remove dirt and contaminants. Dry the pods completely at room temperature or in a hot air oven at 40–50°C. Pulverize the pods into coarse powder using a grinder or mortar and pestle.

2.3.2. Weighing the Sample: Weigh approximately 50 grams of the powdered pod material for extraction.

2.3.3. Boiling the Pods: Place the powdered pods in a beaker containing 500 mL of distilled water (ratio 1:10 w/v). Heat the mixture on a hot plate or water bath and bring it to a boil. Simmer the mixture for 30–60 minutes to extract the bioactive compounds effectively.
2.3.4. Cooling: Allow the decoction to cool to room temperature. Filtration: Filter the cooled mixture through a fine muslin cloth or Whatman No. 1 filter paper to remove the solid residue. Collect the filtrate, which is the aqueous pod extract.

2.3.5. Concentration: Concentrate the extract by evaporating excess water using a water bath at 40–50°C.

2.3.6. Storage: Store the prepared extract in a clean, airtight container at 4°C for further use **(13,14)**.

2.4. Phytochemical Analysis

2.4.1. Test for Phenolic Compounds
Ferric Chloride Test: Add 2-3 drops of 5% ferric chloride solution to 2 mL of plant extract. Observe the color change. A dark green or blue-black color indicates the presence of phenolic compounds **(15)**

2.4.2. Test for Flavonoids
Shinoda Test: Mix 2 mL of plant extract with a small piece of magnesium ribbon. Add a few drops of concentrated hydrochloric acid. Observe the color change. A pink, orange, or red color indicates the presence of flavonoids **(16).**.

2.4.3. Test for Tannins
Gelatin Test: Mix 2 mL of plant extract with 2 mL of a 1% gelatin solution containing 10% sodium chloride. Formation of a precipitate confirms the presence of tannins **(17).**.

2.4.4. Test for Saponins
Foam Test: Mix 2 mL of plant extract with 5 mL of distilled water in a test tube. Shake vigorously and let it stand for 10 minutes. Persistent foam formation indicates the presence of saponins **(18)**.

2.4.5. Test for Alkaloids
Dragendorff's Test: Add 2 mL of Dragendorff's reagent (potassium bismuth iodide) to 2 mL of plant extract. Observe for a reddish-brown precipitate, indicating the presence of alkaloids **(19)**.

2.4.6. Test for Glycosides
Keller-Killiani Test: Mix 2 mL of plant extract with 2 mL of glacial acetic acid containing a drop of ferric chloride.

Carefully add 1 mL of concentrated sulfuric acid by the side of the test tube. A blue-green ring at the interface indicates the presence of glycosides **(20)**.

2.4.7. Test for Terpenoids
Salkowski Test: Add 2 mL of chloroform to 2 mL of plant extract, followed by a few drops of concentrated sulfuric acid. Observe for a reddish-brown interface, which indicates terpenoids **(21)**.

2.4.8. Test for Steroids
Liebermann-Burchard Test: Add 2 mL of acetic anhydride and 1 mL of concentrated sulfuric acid to 2 mL of plant extract. A blue or green color indicates the presence of steroids **(22)**.

2.5. Antibacterial Activity of Prosopis juliflora and Prosopis cineraria Pod Extracts by Turbidity Method

2.5.1. Preparation of Extract Solutions: Dissolve the extracts in a sterile solvent (e.g., dimethyl sulfoxide (DMSO)) to prepare stock solutions at a known concentration (e.g., 10 mg/mL).
2.5.2. Bacterial Inoculum: Prepare bacterial suspensions in sterile saline to match a 0.5 McFarland standard (approximately 1 × 1 0 8 1×10 8 CFU/mL). Dilute the bacterial suspension to 1 × 1 0 6 1×10 6 CFU/mL using nutrient broth.
2.5.3. Broth Dilution Setup: Dispense 1 mL of nutrient broth into each test tube or well. Add varying concentrations of the extract (100, 200, 300, 400, and 500µg/mL) to the test tubes. Add 100 µL of bacterial inoculum to each test tube. Include a positive control with antibiotic and a negative control with the solvent.

2.5.4. Incubation: Incubate the test tubes or plates at 37 ◦ C for 18–24 hours.

2.5.5. Measurement of Turbidity: After incubation, measure the turbidity of each sample at 600 nm using a spectrophotometer or turbidimeter.
Turbidity is inversely proportional to antibacterial activity (less turbidity indicates higher bacterial inhibition) **(23. 24)**.

3.RESULTS AND DISCUSSION

3.1. Aqueous pod extracts of Prosopis juliflora and Prosopis cineraria

About 50g of pod powder of each species of Prosopis was taken and aqueous extract was prepared using sterile distilled water (500mL) by decotion method. Prosopis juliflora pod extract was dark brown, and P. cineraria was light brown in color. Both extracts were slightly viscous.

3.2. Phtochemical analysis of aqueous pod extracts of Prosopis juliflora and Prosopis cineraria

Phytochemical screening was performed using standard qualitative tests for phenolics, flavonoids, tannins, saponins, alkaloids, glycosides, terpenoids, and steroids.

Phytochemical	Test Performed	Observation for *P. juliflora*	Observation for *P. cineraria*	Inference
Phenolics	Ferric Chloride Test	Greenish-black coloration	Greenish-black coloration	Phenolics are present in both extracts.
Flavonoids	Shinoda Test	Pink coloration	Light pink coloration	Flavonoids are present in both, but possibly higher in *P. juliflora*.
Tannins	Gelatin Test	Precipitate formed	Precipitate formed	Tannins are present in both extracts.

Saponins	Foam Test	Persistent foam observed	Foam formed but dissipated quickly	Saponins are more prominent in *P. juliflora* compared to *P. cineraria*.
Alkaloids	Dragendorff's Test	Reddish-brown precipitate	Light brown precipitate	Alkaloids are present in both, with higher levels in *P. juliflora*.
Glycosides	Keller-Killiani Test	Blue-green ring at interface	Faint green ring	Glycosides are present, with higher intensity in *P. juliflora*.
Terpenoids	Salkowski Test	Reddish-brown interface	Yellowish-brown interface	Terpenoids are present in both, but more pronounced in *P. juliflora*.

Table 1. Phytochemical screening of aqueous pod extracts of Prosopis juliflora and Prosopis cineraria

Both species showed the presence of major phytochemicals, such as phenolics, flavonoids, tannins, saponins, and alkaloids. P. juliflora had relatively higher levels of saponins, alkaloids, glycosides, terpenoids, and steroids. P. cineraria showed the presence of these compounds but at lower intensities. The phytochemical profile suggests both species possess antioxidant, antibacterial, and potential therapeutic properties. The aqueous pod extracts of P. juliflora and P. cineraria are rich in bioactive phytochemicals, with P. juliflora showing a slightly higher concentration of most compounds. These findings support their traditional use in medicine and potential for further pharmacological studies.

Antibacterial Activity of Prosopis juliflora and Prosopis cineraria Pod Extracts by Turbidity Method

juliflora and P. cineraria pod extracts demonstrated significant antibacterial activity. P. juliflora exhibited greater inhibition across all tested bacterial strains and concentrations compared to P. cineraria (Figure 1 and 2). The higher activity of P. juliflora could be due to higher concentrations of alkaloids, saponins, and phenolic compounds, as identified in the phytochemical analysis. Although both extracts exhibited potent antibacterial activity, their inhibition percentages were slightly lower than the antibiotic standard, suggesting potential for synergistic use. The

aqueous pod extracts of P. juliflora and P. cineraria demonstrate strong antibacterial activity, with P. juliflora being more effective. These findings validate their traditional use in treating bacterial infections and support further investigation into their bioactive compounds and pharmacological potential.

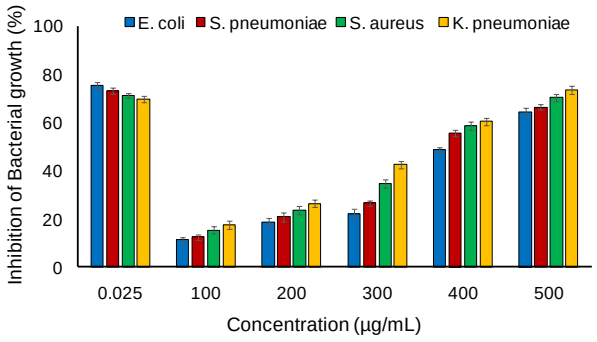

Figure 1. Antibacterial Activity of Prosopis juliflora against E. coli, S. pneumonia, S. aureus, and K. pneumonia

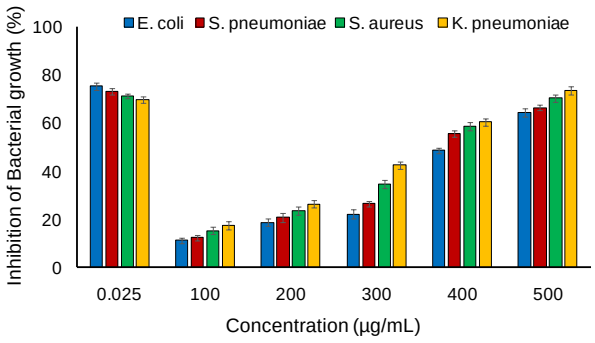

Figure 2. Antibacterial Activity of Prosopis cineraria against E. coli, S. pneumonia, S. aureus, and K. pneumonia.

4.CONCLUSION

The study on the aqueous pod extracts of Prosopis juliflora and Prosopis cineraria highlights their significant potential as sources of bioactive compounds with antibacterial properties. The phytochemical analysis revealed the presence of key secondary metabolites, including phenolics, flavonoids, tannins, saponins, alkaloids, glycosides, terpenoids, and steroids, which contribute to their biological activity. The preparation of extracts using the decoction method yielded satisfactory results, with P. juliflora showing slightly higher extraction efficiency compared to P. cineraria. The antibacterial activity, evaluated using the turbidity method, demonstrated that both extracts exhibit broad-spectrum activity against Gram-positive and Gram-negative bacteria. Notably, P. juliflora showed superior inhibitory effects, likely due to its higher phytochemical content. These findings validate the traditional use of Prosopis species in folk medicine and underscore their potential for developing natural antibacterial agents. Future work should focus on the isolation and characterization of the active compounds, as well as exploring synergistic applications with conventional antibiotics. The study reinforces the importance of Prosopis as a sustainable resource with therapeutic and ecological benefits.

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