



Evaluation of Pharmacological Properties of *Premna Serratifolia* L. Seed Extract

Panchami B and Rama Bhat P*

Dept of PG Studies and Research in Biotechnology, Alva's College (Autonomous), Moodbidri-574 227, Karnataka, India

*Corresponding author: Rama Bhat P, Dept of PG Studies and Research in Biotechnology, Alva's College (Autonomous), Moodbidri-574 227, Karnataka, India.

To Cite This article: Panchami B and Rama Bhat P*, Evaluation of Pharmacological Properties of *Premna Serratifolia* L. Seed Extract. Am J Biomed Sci & Res. 2026 30(5) AJBSR.MS.ID.003963, DOI: 10.34297/AJBSR.2026.30.003963

Received: 📅 March 19, 2026; Published: 📅 April 02, 2026

Abstract

Premna serratifolia L., a pharmacologically significant species within the Lamiaceae family, exhibits a broad spectrum of bioactivities including anti-inflammatory, antioxidant, antimicrobial, and hepatoprotective effects. While its aerial and root parts have been extensively characterized, the seeds remain under-investigated despite their potential as a reservoir of bioactive metabolites. This study presents a rigorous phytochemical profiling and comprehensive bioactivity evaluation of ethanolic and aqueous seed extracts of *P. serratifolia*. Advanced qualitative and quantitative phytochemical analyses identified a diverse array of secondary metabolites, prominently alkaloids, flavonoids, phenolic compounds, tannins, and steroids. Spectroscopic investigation via FTIR delineated functional group signatures corroborative of these compounds. Functional assays demonstrated pronounced free radical scavenging activity, robust inhibition of protein denaturation and α -amylase enzyme, reflecting significant antioxidant, anti-inflammatory, and antidiabetic potentials. Furthermore, the extracts exhibited potent antimicrobial efficacy against multiple pathogenic strains, encompassing both bacterial (*Escherichia coli*, *Staphylococcus aureus*) and fungal (*Aspergillus niger*) species. The integrative biochemical and pharmacological findings underscore the therapeutic promise of *P. serratifolia* seeds and advocate their strategic incorporation into drug discovery and nutraceutical formulations. This work not only delineates the phytochemical complexity of these seeds but also contributes to the valorization of an underutilized botanical resource with substantial clinical relevance.

Keywords: *Premna serratifolia*, Seed extract, Phytochemicals, Antioxidant, Anti-inflammatory, Antidiabetic

Introduction

Medicinal plants have been central to traditional medicine across cultures, offering therapeutic potential through their bioactive compounds. *Premna serratifolia* L. (*Lamiaceae*), native to tropical and subtropical regions such as India, Sri Lanka, and Southeast Asia, is a widely used medicinal plant valued for its anti-inflammatory, antimicrobial, antioxidant, and hepatoprotective properties [1,2]. The genus *Premna*, which includes over 200 species distributed across Asia, Africa, and Oceania, is an important source of phytochemicals with diverse pharmacological applications [3]. Traditionally, different parts of *P. serratifolia* roots, leaves, bark, and fruits have been used to treat fever, inflammation, liver disorders, respiratory issues, and wound infections [4,5]. Phytochemical studies have revealed the presence of flavonoids, tannins, alkaloids,

glycosides, and phenolic compounds contributing to its therapeutic efficacy [6]. Despite extensive research on its roots and leaves, scientific studies on its seeds remain limited, although they may hold unexplored pharmacological potential [7]. Given the rising interest in plant-derived anticancer agents and the traditional reputation of *P. serratifolia*, its seeds may represent a novel source of bioactive compounds with hepatoprotective and anticancer activity [8, 9]. There are few earlier reports on antibacterial, antioxidant and antidiabetic studies on leaf and roots of *Premna* species [10-14]. Based on the above literature an investigation was carried out on seed extracts of *Premna serratifolia* with the objectives: i) qualitative and quantitative analysis of seed extracts, ii) identification and characterization of the phytochemical constituents of seed extract using FTIR spectroscopy, iii) evaluation of in vitro antioxidant, an-

ti-inflammatory, antidiabetic, and antimicrobial activity of seed extracts.

Materials and Methods

Sample Collection

Seeds of *Premna serratifolia* were collected from Mangalore, Karnataka, India. The seeds were shade-dried for one week and then ground into a coarse powder for further use.

Extract Preparation

Aqueous Extract: 10 g of seed powder was mixed with 200 mL of distilled water and heated at 90°C for 1 h. The volume was reduced to 100 mL, filtered through Whatman No. 1 filter paper, evaporated, dried at 40°C, and stored at 4°C for subsequent analysis [15].

Ethanolic Extract: 10 g of seed powder was soaked in 100 mL of ethanol and kept at room temperature in the dark for three days. The mixture was filtered, dried at 50°C, and stored at 4°C [15].

Preliminary Phytochemical Screening

Qualitative analysis of bioactive compounds including alkaloids, phenolics, proteins, flavonoids, tannins, terpenoids, saponins, and steroids was performed using standard protocols and specific reagents (Mayer's for alkaloids, Molisch's for glycosides, Biuret for proteins, etc.) as described by *Kancherla et al.*, [7].

Quantitative Analysis

- Protein Content:** Determined using the Biuret method with Bovine Serum Albumin (BSA) as standard; absorbance measured at 520 nm [16].
- Total Phenolics:** Estimated using Folin-Ciocalteu colorimetric method with gallic acid as standard; absorbance recorded at 760 nm [17].
- Total Flavonoids:** Measured using aluminium chloride colorimetric assay with rutin as standard; absorbance recorded at 510 nm [18].

FTIR Spectroscopy: Dried extracts were analyzed using a Bruker OPUS 8.7.14 spectrophotometer in the range of 4000–500 cm^{-1} to identify functional groups such as O–H, C–H, C=O, and C–O [19].

Biological Assays

- Antioxidant Activity:** DPPH radical scavenging assay was

performed, absorbance measured at 517 nm, and IC_{50} values determined [15].

- Reducing Power Assay:** Conducted using ferricyanide-phosphate buffer method; absorbance measured at 700 nm [20].
- Anti-Inflammatory Activity:** Protein denaturation assay using Bovine Serum Albumin (BSA); absorbance measured at 660 nm and % inhibition calculated with aspirin as standard [21].
- Antidiabetic Activity:** α -Amylase inhibition assay; absorbance measured at 540 nm and % inhibition calculated using acarbose as standard [22].
- Antibacterial Activity:** Agar well diffusion method against *Escherichia coli* and *Staphylococcus aureus*; zones of inhibition compared with streptomycin control [23].
- Antifungal Activity:** Poison bait method using *Aspergillus niger*; dry biomass measured with Bavistin as control [24].

Statistical Analysis: All experiments were performed in triplicate. Results are expressed as mean \pm Standard Deviation (SD).

Results and Discussion

Extraction Yield of Seed Extracts

The extraction yield of *Premna serratifolia* seed extracts varied depending on the solvent used. The aqueous extract (10%) exhibited a higher yield than the ethanolic extract (2.7%), indicating that water efficiently extracted more soluble polar constituents such as proteins, flavonoids, and phenolic acids (Figure 1). Similar observations were made by *Singh et al.*, [25], who reported higher yields in water-based extractions of *P. serratifolia* roots. The brownish semi-solid appearance of both extracts signifies the presence of diverse phytoconstituents, irrespective of solvent polarity.

Qualitative Phytochemical Screening

The preliminary phytochemical screening confirmed the presence of several major bioactive compounds in both seed extracts (Table 1). Alkaloids, phenolics, flavonoids, tannins, terpenoids, saponins, and steroids were detected, while glycosides were absent. These compounds are pharmacologically relevant due to their antioxidant, antimicrobial, and anti-inflammatory roles. The detection of multiple classes of secondary metabolites supports the therapeutic potential of *P. serratifolia* seeds, aligning with similar findings in *Premna latifolia* and *P. integrifolia* [2,5].

Table 1: Preliminary phytochemical screening in seed extracts of *P. serratifolia*.

Phytochemicals	Test	Seed Ethanol	Seed Aqueous
Alkaloids	Mayer's test	+	+
Glycosides	Molisch's test	-	-

	Bromine water test	-	-
Phenolics	Lead acetate test	+	+
	Follin Ciocaltaeu test	+	+
Protein	Biuret test	+	+
Flavonoids	Aluminium chloride test	+	+
	Sodium hydroxide test	+	+
Tannins	Braymer's test	+	+
Terpenoids	Salkowski test	+	+
Saponins	Foam formation test	+	+
Steroids	Hesse's response	+	+

'+' = present '-' = absent

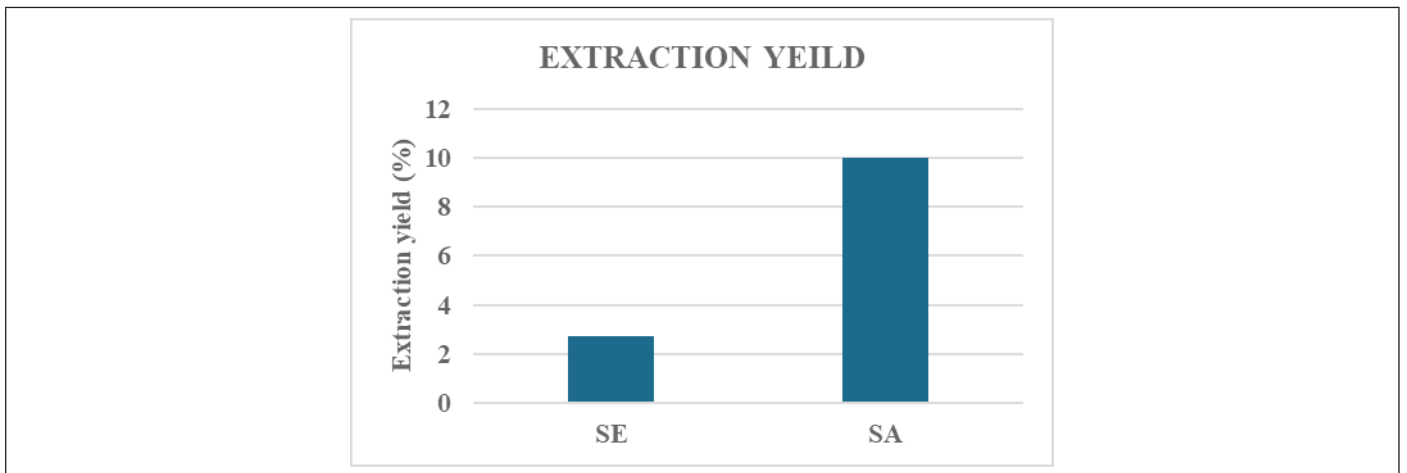
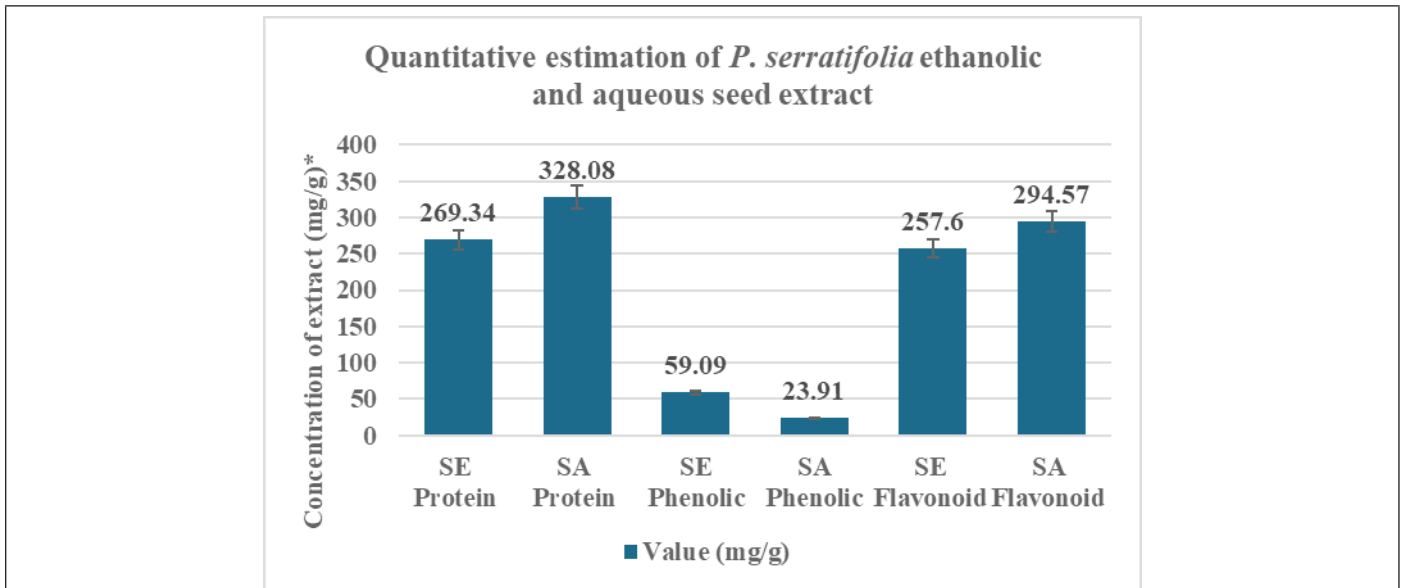


Figure 1: Ethanolic and aqueous seed extract of *P. serratifolia*.

Quantitative Phytochemical Contents



Note*: Mean ± standard deviation, N=3

Figure 2: Quantitative estimation of seed extracts of *P. serratifolia*.

The quantitative analysis revealed that the aqueous extract contained higher levels of proteins (328.08 mg/g) and flavonoids (298.33 mg/g), while the ethanolic extract showed higher phenolic content (59.09 mg/g). This indicates that water is more efficient in extracting hydrophilic compounds such as proteins and flavonoid glycosides, whereas ethanol extracts more semi-polar phenolic constituents (Figure 2). Similar solvent-dependent variations in phenolic and flavonoid content were reported in *Premna integrifolia* [26] and *Premna latifolia* [27].

lia [26] and *Premna latifolia* [27].

FTIR Spectral Analysis

FTIR spectral analysis of *P. serratifolia* seed extracts confirmed the presence of diverse functional groups such as hydroxyl (O-H), carbonyl (C=O), and aromatic C=C bonds, suggesting the existence of phenolic and flavonoid compounds (Figure 3,4).

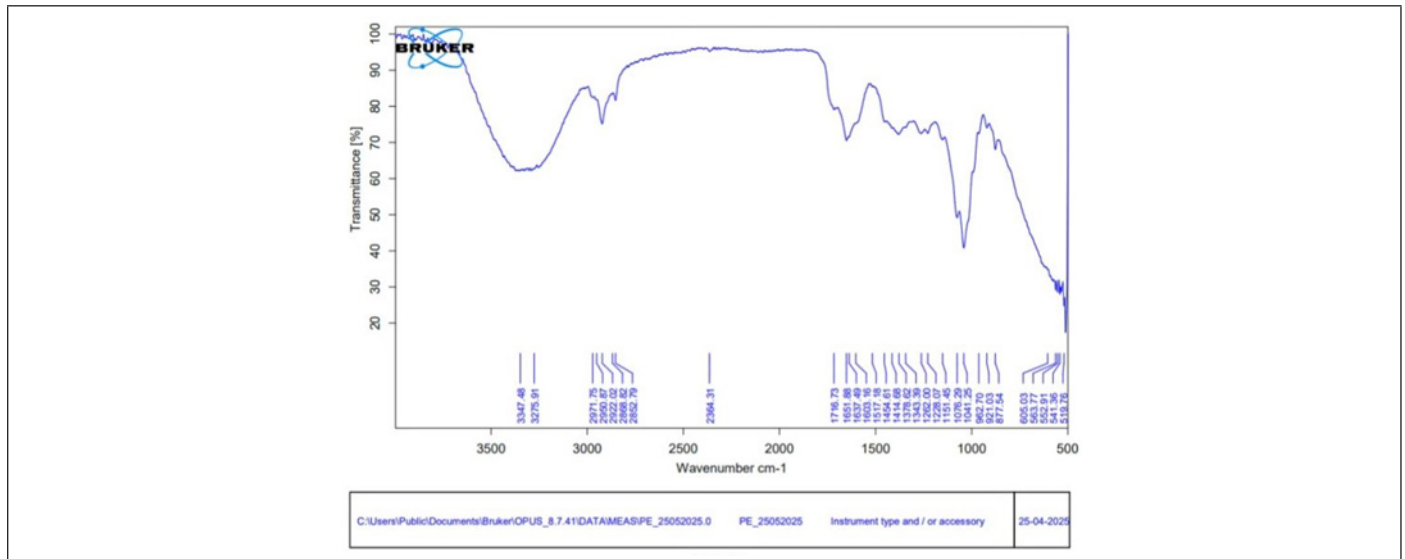


Figure 3: FTIR spectrum of ethanolic extract of *Premna serratifolia* seeds.

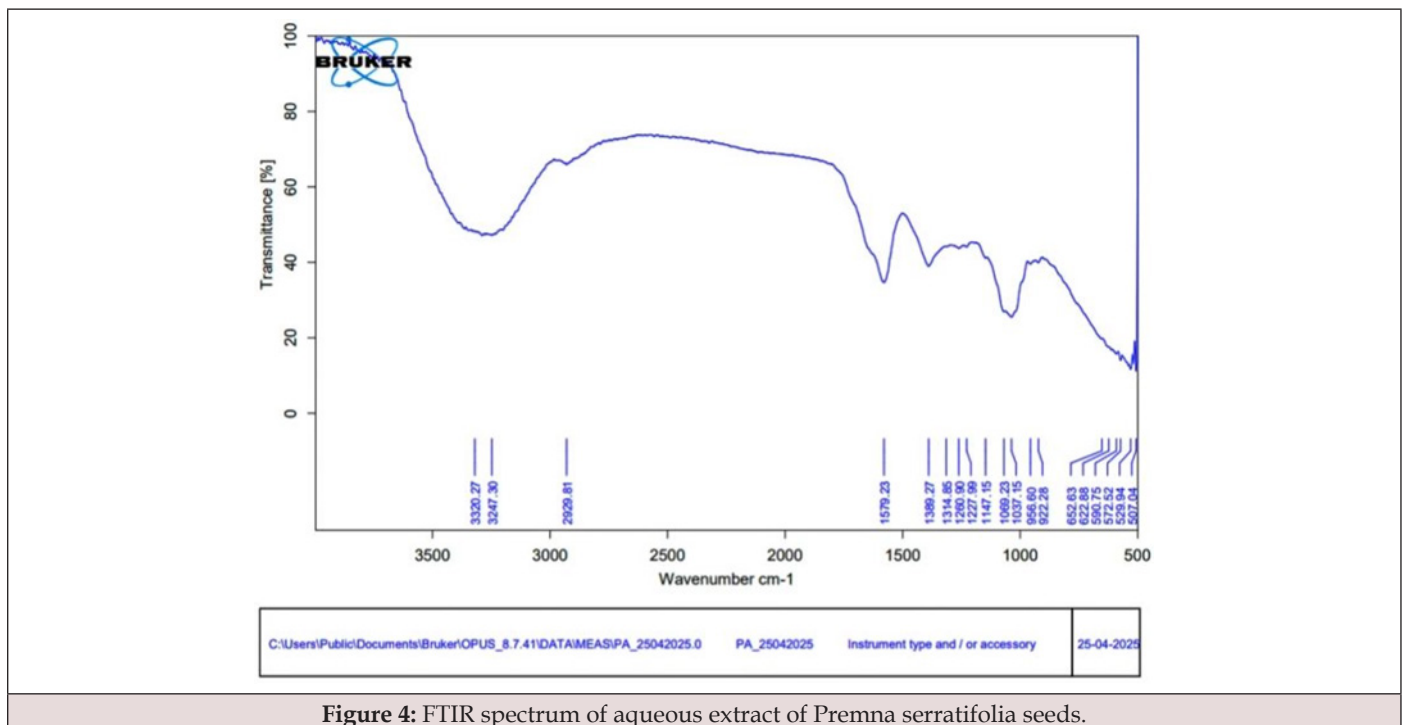


Figure 4: FTIR spectrum of aqueous extract of *Premna serratifolia* seeds.

The ethanol extract exhibited stronger peaks for C–O and C=C stretching, which correlate with high phenolic content, while the aqueous extract showed a prominent C=O band unique to carbonyl compounds (Table 2). Comparable FTIR profiles were document-

ed for *Premna integrifolia* leaves and bark extracts by Kumar *et al.* [19] and Singh and Devi [28], emphasizing the consistency in phytochemical structure among *Premna* species.

Table 2: Comparative FTIR peak analysis of aqueous and ethanolic seed extracts.

Functional Group	SA (Aqueous Extract) Wavenumber (cm ⁻¹)	SE (Ethanolic Extract) Wavenumber (cm ⁻¹)
O–H stretching (alcohols, phenolics)	3320.27, 3247.30	3375.48, 3275.91
C–H stretching (alkanes)	2929.81	2971.75, 2922.02
C=O stretching (esters, acids)	1579.23	—
C=C stretching (aromatics, alkenes)	1600–1650	1616.73, 1651.88
C–O stretching (esters/ethers)	1230.99, 1260.90	1262.00, 1343.39

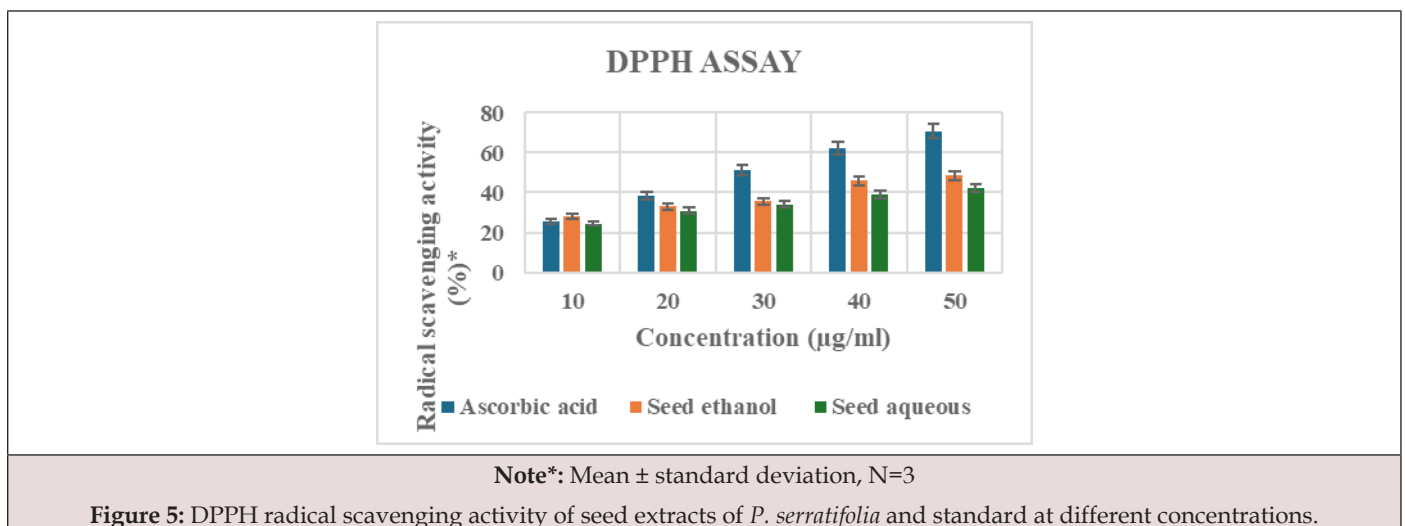
Antioxidant Assay

DPPH Radical Scavenging Activity: Both seed extracts exhibited concentration-dependent DPPH radical scavenging activity (Figure 5). The ethanolic extract demonstrated greater antioxidant potential (IC₅₀ = 57 µg/mL) than the aqueous extract

(IC₅₀ = 65 µg/mL), although both were less potent than ascorbic acid (31 µg/mL) (Table 3). This difference may be attributed to the higher phenolic content in ethanol extracts, which enhances hydrogen-donating ability. Comparable findings were reported previously by in ethanol-based *Premna* leaf extracts [9,15].

Table 3: IC₅₀ values of DPPH radical scavenging activity.

Sample	IC ₅₀ value (µg/mL)
Ascorbic acid	31
Seed ethanol extract	57
Seed aqueous extract	65



Reducing Power Assay: The reducing power assay of *Premna serratifolia* seed extracts showed a concentration-dependent increase in antioxidant activity, with higher absorbance values at 700 nm from 100 to 500 $\mu\text{g/mL}$. The ethanol extract exhibited greater reducing potential (0.02 ± 0.001 to 0.09 ± 0.001) than the aqueous extract (0.01 ± 0.001 to 0.08 ± 0.001), while ascorbic acid showed the highest activity (0.20 ± 0.01 to 0.60 ± 0.01) (Figure 6). These results indicate that ethanol effectively extracts antioxidant compounds such as phenolics and flavonoids, aligning with previous findings by Sharma *et al.*, [29] and Raju and Suresh [30], and confirm the presence of bioactive constituents responsible for the plant's antioxidant potential.

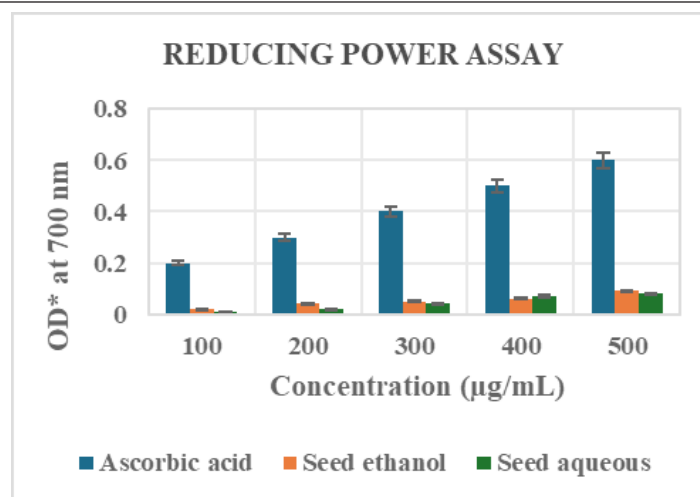
Anti-Inflammatory Assay

Protein Denaturation Assay: The results revealed a concentration-dependent anti-inflammatory response, with the ethanolic

extract showing superior protein denaturation inhibition (80.5% at 1000 $\mu\text{g/mL}$) compared to the aqueous extract (68.8% at 1000 $\mu\text{g/mL}$) (Figure 7). This significant inhibition implies that bioactive molecules such as flavonoids and terpenoids are responsible for membrane stabilization and anti-inflammatory effects, corroborating the findings of Meena *et al.*, [31], in *P. corymbosa* extracts.

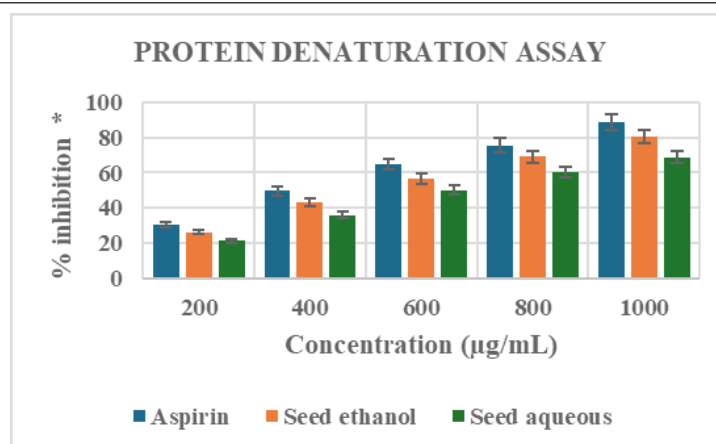
Antidiabetic Assay

α -Amylase Inhibitory Activity: Both extracts exhibited α -amylase inhibitory potential in a dose-dependent manner. The ethanolic extract showed the highest inhibition (82.4% at 1000 $\mu\text{g/mL}$), closely approaching the standard drug acarbose (Figure 8). These results suggest the presence of flavonoids, tannins, and polyphenols capable of modulating carbohydrate digestion. Similar inhibitory patterns were reported earlier in other medicinal plant extracts [22,32].



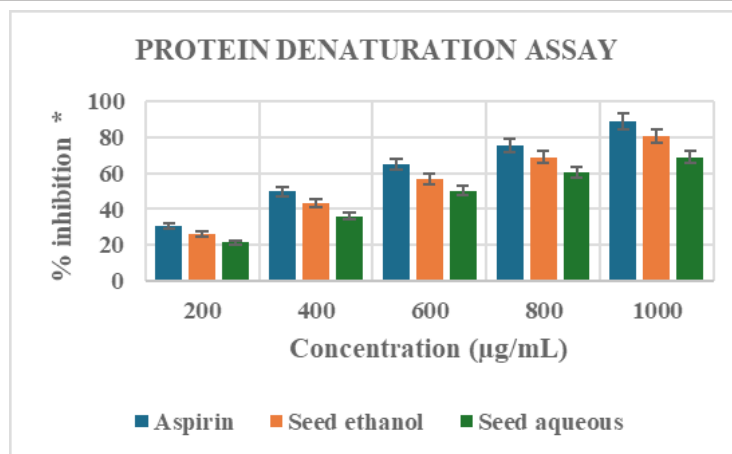
Note*: Mean \pm standard deviation, N=3

Figure 6: Reducing power activity of seed extracts of *P. serratifolia* and standard at different concentrations.



Note*: Mean \pm standard deviation, N=3

Figure 7: Anti-inflammatory activity of seed extracts of *P. serratifolia* and standard at different concentrations.



Note*: Mean ± standard deviation, N=3

Figure 8: α-Amylase Inhibitory Activity of seed extracts of *P. serratifolia* and standard at different concentrations.

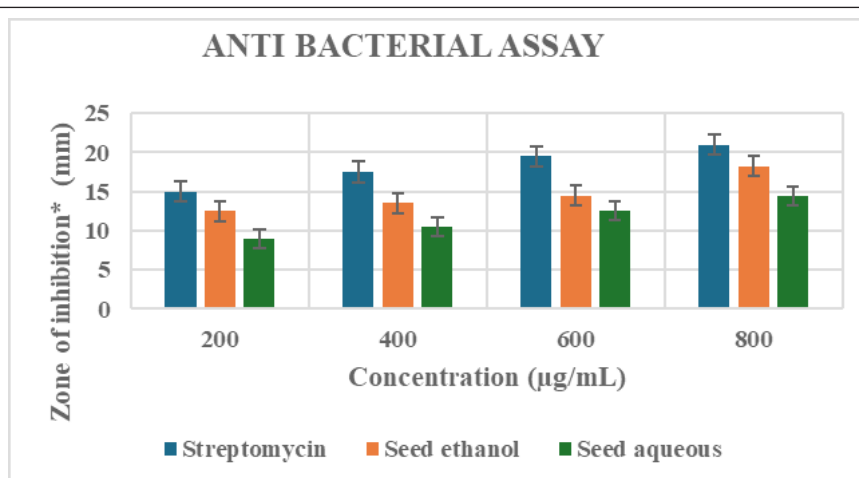
Antibacterial Activity- Agar Well Diffusion

The ethanol and aqueous seed extracts of *Premna serratifolia* showed dose-dependent antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. The ethanol extract exhibited larger inhibition zones (12.25–18.25 mm for *E. coli*; 8.5–11.75 mm for *S. aureus*) than the aqueous extract (9.0–14.5 mm and 7.0–10.0 mm, respectively), while Streptomycin showed the highest activity (Ta-

ble 4 and Figures 9,10). The ethanol extract also showed lower IC₅₀ values (140.2 µg/mL for *E. coli*) compared to the aqueous extract (175.4 µg/mL), indicating higher potency. These findings agree with Singh [33], *Bhalerao et al.*, [34] and Kumar [35], confirming the superior antibacterial efficiency of ethanol-based extracts. In a recent study by *Saptu et al* [36] indicated the good antibacterial and antioxidant activity in the extract of *P. serratifolia* leaf obtained by cold extraction method.

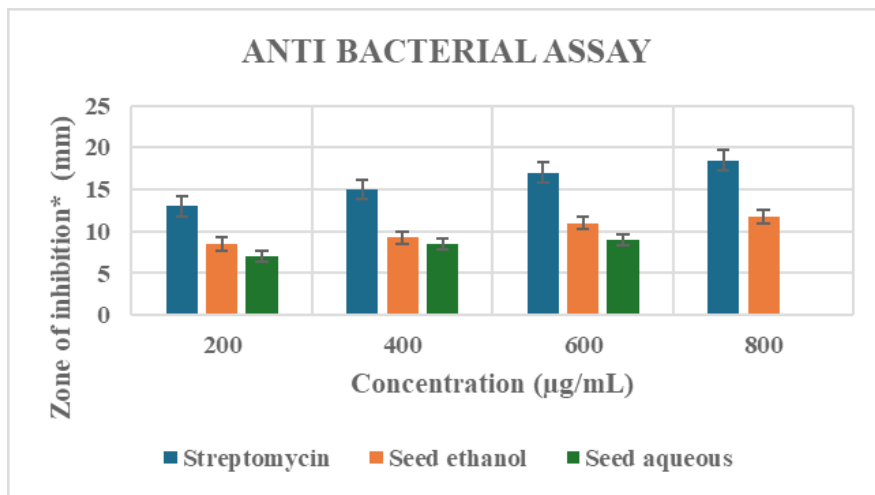
Table 4: IC₅₀ values of seed extracts against bacterial strains.

Extract Type	Bacterial Strain	IC ₅₀ value (µg/mL)
Seed Ethanol (SE)	<i>Escherichia coli</i>	140.2
Seed Ethanol (SE)	<i>Staphylococcus aureus</i>	165.8
Seed Aqueous (SA)	<i>Escherichia coli</i>	175.4
Seed Aqueous (SA)	<i>Staphylococcus aureus</i>	185.7



Note*: Mean ± standard deviation, N=3

Figure 9: Antibacterial activity of seed extracts of *P. serratifolia* and standard at different concentrations against *E.coli*.



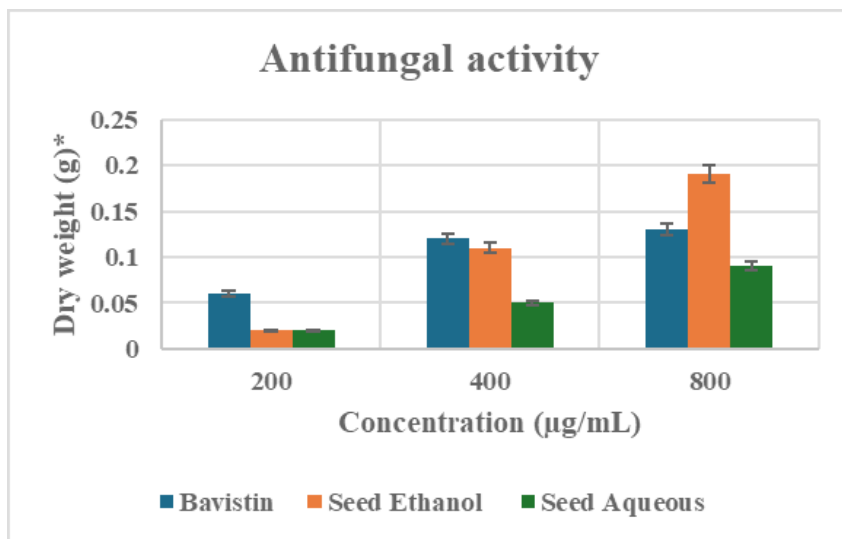
Note*: Mean ± standard deviation, N=3

Figure 10: Antibacterial activity of seed extracts of *P. serratifolia* and standard at different concentration against *S. aureus*.

Antifungal Activity

The antifungal activity of *Premna serratifolia* seed extracts against *Aspergillus niger* was evaluated using the poison bait method. Both extracts showed concentration-dependent inhibition, with the highest activity at 800 µg/mL (Figure 11).

The of *P. serratifolia* seed extracts as effective natural antifungal agents. ethanolic extract exhibited stronger antifungal effects (0.02 to 0.19 ± 0.006 g) compared to the aqueous extract (0.02 ± 0.003 g to 0.09 ± 0.005 g), while Bavistin showed moderate inhibition. These findings align with *Ginthujah et al.*, [3], confirming the potential.



Note*: Mean ± standard deviation, N=3

Figure 11: Antifungal activity of seed extracts of *P. serratifolia* and standard at different concentration against *Aspergillus niger*.

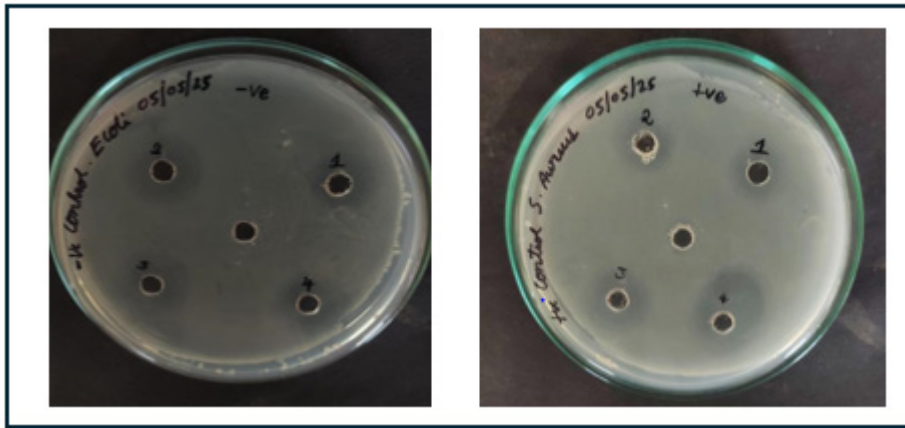


Plate 1: Anti-bacterial assay of streptomycin at different concentration against *Escherichia coli* and *Staphylococcus aureus*.

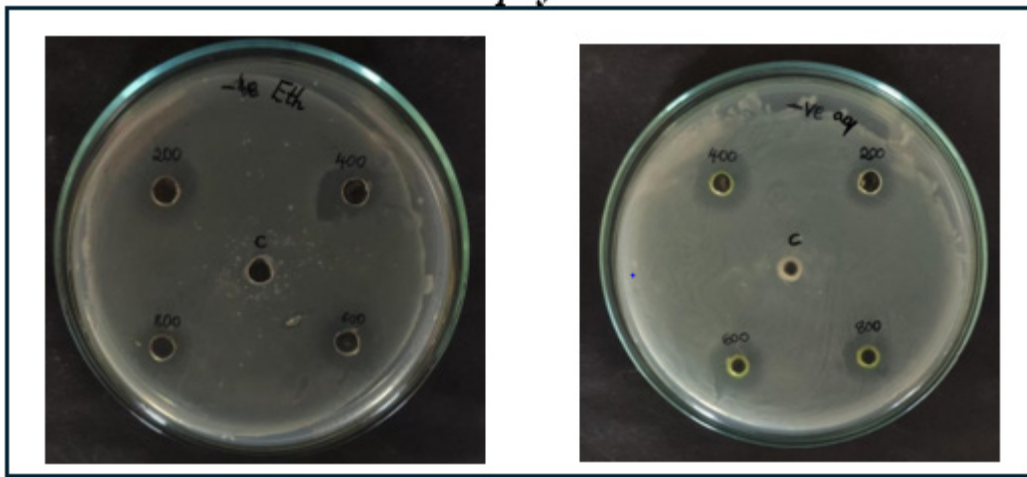


Plate 2: Anti-bacterial assay of extracts at different concentration against *E. coli*.

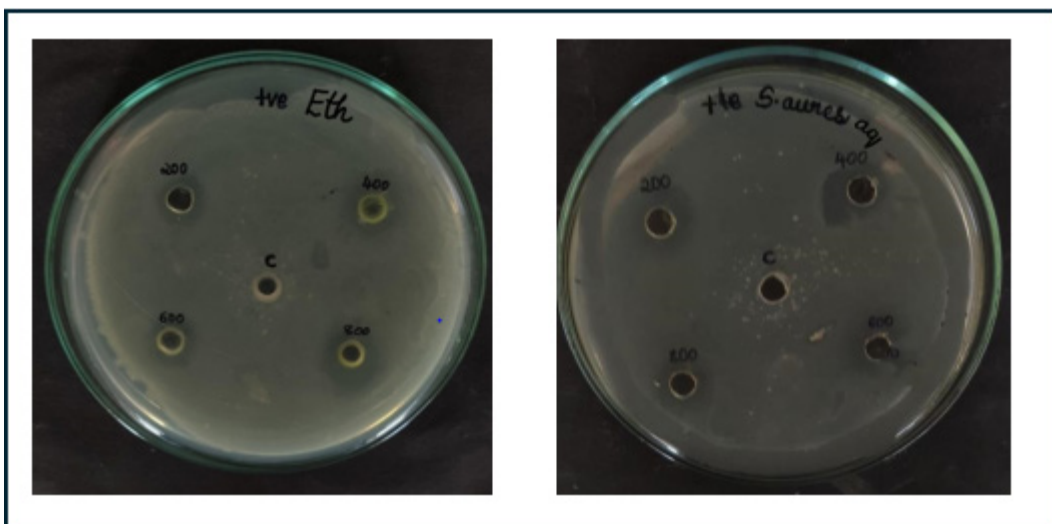


Plate 3: Anti-bacterial assay of extracts at different concentration against *S. aureus*.

Conclusions

Premna serratifolia seed extracts exhibited diverse bioactive compounds with notable pharmacological activities. The ethanolic extract demonstrated superior antioxidant, anti-inflammatory, antidiabetic, and antibacterial properties compared to the aqueous extract, suggesting its greater therapeutic potential. These findings support the use of *P. serratifolia* seeds as a promising source of natural bioactive agents for pharmaceutical applications.

Acknowledgement

None.

Competing of Interest

None.

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