RESEARCH PAPER

Phytochemical Profiling and Evaluation of in vitro Antioxidant, Antimicrobial, and Anti-inflammatory Activities of Ethanol Extracts and Fractions of Spondias pinnata (L. f.) Kurz. Leaves

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ABSTRACT

Spondias pinnata (L. f.) Kurz., belonging to Anacardiaceae family, is being traditionally employed in folk medicine to treat inflammatory and infectious conditions. The current investigation aimed to analyse the phytochemical profile of S. pinnata and investigate its in vitro antioxidant, antibacterial and anti-inflammatory activities using ethanol extract and various solvent fractions of its leaves. Phytochemical screening identified key secondary metabolites such as phenolics, flavonoids, tannins, and terpenoids in all samples. Quantitative analysis showed that the ethyl acetate (EA) fraction contained the maximum levels of phenolics and flavonoids, indicating a rich presence of polyphenols. The DPPH assay revealed IC_{50} values of 68.88 ± 0.11 µg/mL for the ethanol (EtOH) extract and 60.43 ± 0.41 µg/mL for the EA fraction, demonstrating notable antioxidant activity. Both of them also exhibited significant anti-inflammatory effects in a heat-induced bovine serum albumin denaturation assay, with IC_{50} values of 96.99 ± 0.29 µg/mL (extract) and 88.71 ± 0.57 µg/mL (fraction). The antibacterial study indicated promising activity against E. coli, with zones measuring 13 mm for the EtOH extract and 15 mm for the EA fraction. Collectively, the findings indicate that the leaves of S. pinnata constitute a promising reservoir of phytoconstituents exhibiting significant antioxidant, anti-inflammatory, and antibacterial activities. Further research on isolating bioactive constituents, elucidating mechanisms, and conducting in vivo studies is needed to confirm its therapeutic potential.

Keywords: Spondias pinnata; Fractionation; Antioxidant; Antibacterial; Anti-inflammatory

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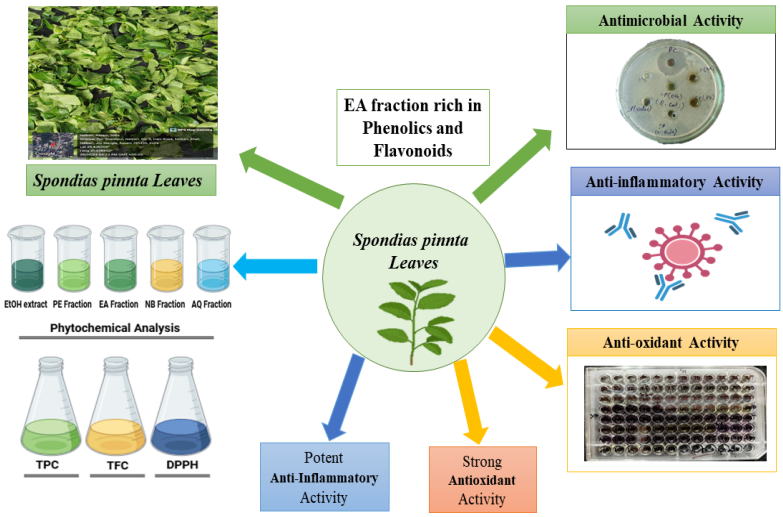

1. Introduction

Oxidative stress and chronic inflammation are central mechanisms in many disorders, including malignancy, cardiac diseases, metabolic syndrome, type 2 diabetes, obesity, and age-related complications (Nakadate et al., 2025). It results from an disproportion among reactive oxygen species (ROS) production and antioxidant defenses, leading to damage of essential macromolecules, and triggering inflammatory pathways (Tumilaar et al., 2024; Chandimali *et al*., 2025).

Furthermore, infectious diseases remain a major global challenge, further complicated by antimicrobial resistance, which has intensified the search for novel or multi-target therapeutics (Pérez-Flores *et al*., 2025). In this regard, plant-derived natural products are of particular interest due to their chemical diversity, broad biological activities, and long-standing traditional use, with majority of the world's population trusting on herbal therapies for principal healthcare (Lee & Barnes, 2022).

The genus *Spondias* (family Anacardiaceae) comprises about 12 species distributed mainly in tropical and subtropical regions (The Plant List). *Spondias pinnata* (L. f.) Kurz., widely found in South and Southeast Asia, is valued in traditional medicine, where its leaves, bark, fruits, and seeds are used to treat gastrointestinal disorders, infections, inflammation, diabetes, and oxidative stress–related conditions (Sujarwo & Keim, 2019). Phytochemical studies recognised various secondary metabolites, including phenolics, flavonoids, tannins, saponins, terpenoids, and sterols, along with phyto-constituents such as caffeic acid, gallic acid, β-sitosterol, alantolactone, α-pinene, caryophyllene, and geraniol, which contribute to its antioxidant, antimicrobial, and anti-inflammatory activities (Devkota & Sai, 2023; Balaji et al., 2024).

Despite its extensive traditional application, scientific studies on *S. pinnata* have primarily examined individual plant parts or specific activities. The bark possesses the anti-inflammatory effects (Ghate et al., 2018), fruit peel essential oil shows cytotoxic, antimicrobial, and anti-inflammatory effects (Li et al., 2020), seed extracts demonstrate antimalarial activity (Chaniad *et al.,* 2022), and hydroalcoholic leaf extracts display antihyperglycemic potential (Sai *et al.,* 2021). However, integrated studies combining phytochemical profiling with antioxidant, anti-inflammatory, and antibacterial evaluation of leaf ethanol (EtOH) extracts and their solvent fractions remain scarce. Accordingly, this study investigates the phytochemical composition of *S. pinnata* leaf EtOH extract and selected fractions, and evaluates their *in vitro* antioxidant, antibacterial, and anti-inflammatory activities, correlating bioactivity with phenolic and flavonoid content to substantiate its traditional use and identify therapeutically relevant fractions.

2. Materials and Methods

2.1. Chemicals and Solvents

2,2-Diphenyl-1-picrylhydrazyl (DPPH), gallic acid, and quercetin were obtained from SRL Pvt. Ltd., Mumbai, India. All other chemicals, culture media, reagents, and solvents employed in our study were of analytical grade and sourced from SRL, HiMedia Laboratories Pvt. Ltd., and Finar Chemicals Pvt. Ltd., India.

2.2. Collection, Identification, and Processing of Plant Materials

Fresh *S. pinnata* leaves were collected in April and May 2024 from Nalbari, Assam, India, and authenticated in the Botany department at Gauhati University, Assam, India, where a voucher specimen (Herb. /GUBH/2024/067) was deposited for future reference. The *S. pinnata* leaves were shade-dried, coarsely powdered, and stored for later use.

2.3 Pharmacognostic Evaluation

2.3.1 Morphological and Organoleptic Evaluation

Morphological and organoleptic characteristics of *S. pinnata* leaves, including colour, taste, odour, shape, size, margin, and apex, were evaluated using standard pharmacognostic procedures (Prakash *et al*., 2019).

2.3.2 Microscopic Evaluation

Thin transverse sections of fresh *S. pinnata* leaves were prepared, cleared with glycerine, stained using safranin and picric acid, and observed under a microscope at 40X magnification to observe anatomical features such as epidermis, mesophyll, vascular tissues, and stomata (Prakash *et al*., 2019).

2.3.3 Powder Microscopy

Dried leaf powder (sieved through mesh no. 40) was subjected to microscopic examination using phloroglucinol–hydrochloric acid reagent to identify diagnostic characters such as fibres, parenchyma cells, and calcium oxalate crystals (Singh *et al*., 2014).

2.3.4 Physicochemical Analysis

Physicochemical parameters, including total ash, acid-insoluble ash, water-soluble ash, loss on drying (LOD), and foaming index, were determined following standard procedures (Prakash *et al*., 2019; Singh *et al*., 2014).

2.3. Preparation of EtOH Extract and Different Fractions of *S. pinnata* leaves

Dried *S. pinnata* leaf powder (2.0 kg) was extracted by cold maceration with EtOH (20 L) for 72 h with intermittent stirring. The filtrate was evaporated to dryness under reduced pressure at 50 °C using a rotary evaporator (Buchi Rotavapor R100). Extraction was repeated until the solvent became colourless. The crude extract was reconstituted in distilled water and successively partitioned with solvents of different polarity to obtain petroleum ether (PE), ethyl acetate (EA), n-butanol (NB), and aqueous (AQ) fractions (Dutta *et al*., 2018; Abubakar & Haque, 2020). All fractions were concentrated and stored at 4 °C until analysis.

2.4. Phytochemical Screening

The qualitative phytoconstituent assessment of *S. pinnata* extract and fractions was conducted using a standard procedure to identify the existence of alkaloids, flavonoids, glycosides, steroids, phenolics, tannins, saponins, terpenoids, and carbohydrates (Chaudhary *et al*., 2020).

2.5. Estimation of Total Phenolic (TPC) and Flavonoid Content (TFC)

TPC was estimated using the Folin–Ciocalteu method and expressed as mg gallic acid equivalents (GAE)/g extract (Noreen *et al*., 2017). TFC was measured using the aluminium chloride colorimetric method and expressed as mg quercetin equivalents (QE)/g extract (Shraim *et al*., 2021).

2.6. *In vitro* Antioxidant Activity

Antioxidant activity was measured using the DPPH radical scavenging (RS) assay (Saeed *et al*., 2012; Mushtaq *et al*., 2021). In brief, a 0.1 mM DPPH solution in EtOH was mixed with different concentrations (10-200 µg/mL) of the samples, then incubated in darkness at room temperature for 30 minutes. Absorbance was measured at 517 nm with a UV-Visible spectrophotometer (Shimadzu UV-1900). Ascorbic acid was used as standard reference. The RS percentage was calculated, and IC_{50} values were derived from dose-response curves.

2.7. *In vitro* Antibacterial Properties

Staphylococcus aureus and *Escherichia coli* were cultured on nutrient agar at 37°C for 24 h. The inoculum was adjusted to 0.5 McFarland standard (≈1.5 × 10^8 CFU/mL) using sterile distilled water (Manandhar *et al*., 2019). Muller–Hinton agar plates were evenly inoculated using sterile swabs. Wells of 6 mm diameter were punched, and each well received 50 µL of extract or fraction solution (10 µg/mL) (Dahiya & Purkayastha, 2012). Sterile EtOH served as the negative control, while amikacin (30 µg) served as the positive control. Following incubation at 37°C for 24 h, zones of inhibition were measured (Akwongo *et al*., 2024).

2.8. *In vitro* Anti-Inflammatory Activity (Protein Denaturation Assay)

Anti-inflammatory activity was assessed using the heat-induced bovine serum albumin (BSA) denaturation assay (Bailey-Shaw *et al*., 2017). A 0.4% (w/v) BSA solution was prepared in Tris-buffered saline (pH 6.4). Extracts and fractions of *S. pinnata* were prepared in EtOH (10 mg/mL) and diluted to the required concentrations. Reaction mixtures were incubated at 72 °C for 10 min, cooled, and absorbance was measured at 660 nm in a UV–Vis spectrophotometer (Shimadzu UV-1900). EtOH and diclofenac sodium served as the negative control and reference standard, respectively. Percentage inhibition of protein denaturation was calculated, and IC_{50} values were determined from dose–response curves.

2.9. Statistical analysis

All experiments were conducted in triplicate for each sample (n = 3), and the results were presented as mean ± SD. Statistical analysis was conducted using SPSS version 13 and Microsoft Excel 2010.

3. Results and Discussions

3.1. Pharmacognostic Evaluation

Macroscopic examination showed that *S. pinnata* leaves are compound and imparipinnate, bearing 4–5 pairs of opposite oblong to oblong-elliptic leaflets with entire margins and acuminate to caudate apices. Fresh leaves were dark green, aromatic, and sour-astringent in taste, indicating the possible presence of tannins and phenolics. These features are in agreement with previously reported descriptions and confirm correct plant identification (George & Joseph, 2013).

Microscopic analysis of leaf transverse sections revealed a well-defined cuticle, upper and lower epidermis, palisade and spongy mesophyll, vascular bundles, and stomata. Powder microscopy showed calcium oxalate crystals and fibres, providing diagnostic markers consistent with pharmacognostic characteristics of Anacardiaceae (George & Joseph, 2013).

Physicochemical parameters, including ash values, LOD, and foaming index, were within acceptable limits, indicating low inorganic contamination and controlled moisture content. Overall, these pharmacognostic and physicochemical findings support the suitability of *S. pinnata* leaves for further phytochemical and biological studies.

Table 1: Morphological characterization of the leaves of *S. pinnata*

Particulars	Observation

Colour	Fresh leaves are dark green. Turn brownish green when they dry.
Odour	Aromatic
Taste	Sour (astringent)
Length	15-20 cm
Margin	Entire (smooth)
Apex	Caudate or acuminate
Shape	Oblong to oblong elliptic
Petioles	5-15 cm long
Surface	Smooth and flat

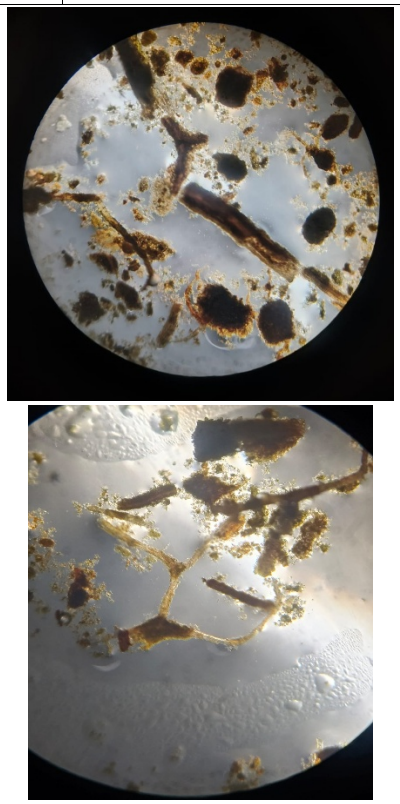

Figure 1: Microscopic characteristics of *S. pinnata* leaves dried powder observed under 10X magnification

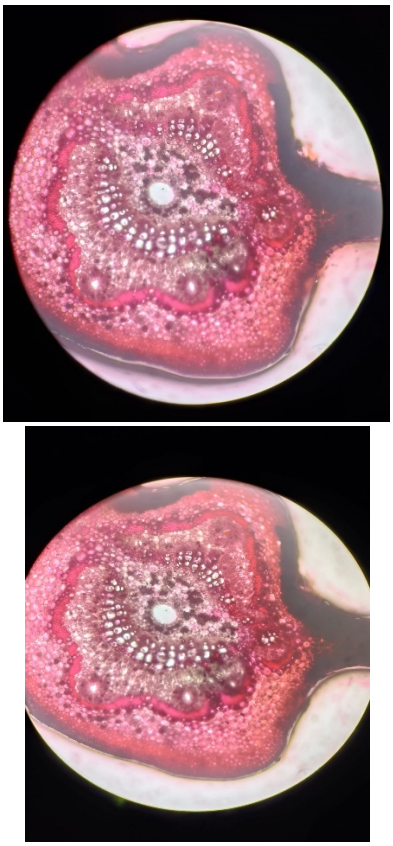

Figure 2: Leaf sections of *S. pinnata* observed under 40X magnification

Table 2: Physicochemical characterization of *S. pinnata* leaves

Plant Name	Parameters	Value (w/w %)
S. pinnata	Total ash value	7.12
	Loss of Drying	14.8
	Ash content (Water-soluble)	2.35
	Ash content (Acid-insoluble)	1.62
	Foaming index	<100

3.2. Extraction and Fractionation

Cold maceration of *S. pinnata* leaf powder with EtOH produced an extract yield of 8.58% (w/w), indicating efficient recovery of EtOH-soluble constituents. EtOH extracts a wide range of polar and moderately non-polar phytochemicals, many associated with antioxidant and anti-inflammatory activities (Khade *et al*., 2023). Sequential fractionation yielded PE (2.26% w/w), EA (2.57% w/w), NB (1.92% w/w), and AQ (1.65% w/w) fractions. Variations in yield reflect differences in phytochemical solubility based on solvent polarity, with relatively higher EA and NB yields suggesting enrichment of moderately polar compounds such as phenolics and flavonoids. The

near-neutral pH of the extract and fractions indicates chemical stability and suitability for biological assays.

3.4. Phytochemical Constituents

Preliminary phytochemical analysis revealed diverse secondary metabolites in the EtOH extract and solvent fractions of *S. pinnata* leaves (Table 3). The EtOH extract contained flavonoids, alkaloids, glycosides, saponins, tannins, phenolics, and steroids, indicating broad biological potential. Fraction-wise analysis showed that the EA and NB fractions were richer in phenolics and flavonoids, the PE fraction mainly contained nonpolar constituents such as steroids and terpenoids, and the AQ fraction was enriched in carbohydrates and polar glycosides. This distribution highlights the effectiveness of solvent fractionation in concentrating specific phytochemical classes. The higher phenolic and flavonoid content in the EA and NB fractions provides a plausible explanation for their stronger antioxidant, anti-inflammatory, and antimicrobial activities observed in subsequent assays, supporting their selection for further pharmacological evaluation.

Table 3: Phytochemical analysis of EtOH extract and fractions of *S. pinnata* leaves

Sl. No.	Phytoconstituents	*S. pinnata* EtOH Extract	*S. pinnata* PE fraction	*S. pinnata* EA fraction	*S. pinnata* NB fraction	*S. pinnata* AQ fraction
1.	Alkaloids	+	−	+	+	−
2.	Flavonoids	+	−	+	+	−
3.	Glycosides	+	−	+	+	+
4.	Steroids	+	+	−	−	−
5.	Phenols	+	−	+	−	+
6.	Carbohydrate	+	−	−	−	+
7.	Saponin	+	−	+	+	+
8.	Terpenoids	−	+	+	−	−
9.	Tannins	+	−	+	+	−

("+" indicates present and "–" indicates absent)

3.5. TPC and TFC

TPC and TFC showed significant variation ($p < 0.05$) among the EtOH extract and solvent fractions of *S. pinnata* leaves (Table 4). The EtOH extract contained 273.14 ± 1.83 mg GAE/g extract (TPC) and 53.15 ± 2.07 mg QE/g extract (TFC), while the EA fraction showed the highest values, 325.42 ± 3.52 mg GAE/g extract and 65.27 ± 2.06 mg QE/g extract, respectively, significantly exceeding the PE, NB, and AQ fractions. This indicates preferential partitioning of phenolics and flavonoids into the EA fraction. The enrichment of polyphenols in the EA fraction is attributable to the intermediate polarity of EA, which efficiently extracts moderately polar compounds such as flavonoid aglycones and phenolic acids. Similar enrichment of polyphenols in EA fractions were reported in medicinal plants, and earlier reports have also showed substantial TPC and TFC content in *S. pinnata* extracts (Hazra *et al*., 2008). Phenolic acids and flavonoids are known for antioxidant and anti-inflammatory effects through RS, metal chelation, and modulation of inflammatory pathways. The strong association between higher TPC and TFC values and the enhanced biological activities of the EA fraction in this study underscores the key role of polyphenolic constituents in the observed bioactivity of S. *pinnata* leaves.

Table 4: Evaluation of TPC, TFC, Antioxidant and Anti-inflammatory activity of the EtOH extract and fractions of *S. pinnata* leaves

Extract/Fraction	*S. pinnata* leaves			
	TPC Value (GAE/g)	TFC Value (QE/g)	Antioxidant activity (IC_{50}) (µg/mL)	Anti-inflammatory activity (IC_{50}) (µg/mL)
S. pinnata EtOH extract	273.14 ± 1.83^{c}	53.15 ± 2.07^{d}	68.88 ± 0.11^{c}	96.99 ± 0.29^{c}
S. pinnata PE fraction	31.03 ± 1.07^{a}	13.49 ± 0.65^{a}	133.10 ± 0.33^{f}	112.15 ± 0.30^{f}
S. pinnata EA fraction	325.42 ± 3.52^{d}	65.27 ± 2.06^{e}	60.43 ± 0.41^{b}	88.71 ± 0.57^{b}
S. pinnata NB fraction	55.29 ± 2.67^{b}	28.87 ± 1.66^{c}	79.14 ± 0.47^{d}	102.03 ± 0.72^{d}
S. pinnata AQ fraction	49.50 ± 2.18^{b}	22.705 ± 0.53^{b}	119.21 ± 0.11^{e}	108.65 ± 0.64^{e}

Ascorbic acid	ND	ND	18.22 ± 0.38[a]	ND
Diclofenac Sodium	ND	ND	ND	73.08 ± 0.19[a]

Values are means of triplicates ± SD; Means within each row that do not share a common letter were significantly different at $p < 0.05$; ND: Not detected; QE: Quercetin Equivalent; GA: Gallic Acid Equivalent

3.6. Antioxidant Activity

The antioxidant activity of EtOH extract and its selected fractions of *S. pinnata* leaves was determined through the DPPH RS assay. In this assay, antioxidants reduce the stable DPPH radical from a deep violet colour to a pale yellow form, with the extent of discoloration being directly proportional to RS activity. The EtOH extract and different solvent fractions of *S. pinnata* were tested at concentrations of 10-200 µg/mL, using ascorbic acid as reference (Table 4). The EtOH extract demonstrated a moderate antioxidant activity, with an IC_{50} value of 68.88 ± 0.11 µg/mL. The IC_{50} values of the solvent fractions ranged from 60.43-133.10 µg/mL, representing differential antioxidant capacities based on solvent polarity. Among all tested fractions, the EA exhibited the maximum antioxidant activity, with an IC_{50} value of 60.43 ± 0.41 µg/mL, which was significantly lower ($p < 0.05$) than those of the PE, NB, and AQ fractions. This enhanced RS activity may be due to the higher TPC and TFC in the EA fraction, as polyphenolic compounds are known to effectively neutralize free radicals via electron donation and resonance stabilization. The observed antioxidant trend aligns with earlier findings by Hazra *et al*. (2008), who demonstrated substantial antioxidant potential in *S. pinnata* extracts rich in phenolic constituents. Collectively, these results suggest a strong correlation between polyphenolic enrichment and antioxidant efficacy, further supporting the role of phenolic and flavonoid compounds as primary contributors to the antioxidant effects of *S. pinnata*.

3.7. Anti-Inflammatory Activity

The anti-inflammatory effects of the EtOH extract and its fractions of *S. pinnata* leaves were evaluated using the heat-induced bovine serum albumin (BSA) protein denaturation assay, a well-established method to screen the capacity of stabilising proteins against inflammatory damage. Protein denaturation is associated with the inflammatory response, and compounds capable of inhibiting this process are regarded as having anti-inflammatory potential. As presented in Table 4, the EtOH extract and different solvent fractions exhibited significant differences ($p < 0.05$) in their protein denaturation inhibitory activity. The EtOH extract exhibited an IC_{50} value of 96.99 ± 0.29 µg/mL, indicating moderate anti-inflammatory activity. The IC_{50} values of the solvent fractions ranged from 88.71 to 112.15 µg/mL, reflecting variation in activity based on phytochemical composition.

Among the tested fractions, the EA fraction showed highest anti-inflammatory activity, with an IC_{50} value of 88.71 ± 0.57 µg/mL, which was significantly lower ($p < 0.05$) than those recorded for the PE, NB, and AQ fractions. The superior activity of the EA fraction is likely related to its greater abundance of phenolic and flavonoid constituents, which are widely reported to mitigate inflammation by stabilizing proteins, suppressing the formation of pro-inflammatory mediators, and alleviating oxidative stress.

The results are in agreement with earlier studies on *S. pinnata*. Li *et al*. (2020) demonstrated that fruit peel extracts of *S. pinnata* significantly inhibited lipopolysaccharide-induced nitric oxide production in RAW 264.7 macrophage cells, indicating potent anti-inflammatory activity at the cellular level. Although differences in plant parts, extraction methods, and assay models account for variations in IC_{50} values, the present results corroborate the intrinsic anti-inflammatory potential of *S. pinnata* and further emphasizing polyphenolic constituents as key bioactive agents.

3.8. Antibacterial Activity

The antibacterial activity of S. *pinnata* EtOH extract and its fractions was assessed against *S. aureus* (Gram-positive) and *E. coli* (Gram-negative), with sterile EtOH /distilled water as negative controls and amikacin as a reference. All samples showed limited activity against *S. aureus*, with the EtOH and EA fractions producing inhibition zones of 7 mm and 8 mm, respectively, while PE and AQ fractions showed minimal effects (2 mm). In contrast, *E. coli* was found to be more susceptible. The EA fraction produced the largest zone of inhibition (15 mm), followed by the EtOH extract (13 mm) and NB fraction (11 mm), comparable to amikacin (17 mm). PE and AQ fractions exhibited weaker activity (7 mm and 3 mm). The stronger effect against *E. coli* likely reflects enrichment of moderately polar bioactive compounds, such as phenolic acids and flavonoids, which act by disrupting bacterial membranes, inhibiting enzymes, and interfering with nucleic acid synthesis. Lower activity against *S. aureus* may be due to its thicker cell wall. The findings are in agreement with earlier reports demonstrating antimicrobial potential of *S. pinnata* extracts and essential oils (Li et al., 2020; Suryanditha

et al., 2024), supporting its traditional use against infections.

Table 5: Antibacterial activity of EtOH extract and fractions of *S. pinnata* leaves

Extract	ZOI (*S. aureus*)	ZOI (*E.coli*)
Standard(Amikacin)	18 mm	17 mm
S. pinnata EtOH extract	7 mm	13 mm
S. pinnata PE	2 mm	7 mm
S. pinnata EA	8 mm	15 mm
S. pinnata NB	5 mm	11 mm
S. pinnata AQ	2 mm	3 mm

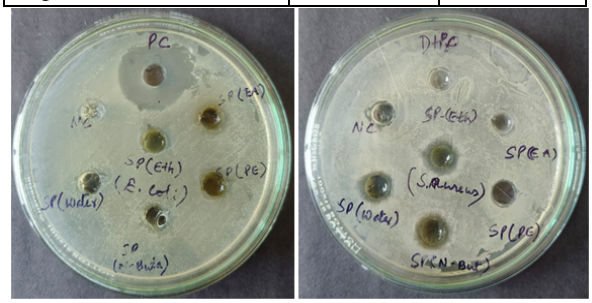

Figure 3: Antibacterial activity of EtOH extract and fractions of *S. pinnata* leaves

Conclusion

Our study gives a systematic assessment of the phytochemical composition and biological activities of *S. pinnata* leaf EtOH extract and its fractions. Quantitative analysis revealed high TPC and TFC, particularly in the EA fraction, indicating effective enrichment of bioactive polyphenols. Correspondingly, the EA fraction showed the strongest antioxidant activity and significant anti-inflammatory effects through protein denaturation inhibition. The EtOH extract and EA fraction also exhibited moderate antibacterial activity, with notable efficacy against *E. coli*. Biological effects strongly correlated with phenolic and flavonoid content, highlighting their key role in antioxidant, anti-inflammatory, and antibacterial activities. Differences in activity between Gram-positive and Gram-negative bacteria likely reflect variations in cell envelope structure affecting susceptibility. To the best of our knowledge, this is the first report of the antioxidant and antibacterial activities of *S. pinnata* leaves, expanding the pharmacological profile of this underexplored plant part. The findings of the present study support its traditional use and suggest that the leaves may serve as a sustainable source of bioactive compounds for nutraceutical, pharmaceutical, and functional food applications. Further studies are necessary to isolate active constituents, elucidate mechanisms, and validate effects *in vivo*.

References

1. Nakadate, K., Ito, N., Kawakami, K., & Yamazaki, N. (2025). Anti-Inflammatory Actions of Plant-Derived Compounds and Prevention of Chronic Diseases: From Molecular Mechanisms to Applications. *International Journal of Molecular Sciences*, *26*(11), 5206.

2. Tumilaar, S. G., Hardianto, A., Dohi, H., & Kurnia, D. (2024). A comprehensive review of free radicals, oxidative stress, and antioxidants: Overview, clinical applications, global perspectives, future directions, and mechanisms of antioxidant activity of flavonoid compounds. *Journal of Chemistry*, *2024*(1), 5594386.

3. Chandimali, N., Bak, S. G., Park, E. H., Lim, H. J., Won, Y. S., Kim, E. K., ... & Lee, S. J. (2025). Free radicals and their impact on health and antioxidant defenses: A review. *Cell death discovery*, *11*(1), 19.

4. Pérez-Flores, J. G., García-Curiel, L., Pérez-Escalante, E., Contreras-López, E., Aguilar-Lira, G. Y., Ángel-Jijón, C., ... & Portillo-Torres, L. A. (2025). Plant antimicrobial compounds and their mechanisms of action on spoilage and pathogenic bacteria: A bibliometric study and literature review. *Applied Sciences*, *15*(7), 3516.

5. Lee, E. L., & Barnes, J. (2022). Prevalence of use of herbal and traditional medicines. In *Pharmacovigilance for Herbal and Traditional Medicines: Advances, Challenges and International Perspectives* (pp. 15-25). Cham: Springer International Publishing.

6. http://www.theplantlist.org/1.1/browse/A/Anacardiaceae/Spondias/

7. Sujarwo, W., & Keim, A. P. (2019). Spondias pinnata (L. f.) Kurz.(Anacardiaceae): profiles and applications to diabetes. In *Bioactive food as dietary interventions for diabetes* (pp. 395-405). Academic Press.

8. Devkota, H. P., & Sai, K. (2023). Spondias pinnata (Lf) Kurz. In *Himalayan fruits and berries* (pp. 395-402). Academic Press.

9. Balaji, R., Nitheesh, S., & Devanathan, K (2024). A Review of Spondias pinnata (Lf) Kurz's Phytochemical Constituents, Traditional Uses, and Pharmacological Activities: An Important Medicinal Plant in Ayurveda. *Texila International Journal of Public Health*.

10. Ghate, N. B., Chaudhuri, D., Panja, S., Singh, S. S., Gupta, G., Lee, C. Y., & Mandal, N. (2018). In vitro mechanistic study of the anti-inflammatory activity of a quinoline isolated from Spondias pinnata Bark. *Journal of Natural Products*, *81*(9), 1956-1961.

11. Li, R., Yang, J. J., Song, X. Z., Wang, Y. F., Corlett, R. T., Xu, Y. K., & Hu, H. B. (2020). Chemical composition and the cytotoxic, antimicrobial, and anti-inflammatory activities of the fruit peel essential oil from Spondias pinnata (Anacardiaceae) in Xishuangbanna, southwest China. *Molecules*, *25*(2), 343.

12. Chaniad, P., Phuwajaroanpong, A., Plirat, W., Techarang, T., Chukaew, A., & Punsawad, C. (2022). In vivo assessment of the antimalarial activity and acute oral toxicity of an ethanolic seed extract of Spondias pinnata (Lf) Kurz. *BMC complementary medicine and therapies*, *22*(1), 72.

13. Sai, K., Chhetri, S. B. B., Devkota, S. R., & Khatri, D. (2021). Evaluation of the hypoglycemic potential of leaves extract of Spondias pinnata (Lf) Kurz. from Nepal. *The Scientific World Journal*, *2021*(1), 3230351.

14. Prakash, A., Janmeda, P., Pathak, P., Bhatt, S., & Sharma, V. (2019). Development and standardization of quality control parameters of different parts of *Trianthema portulacastrum* L. *SN Applied Sciences*, *1(9).*

15. Singh, P., Khosa, R. L., Srivastava, S., Mishra, G., Jha, K. K., Srivastava, S., Sangeeta, Verma, R. K., & Tahseen, M. A. (2014). Pharmacognostical study and establishment of quality parameters of aerial parts of *Costus speciosus*–a well known tropical folklore medicine. *Asian Pacific Journal of Tropical Biomedicine*, *4*(6), 486–491.

16. Dutta, P. P., Bordoloi, M., Roy, S., Narzary, B., Gogoi, K., Bhattacharyya, D. R., ... & Mazumder, B. (2018). Antiplasmodial activity of Gnetum gnemon leaves and compounds isolated from them. *Natural Product Communications*, *13*(10), 1934578X1801301007.

17. Abubakar, A. R., & Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy and Bioallied Sciences*, *12*(1), 1-10.

18. Chaudhary, A., Sharma, S., Mittal, A., Gupta, S., & Dua, A. (2020). Phytochemical and antioxidant profiling of Ocimum sanctum. *Journal of Food Science and technology*, *57*(10), 3852-3863.

19. Noreen, H., Semmar, N., Farman, M., & McCullagh, J. S. (2017). Measurement of total phenolic content and antioxidant activity of aerial parts of medicinal plant Coronopus didymus. *Asian Pacific journal of tropical medicine*, *10*(8), 792-801.

20. Shraim, A. M., Ahmed, T. A., Rahman, M. M., & Hijji, Y. M. (2021). Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. *Lwt*, *150*, 111932.

21. Saeed, N., Khan, M. R., & Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. BMC complementary and alternative medicine, 12(1), 221.

22. Mushtaq, M. M., Peerzada, S., & Ishtiaq, S. (2021). Pharmacognostic features, preliminary phytochemical screening and in vitro antioxidant studies of calotropis gigantea. Asian Journal of Hospital Pharmacy, 11-16.

23. Manandhar, S., Luitel, S., & Dahal, R. K. (2019). In vitro antimicrobial activity of some medicinal plants against human pathogenic bacteria. *Journal of tropical medicine*, *2019*(1), 1895340.

24. Dahiya, P., & Purkayastha, S. (2012). Phytochemical screening and antimicrobial activity of some medicinal plants against multi-drug resistant bacteria from clinical isolates. *Indian journal of pharmaceutical sciences*, *74*(5), 443.

25. Akwongo, B., Kakudidi, E. K., Nsubuga, A. M., Andama, M., Namaganda, M., Tugume, P., ... & Katuura, E. (2024). In vitro antifungal activities of medicinal plants used for treatment of candidiasis in Pader district, Northern Uganda. *Tropical Medicine and Health*, *52*(1), 84.

26. Bailey-Shaw, Y. A., Williams, L. A., Green, C. E., Rodney, S., & Smith, A. M. (2017). In-vitro evaluation of the anti-inflammatory potential of selected Jamaican plant extracts using the bovine serum albumin protein denaturation assay. *International Journal of Pharmaceutical Sciences Review and Research*, *47*(1), 145-153.

27. George, M., & Joseph, L (2013). Pharmacognostical and Phytochemical Studies on Spondia pinnata Leaf. *Annals of Plant Sciences*, 03 (1), 575-581.

28. Khade, O. S., Sruthi, K., Sonkar, R. M., Gade, P. S., & Bhatt, P. (2023). Plant secondary metabolites: extraction, screening, analysis and their bioactivity. *International Journal of Herbal Medicine*, *11*(2), 01-17.

29. Hazra, B., Biswas, S., & Mandal, N. (2008). Antioxidant and free radical scavenging activity of Spondias pinnata. *BMC complementary and Alternative Medicine*, *8*(1), 63.

30. Li, R., Yang, J. J., Song, X. Z., Wang, Y. F., Corlett, R. T., Xu, Y. K., & Hu, H. B. (2020). Chemical composition and the cytotoxic, antimicrobial, and anti-inflammatory activities of the fruit peel essential oil

from Spondias pinnata (Anacardiaceae) in Xishuangbanna, southwest China. *Molecules*, *25*(2), 343.

31. Suryanditha, P. A., Widhidewi, N. W., Paramasatiari, A. A. A. L., & Wedari, N. L. P. H. (2024). Antibacterial activity of Spondias pinnata leaf extract on the in-vitro growth of Gram-positive and Gram-negative bacteria. *Indonesia Journal of Biomedical Science*, *18*(1), 90-94.