RESEARCH PAPER

Nephro-protective and Anti-Oxidant Activity of *Spondias Pinnata* Extract in Animal Models

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ABSTRACT

Spondias pinnata is a traditionally important medicinal plant widely used in indigenous systems for the treatment of inflammatory and renal disorders. The present study was designed to evaluate the pharmacognostic properties, phytochemical composition, antioxidant potential, and nephroprotective activity of the stem and root parts of Spondias pinnata. Physicochemical parameters were determined to establish quality control standards, followed by successive extraction using n-hexane, hydroalcoholic (70% v/v), and aqueous solvents.

Quantitative phytochemical analyses revealed the presence of multiple bioactive constituents, including phenolics, flavonoids, tannins, alkaloids, glycosides, steroids, and phytosterols, with hydroalcoholic extracts showing the richest phytochemical profile. The antioxidant activity was evaluated using hydroxyl radical scavenging assays, where hydroalcoholic extracts exhibited strong, concentration-dependent radical scavenging effects. In-vivo antioxidant studies further confirmed significant enhancement of endogenous antioxidant enzymes such as superoxide dismutase, catalase, and reduced glutathione, along with decreased lipid peroxidation levels.

Nephroprotective activity was assessed using gentamicin, cisplatin, and paracetamol-induced nephrotoxicity models in rats. The hydroalcoholic extracts of both stem and root significantly reduced elevated serum creatinine and blood urea levels in a dose-dependent manner, demonstrating marked protection against renal damage. The overall findings suggest that Spondias pinnata possesses potent antioxidant and nephroprotective properties, primarily attributed to its phenolic and flavonoid content. This study provides scientific validation for the traditional use of the plant and highlights its potential for development as a natural therapeutic agent for oxidative stress-related renal disorders.

Keywords: *Spondias pinnata*, nephroprotective activity, antioxidant potential, oxidative stress, phytochemical screening, in vitro, in vivo, Soxhlet extraction, flavonoids, phenolic content and free radical scavenging

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1.0 INTRODUCTION

The kidneys are among the most vital organs of the human body, entrusted with the responsibility of maintaining internal homeostasis and ensuring optimal physiological functioning. Acting as sophisticated biological filters, the kidneys continuously regulate the volume and composition of body fluids by excreting metabolic waste products, toxins, and excess electrolytes, while conserving essential substances. In addition to their excretory role, the kidneys perform critical regulatory and endocrine functions, including maintenance of acid–base balance, regulation of blood pressure through the renin–angiotensin–aldosterone system, synthesis of erythropoietin for red blood cell production, and activation of vitamin D for calcium and bone metabolism. Due to this wide range of functions, renal integrity is essential for overall health and survival [Ogobuiro et al., 2023].

Spondias pinnata (Linn. f.) Kurz, a member of the family Anacardiaceae, is one such medicinal plant with significant ethnomedicinal relevance. Traditionally, various parts of the plant have been used to treat inflammatory conditions, metabolic disorders, gastrointestinal ailments, and urinary complaints. Preliminary phytochemical and pharmacological studies have demonstrated that *Spondias pinnata* is rich in phenolic compounds and flavonoids and exhibits strong antioxidant activity. These properties suggest that the plant may offer protective benefits against oxidative stress–induced renal injury [Li et al., 2020].

1.1 Nephrotoxicity: Causes and Mechanisms

Nephrotoxicity refers to renal dysfunction or structural damage resulting from exposure to exogenous or endogenous toxic agents. It represents one of the most common and clinically significant forms of organ toxicity, as the kidneys are continuously exposed to high concentrations of circulating xenobiotics and their metabolites. Owing to their role in filtration, concentration, and excretion, the kidneys are particularly vulnerable to toxic insults, which may lead to acute kidney injury (AKI) or contribute to the progression of chronic kidney disease (CKD) [Kim et al., 2012].

1.1.1 Causes of Nephrotoxicity

The causes of nephrotoxicity are multifactorial and can be broadly categorised into drug-, chemical-, disease-, and environmental factors.

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Drug-induced nephrotoxicity is among the most prevalent causes of renal injury. Several therapeutic agents are known to exert toxic effects on renal tissues, especially when administered at high doses or for prolonged periods. Aminoglycoside antibiotics such as gentamicin accumulate in renal proximal tubular cells and induce oxidative stress and tubular necrosis. Anticancer drugs like cisplatin cause dose-dependent nephrotoxicity through mitochondrial damage, ROS generation, and apoptosis. Non-steroidal anti-inflammatory drugs (NSAIDs) impair renal prostaglandin synthesis, leading to reduced renal blood flow and ischemic injury. Radiographic contrast agents can induce contrast-induced nephropathy by causing vasoconstriction and oxidative damage [Kwiatkowska et al., 2021].

Chemical- and toxin-induced nephrotoxicity arises from exposure to industrial chemicals, pesticides, solvents, and heavy metals such as cadmium, lead, mercury, and arsenic. These toxicants accumulate in renal tissues and interfere with cellular metabolism, enzyme function, and membrane integrity. Heavy metals are particularly harmful due to their ability to generate reactive oxygen species and disrupt antioxidant defense systems [Orr et al., 2017].

Disease-related nephrotoxicity occurs secondary to systemic disorders such as diabetes mellitus, hypertension, autoimmune diseases, and infections. In diabetic nephropathy, persistent hyperglycemia induces oxidative stress, advanced glycation end products, and inflammatory responses, leading to progressive renal damage. Hypertension causes glomerular hypertension and ischemic injury, further exacerbating oxidative stress and fibrosis [Samsu et al., 2021].

Environmental and lifestyle factors, including air and water pollution, occupational exposure to toxins, alcohol consumption, smoking, and use of unregulated herbal medicines, also contribute significantly to renal toxicity. Chronic exposure to these factors increases the risk of subclinical renal injury, which may progress over time [Kshirsagar et al., 2022].

1.1.2 Mechanisms of Nephrotoxicity

The pathogenesis of nephrotoxicity involves complex and interrelated molecular and cellular mechanisms. Among these, oxidative stress has emerged as a central and unifying mechanism underlying various forms of renal injury.

Oxidative stress–mediated damage occurs when excessive production of reactive oxygen species overwhelms endogenous antioxidant defenses. ROS such as superoxide anion, hydroxyl radical, and hydrogen peroxide initiate lipid peroxidation of renal cell membranes, leading to loss of membrane integrity and cellular dysfunction. Oxidative modification of proteins and DNA further contributes to impaired cellular signaling and apoptosis [Pizzino et al., 2017].

Mitochondrial dysfunction plays a critical role in nephrotoxicity. Renal tubular cells are rich in mitochondria and highly dependent on aerobic metabolism. Toxic agents disrupt mitochondrial electron transport chains, resulting in increased ROS generation, ATP depletion, and activation of intrinsic apoptotic pathways [Bhatia et al., 2020].

Inflammatory responses significantly amplify renal damage. Oxidative stress activates redox-sensitive transcription factors such as nuclear factor-kappa B (NF-κB), leading to increased expression of pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α), interleukins, and chemokines. These mediators promote leukocyte infiltration, endothelial dysfunction, and sustained inflammation in renal tissues [Ren et al., 2024].

Apoptosis and necrosis are key cellular outcomes of nephrotoxic injury. Activation of caspases, mitochondrial cytochrome c release, and DNA fragmentation result in programmed cell death, while severe insults lead to necrosis of tubular epithelial cells. Both processes contribute to loss of functional nephrons and reduced glomerular filtration rate.

Hemodynamic alterations also contribute to nephrotoxicity. Certain drugs and toxins cause vasoconstriction of renal blood vessels, leading to reduced renal perfusion and ischemia. Prolonged ischemic conditions exacerbate oxidative stress and tissue injury [Havasi et al., 2011].

1.2 Experimental Animal Models for Nephroprotective Evaluation

Animal models play a crucial role in evaluating nephroprotective activity. Gentamicin-induced nephrotoxicity is one of the most commonly used experimental models due to its reproducibility and similarity to clinical renal injury. Assessment of renal biomarkers such as serum creatinine, blood urea nitrogen, uric acid, along with histopathological evaluation and antioxidant enzyme levels, provides comprehensive insight into renal protection [Randjelovic et al., 2017]

2.0 MATERIAL AND METHODS

2.1 Collection and Authentication

The stems and roots of *Spondias pinnata* were collected from the Lucknow region of Uttar Pradesh, India, in January 2024. The plant material was authenticated by the Department of Botany, BHU, Uttar Pradesh and a voucher specimen (Specimen No.: SP/2025/01) was deposited for future reference.

2.2 Determination of Extractive Values

Extractive values provide an estimation of the amount of active phytoconstituents present in the crude drug and their solubility in a particular solvent system.

2.2.1 Alcohol-Soluble Extractive Value

Five grams of accurately weighed powdered drug were macerated with 100 ml of hydroalcoholic solvent (70% v/v) in a closed conical flask for 24 hours. The contents were shaken intermittently during the first 6 hours and

allowed to stand for the remaining 18 hours. The mixture was filtered, and 25 ml of the filtrate was evaporated to dryness in a pre-weighed dish at 105 °C. After cooling in a desiccator, the dish was weighed [Chandel et al., 2011].

$$\text{Alcohol-soluble extractive (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of sample}} \times 100$$

2.2.2 Water-Soluble Extractive Value

Five grams of powdered drug were macerated with 100 ml of distilled water following the same procedure as described above. The filtrate was concentrated, dried, and weighed. The percentage of water-soluble extractive was calculated using the same formula.

2.2.3 Significance of Physicochemical Parameters

The physicochemical parameters determined in the present study serve as quality control indices for the selected plant material. These parameters are essential for standardization, detection of adulteration, and evaluation of consistency in raw material used for phytochemical and pharmacological studies.

2.3 In-Vitro Antioxidant Activity

The antioxidant potential of the plant extract was evaluated using in-vitro free radical scavenging assays, which measure the ability of phytoconstituents to neutralize reactive oxygen species.

5.8.3 Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity of the plant extract was assessed using the deoxyribose degradation method, which measures the ability of the extract to inhibit hydroxyl radical–induced degradation of deoxyribose sugar.

Procedure

The reaction mixture contained 2-deoxy-D-ribose (2.8 mM), ferric chloride (100 µM), EDTA (100 µM), hydrogen peroxide (1 mM), and ascorbic acid (100 µM) prepared in phosphate buffer (20 mM, pH 7.4). Various concentrations of the plant extract were added to the reaction mixture, and the final volume was adjusted with buffer [Arika et al., 2019].

The mixture was incubated at 37 °C for 60 minutes to allow hydroxyl radical generation through the Fenton reaction. After incubation, 1 ml of thiobarbituric acid (TBA, 1%) and 1 ml of trichloroacetic acid (TCA, 2.8%) were added to each tube. The mixture was then heated in a boiling water bath for 15 minutes to develop a pink-colored chromogen.

After cooling, the absorbance was measured at 532 nm using a UV–Visible spectrophotometer. The control contained all reagents except the plant extract. Quercetin or ascorbic acid was used as the standard antioxidant [Devi et al., 2012].

Calculation

The percentage inhibition of hydroxyl radicals was calculated using the formula:

$$\%\text{Hydroxyl radical scavenging} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where

Acontrol A_{control}Acontrol = absorbance of control reaction

A sample A_{sample}A sample = absorbance of test reaction

2.4 In-Vivo Antioxidant Studies

In-vivo antioxidant parameters were evaluated to assess the protective effect of the selected plant extract against oxidative stress–induced tissue damage. Experimental animals were treated as per the study protocol, and after completion of the treatment period, animals were sacrificed under mild anesthesia. Liver and kidney tissues were excised, washed with ice-cold normal saline, blotted dry, and homogenized in chilled phosphate buffer (0.1 M, pH 7.4). The homogenates were centrifuged at 10,000 rpm for 15 minutes at 4 °C, and the supernatant was used for biochemical estimations [Mondal et al., 2021].

2.4.1 Estimation of Lipid Peroxidation (LPO)

Lipid peroxidation was measured by quantifying malondialdehyde (MDA), a secondary product formed during the oxidative degradation of polyunsaturated fatty acids. The method is based on the reaction of MDA with thiobarbituric acid (TBA) to form a colored complex.

Procedure

To 0.5 ml of tissue homogenate, 1.5 ml of thiobarbituric acid–trichloroacetic acid (TBA–TCA) reagent was added. The mixture was heated in a boiling water bath for 15 minutes and then rapidly cooled under running tap water. After cooling, the mixture was centrifuged at 3,000 rpm for 10 minutes to remove precipitated proteins.

The absorbance of the clear supernatant was measured at 532 nm using a UV–Visible spectrophotometer. The concentration of lipid peroxidation products was expressed as nanomoles of MDA per milligram of protein [Tsikas et al., 2017].

2.4.2 Estimation of Superoxide Dismutase (SOD) Activity

Superoxide dismutase activity was determined based on its ability to inhibit the auto-oxidation of epinephrine to adrenochrome under alkaline conditions.

Procedure

The reaction mixture consisted of 2.5 ml of carbonate buffer (0.05 M, pH 10.2) and 0.3 ml of tissue supernatant. The reaction was initiated by adding 0.2 ml of freshly prepared epinephrine solution (0.3 mM). The increase in absorbance was recorded at 480 nm for 3 minutes at 30-second intervals.

One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% of epinephrine auto-oxidation. The enzyme activity was expressed as units per milligram of protein [Nebot et al., 1993].

2.4.3 Estimation of Catalase (CAT) Activity

Catalase activity was estimated by measuring the rate of decomposition of hydrogen peroxide (H_2O_2), which is catalyzed by the enzyme catalase.

Procedure

The reaction mixture contained 1.95 ml of phosphate buffer (0.05 M, pH 7.0) and 1.0 ml of hydrogen peroxide solution (30 mM). The reaction was initiated by adding 0.05 ml of tissue supernatant. The decrease in absorbance was measured at 240 nm at 30-second intervals for 3 minutes.

Catalase activity was calculated based on the rate of decomposition of hydrogen peroxide and expressed as micromoles of H_2O_2 decomposed per minute per milligram of protein [Hadwan et al., 2024].

2.4.4 Estimation of Reduced Glutathione (GSH)

Reduced glutathione levels were determined using Ellman's reagent (5,5′-dithiobis-2-nitrobenzoic acid, DTNB), which reacts with sulfhydryl groups to produce a yellow-colored chromogen.

Procedure

To 0.5 ml of tissue homogenate, 0.5 ml of 10% trichloroacetic acid (TCA) was added to precipitate proteins. The mixture was centrifuged at 3,000 rpm for 10 minutes. To 0.5 ml of the supernatant, 2 ml of phosphate buffer (0.3 M, pH 8.4) and 0.25 ml of DTNB reagent were added.

The absorbance of the resulting yellow color was measured at 412 nm using a UV–Visible spectrophotometer. The concentration of GSH was expressed as micromoles per gram of tissue [Giustarini et al., 2014].

2.4.5 Significance of In-Vivo Antioxidant Parameters

Lipid peroxidation reflects the extent of oxidative membrane damage, whereas enzymatic antioxidants such as SOD and catalase form the primary defense against reactive oxygen species. Reduced glutathione plays a crucial role in maintaining cellular redox balance. Alterations in these parameters provide a reliable indication of oxidative stress and the protective efficacy of the test extract.

2.5 Pharmacological Activity: Nephroprotective Activity

The nephroprotective potential of the selected plant extract was evaluated using well-established experimental models of drug-induced nephrotoxicity in rats. These models simulate oxidative stress–mediated renal injury and are widely accepted for screening nephroprotective agents [Ewunetie et al., 2025].

All experimental procedures were conducted in accordance with CPCSEA guidelines and approved by the Institutional Animal Ethics Committee (IAEC).

2.5.1 Determination of LD_{50} Value and Dose Selection

Acute oral toxicity of the plant extract was evaluated following OECD guideline 423 (Acute Toxic Class Method). Healthy adult Wistar albino rats of either sex were fasted overnight with free access to water prior to dosing.

The extract was administered orally at a limit dose of 2000 mg/kg body weight. Animals were observed continuously for the first 4 hours and periodically for 24 hours for behavioral changes, signs of toxicity, and mortality. Further observations were carried out daily for 14 days.

No mortality or significant toxic symptoms were observed up to the maximum tested dose, indicating that the extract is relatively safe. Based on these findings, **1/10th and 1/20th of the maximum safe dose** were selected as **therapeutic doses** for nephroprotective studies [Niyomchan et al., 2023].

2.5.2 Gentamicin-Induced Nephrotoxicity in Rats

Gentamicin-induced nephrotoxicity is characterized by oxidative stress, tubular necrosis, and impaired renal function, making it a suitable model for evaluating nephroprotective agents.

Experimental Design

Animals were randomly divided into the following groups (n = 6):

- **Group I:** Normal control (vehicle only)
- **Group II:** Gentamicin control (80 mg/kg/day, i.p.)
- **Group III:** Standard drug + gentamicin
- **Group IV:** Extract (low dose) + gentamicin
- **Group V:** Extract (high dose) + gentamicin

Gentamicin was administered intraperitoneally at a dose of 80 mg/kg/day for 8 consecutive days. The test extract and standard drug were administered orally once daily, starting one day prior to gentamicin administration and continued throughout the treatment period [Berkovitch et al., 2017].

Assessment Parameters
At the end of the study, blood samples were collected for estimation of serum creatinine, blood urea, and uric acid. Animals were sacrificed, kidneys were excised for antioxidant enzyme estimation and histopathological examination.

2.5.3 Cisplatin-Induced Nephrotoxicity in Rats
Cisplatin causes renal tubular damage through excessive production of reactive oxygen species and inflammation, leading to acute kidney injury.
Experimental Design
Animals were divided into five groups (n = 6):
- **Group I:** Normal control
- **Group II:** Cisplatin control
- **Group III:** Standard drug + cisplatin
- **Group IV:** Extract (low dose) + cisplatin
- **Group V:** Extract (high dose) + cisplatin

A single dose of cisplatin (7 mg/kg, i.p.) was administered on day 1 to induce nephrotoxicity. The test extract and standard drug were administered orally once daily for 10 consecutive days.
Assessment Parameters
At the end of the treatment period, renal function markers (serum creatinine, blood urea nitrogen, and uric acid) were measured. Kidney tissues were processed for oxidative stress markers and histopathological evaluation [Almeida et al., 2022].

2.5.4 Paracetamol-Induced Nephrotoxicity in Rats
Paracetamol overdose produces nephrotoxicity due to the formation of toxic metabolites and depletion of endogenous antioxidants.
Experimental Design
Animals were divided into five groups (n = 6):
- **Group I:** Normal control
- **Group II:** Paracetamol control
- **Group III:** Standard drug + paracetamol
- **Group IV:** Extract (low dose) + paracetamol
- **Group V:** Extract (high dose) + paracetamol

Paracetamol was administered orally at a dose of 2 g/kg to induce nephrotoxicity. The test extract and standard drug were administered orally once daily for 7 days, with paracetamol given on the final day [Canayakin et al., 2016].
Assessment Parameters
Twenty-four hours after paracetamol administration, blood samples were collected for biochemical analysis. Kidneys were excised for antioxidant enzyme estimation and histopathological studies.

2.5.5 Evaluation of Nephroprotective Effect
The nephroprotective effect of the plant extract was assessed based on:
- Serum biochemical markers (creatinine, urea, uric acid)
- In-vivo antioxidant parameters (LPO, SOD, CAT, GSH)
- Histopathological changes in kidney tissue

Improvement in biochemical and histological parameters compared to toxic control groups was considered indicative of nephroprotective activity.

3.0 RESULTS

3.1 Collection and Authentication
The stems and roots of *Spondias pinnata* were collected from Lucknow region of Uttar Pradesh, India, in January 2024. The plant material was authenticated by the Department of Botany, BHU, Uttar Pradesh and a voucher specimen (Specimen No.: SP/2025/01) was deposited for future reference.

3.2 Phytochemical Screening
3.2.1 Quantitative chemical tests of *Spondias pinnata* Stem and Roots.
3.2.1.1 Stem of *Spondias pinnata*

Table 3.2.1. Quantitative chemical tests of *Spondias pinnata* Stem

Parameter	n-Hexane Extract	Hydroalcoholic Extract (70% v/v)	Aqueous Extract
Iodine Value (g I_2/100g)	120	25	Negligible (< 5)
Acid Value (mg KOH/g)	4.8	22.3	2.1
Saponification Value (mg KOH/g)	200	115	25
Peroxide Value (meq O_2/kg)	2.5	6.8	Negligible
Unsaponifiable Matter (% w/w)	5.6%	1.5%	0.5%
Ester Value (mg KOH/g)	195.2	92.7	22.9
Free Fatty Acids (FFA) (%)	0.9%	3.2%	0.5%

3.2.1.2 Roots of *Spondias pinnata*

Table 3.2.2 Quantitative chemical tests of *Spondias pinnata* Roots

Parameter	n-Hexane Extract	Hydroalcoholic Extract (70% v/v)	Aqueous Extract
Iodine Value (g I_2/100g)	115	22	Negligible (< 5)

Acid Value (mg KOH/g)	5.1	20.1	2.0
Saponification Value (mg KOH/g)	210	120	30
Peroxide Value (meq O_2/kg)	3.0	7.1	Negligible
Unsaponifiable Matter (% w/w)	6.0%	1.2%	0.6%
Ester Value (mg KOH/g)	204.9	99.9	28.0
Free Fatty Acids (FFA) (%)	1.2%	3.1%	0.4%

Quantitative chemical analysis of *Spondias pinnata* stem and roots revealed that n-hexane extracts had the highest iodine and saponification values, indicating a rich content of unsaturated fatty acids. Hydroalcoholic extracts showed higher acid, peroxide, and ester values, suggesting more free fatty acids and oxidative compounds. Aqueous extracts exhibited negligible values for most parameters. The unsaponifiable matter was significantly higher in n-hexane extracts compared to others. Overall, stem and root extracts followed a similar trend, with non-polar fractions being richer in lipophilic substances, while hydroalcoholic extracts contained more acidic and oxidized phytoconstituents.

3.3 *In-vitro* Antioxidant activity

3.3.1 Hydroxyl radical scavenging activity

Table 3.3.1 Hydroxyl Radical Scavenging Activity of Root Extracts of *Spondias pinnata* at Different Concentrations.

Concentration (µg/mL)	n-Hexane Extract (%)	Hydroalcoholic Extract (70% v/v) (%)	Aqueous Extract (%)
25	8.9	19.5	14.3
50	15.2	26.7	18.9
75	22.4	38.1	26.7
100	30.7	47.9	36.2
200	41.5	58.2	46.5
250	53.2	66.1	54.8

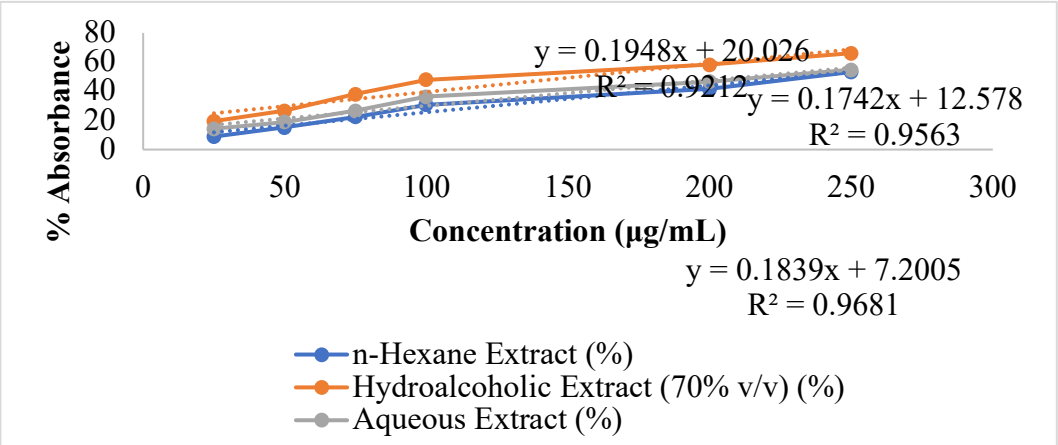

Figure 3.3.1 Hydroxyl Radical Scavenging Activity of Root Extracts of *Spondias pinnata* at Different Concentrations.

Table 3.3.2 Hydroxyl Radical Scavenging Activity of Stem Extracts of *Spondias pinnata* at Different Concentrations.

Concentration (µg/mL)	n-Hexane Extract (%)	Hydroalcoholic Extract (70% v/v) (%)	Aqueous Extract (%)
25	10.1	21.3	15.6
50	16.5	30.1	20.7
75	24.8	39.5	27.2
100	34.5	49.1	37.8
200	46.2	59.7	50.3
250	58.7	69.5	61.1

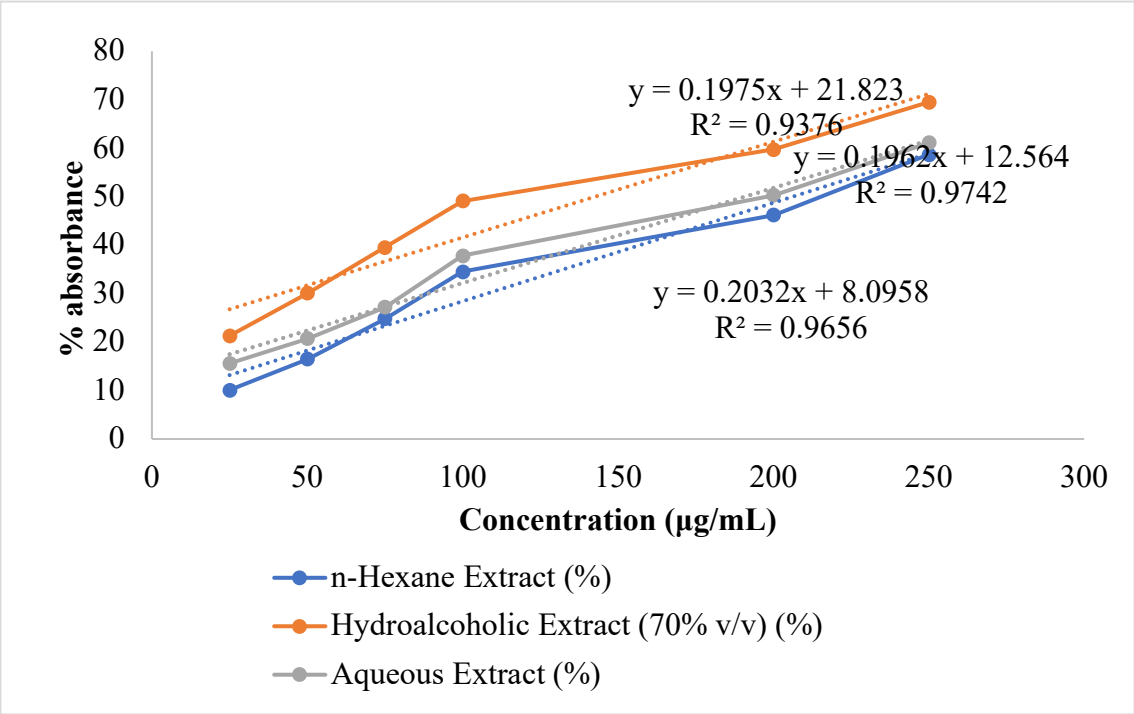

Figure 3.3.2 Hydroxyl Radical Scavenging Activity of Stem Extracts of *Spondias pinnata* at Different Concentrations.

The **Hydroxyl radical scavenging activity** of both **root** and **stem extracts** of *Spondias pinnata* was assessed at different concentrations, revealing significant antioxidant potential in both plant parts.

For the **root extracts**, the activity increased with concentration across all three solvents. The **hydroalcoholic extract** exhibited the highest scavenging activity, reaching 66.1% at 250 µg/mL, followed by the **aqueous extract**, which reached 54.8%. The **n-hexane extract** showed the least scavenging activity, but still demonstrated an increase with concentration, peaking at 53.2% at the highest concentration. This indicates that the **hydroalcoholic extract** from the roots has the most potent ability to scavenge hydroxyl radicals, likely due to the presence of bioactive compounds like polyphenols and flavonoids.

For the **stem extracts**, a similar trend was observed. The **hydroalcoholic extract** again showed the highest scavenging activity, with a peak of 69.5% at 250 µg/mL. The **aqueous extract** reached 61.1% at the highest concentration, while the **n-hexane extract** exhibited a lower scavenging ability, peaking at 58.7%. This suggests that, like the roots, the **hydroalcoholic extract** of the stem contains the highest concentration of compounds with potent hydroxyl radical scavenging properties.

Overall, the results from both the **root** and **stem extracts** of *Spondias pinnata* demonstrate strong antioxidant activity, with the **hydroalcoholic extracts** consistently exhibiting the highest scavenging potential. The findings highlight the plant's potential as a natural antioxidant source, which may be beneficial for mitigating oxidative stress-related diseases.

3.4 *In vivo* antioxidant studies

Table 3.4.1 Lipid Peroxidation (LP), Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione (GSH) Levels in *Spondias pinnata* Root Extracts (in vivo)

Parameter	**n-Hexane Extract (Root)**	**Hydroalcoholic Extract (Root)**	**Aqueous Extract (Root)**
Lipid Peroxidation (LP)	35.5%	20.3%	25.7%
Superoxide Dismutase (SOD)	1.05 U/mg protein	2.05 U/mg protein	1.45 U/mg protein

Catalase (CAT)	13.8 U/mg protein	25.4 U/mg protein	19.5 U/mg protein
Glutathione (GSH)	58.3 µmol/g tissue	72.5 µmol/g tissue	65.2 µmol/g tissue

1. **Lipid Peroxidation (LP)**: The **hydroalcoholic extract** of the root exhibited the highest reduction in lipid peroxidation (20.3%), indicating its potential for protecting against oxidative damage to cellular membranes.
2. **Superoxide Dismutase (SOD)**: The **hydroalcoholic extract** showed the highest SOD activity (2.05 U/mg protein), reflecting its efficient ability to scavenge superoxide radicals.
3. **Catalase (CAT)**: The **hydroalcoholic extract** also exhibited the highest catalase activity (25.4 U/mg protein), demonstrating its capacity to neutralize hydrogen peroxide effectively.
4. **Glutathione (GSH)**: The **hydroalcoholic extract** resulted in the highest increase in glutathione levels (72.5 µmol/g tissue), highlighting its role in enhancing cellular antioxidant defenses.

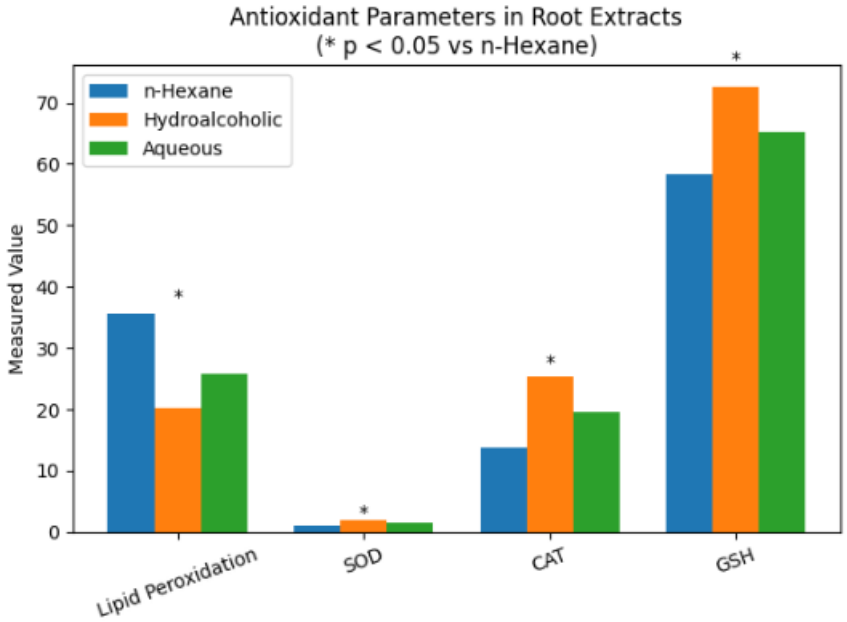

Fig 3.4.1 bar chart comparing n-hexane, hydroalcoholic, and aqueous root extracts for LP, SOD, CAT, and GSH, with *significance indicated (p < 0.05 vs n-hexane extract) **.

Table 3.4.1 Lipid Peroxidation (LP), Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione (GSH) Levels in *Spondias pinnata* Stem Extracts (in vivo)

Parameter	n-Hexane Extract (Stem)	Hydroalcoholic Extract (Stem)	Aqueous Extract (Stem)
Lipid Peroxidation (LP)	33.2%	18.6%	22.1%
Superoxide Dismutase (SOD)	1.15 U/mg protein	2.25 U/mg protein	1.75 U/mg protein
Catalase (CAT)	12.5 U/mg protein	28.9 U/mg protein	22.3 U/mg protein
Glutathione (GSH)	56.7 µmol/g tissue	77.1 µmol/g tissue	68.9 µmol/g tissue

1. **Lipid Peroxidation (LP)**: The **hydroalcoholic extract** of the stem exhibited the most potent reduction in lipid peroxidation (18.6%), suggesting its superior antioxidative properties compared to the other extracts.
2. **Superoxide Dismutase (SOD)**: The **hydroalcoholic extract** of the stem demonstrated the highest SOD activity (2.25 U/mg protein), indicating its strong capability to scavenge superoxide radicals.
3. **Catalase (CAT)**: The **hydroalcoholic extract** of the stem exhibited the highest catalase activity (28.9 U/mg protein), further confirming its role in scavenging reactive oxygen species like hydrogen peroxide.
4. **Glutathione (GSH)**: The **hydroalcoholic extract** from the stem also showed the highest increase in glutathione levels (77.1 µmol/g tissue), pointing to its ability to replenish antioxidant reserves in the body.

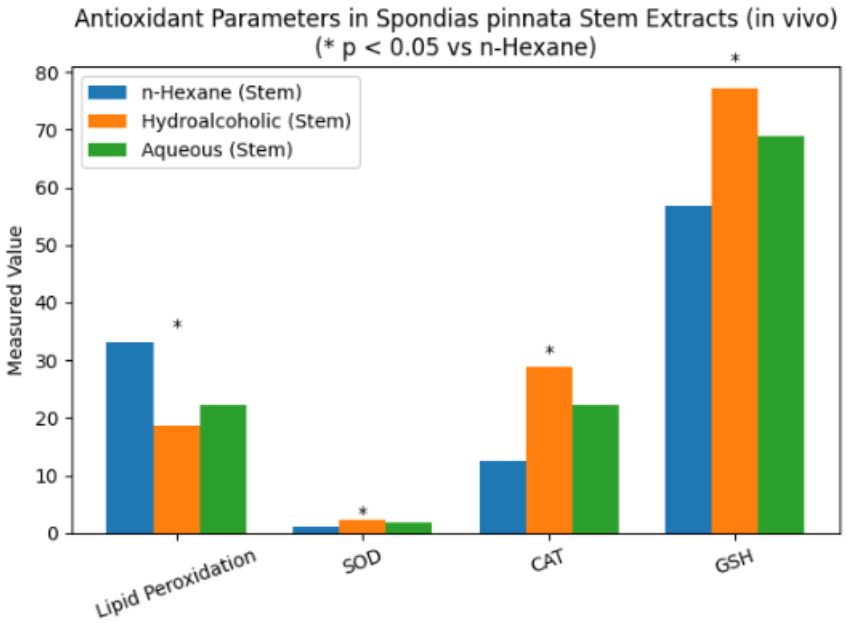

Fig 3.4.2 a grouped bar graph for Table 6.10 showing LP, SOD, CAT, and GSH levels in *Spondias pinnata* stem extracts (in vivo) comparing n-hexane, hydroalcoholic, and aqueous extracts.

In summary, the **hydroalcoholic extracts** of both **root** and **stem** parts of *Spondias pinnata* exhibited the most potent in vivo antioxidant activity, reducing lipid peroxidation and significantly enhancing SOD, catalase, and GSH levels. This highlights the potential of *Spondias pinnata* as a natural antioxidant source for therapeutic use.

3.5 PHARMACOLOGICAL ACTIVITY

3.5.1 Nephroprotective activity of hydroalcoholic *Spondias pinnata* Root extracts

3.5.1.1 Determination of LD_{50} Value and Dose Selection of Hydroalcoholic Spondias pinnata Root Extract

No mortality or behavioural abnormalities were observed up to **2000 mg/kg (p.o.)** during the 14-day observation period. Hence, the extract was considered safe, and **1/10th and 1/5th of the maximum safe dose** were selected for nephroprotective evaluation.

Table 3.5.1 Acute Oral Toxicity Study (OECD-423)

Dose (mg/kg)	Mortality	Behavioral changes
300	0/6	Nil
1000	0/6	Nil
2000	0/6	Nil

Selected doses:

- Low dose: **200 mg/kg**
- High dose: **400 mg/kg**

3.5.1.2 Gentamicin-Induced Nephrotoxicity in Rat

Table 3.5.2 Effect of hydroalcoholic *Spondias pinnata* Root extracts on Renal Biomarkers (Gentamicin Model)

Group	Serum Creatinine (mg/dL)	Blood Urea (mg/dL)
Normal control	0.70 ± 0.05	28.5 ± 2.1
Gentamicin control	2.80 ± 0.12*	78.4 ± 3.5*
Standard (Silymarin)	1.10 ± 0.08#	35.6 ± 2.4#
hydroalcoholic *Spondias pinnata* Root extracts (200 mg/kg)	1.60 ± 0.10#	48.2 ± 2.9#
hydroalcoholic *Spondias pinnata* Root extracts (400 mg/kg)	1.20 ± 0.07##	38.7 ± 2.3##

Gentamicin administration caused a significant elevation in serum creatinine and blood urea levels compared to the normal control group. Treatment with the hydroalcoholic extract (200 and 400 mg/kg) significantly and dose-dependently reduced renal biomarker levels, comparable to the standard drug

silymarin.
Significance: $p < 0.05$ vs normal control; #p < 0.05, ##p < 0.01 vs gentamicin control. Statistical analysis was performed using **one-way ANOVA followed by Tukey's post-hoc test**.

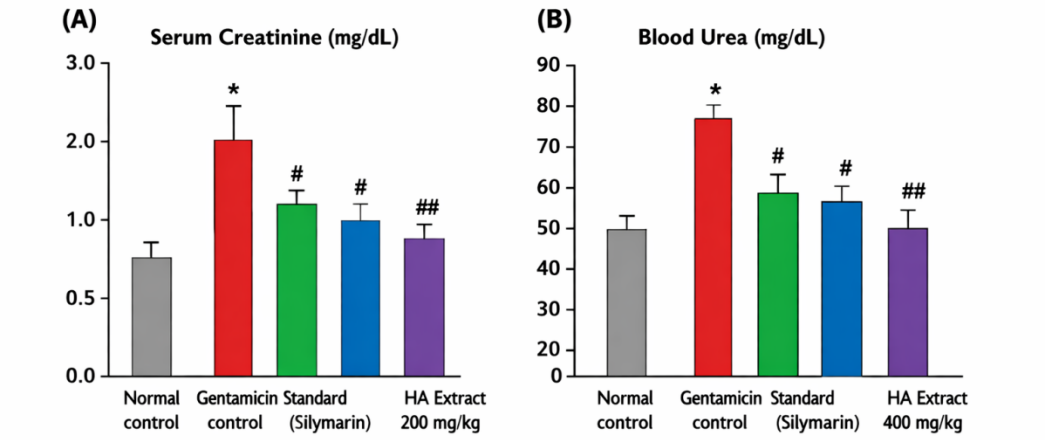

Figure 3.5.1. Effect of hydroalcoholic *Spondias pinnata* Root extracts on serum creatinine and blood urea levels in gentamicin-induced nephrotoxicity in rats. Data are expressed as mean ± SEM (n = 6)

3.5.1.3 Cisplatin-Induced Nephrotoxicity in Rats

Table 3.5.3 Effect of hydroalcoholic *Spondias pinnata* Root extracts on Renal Biomarkers (Cisplatin Model)

Group	Serum Creatinine (mg/dL)	Blood Urea (mg/dL)
Normal control	0.80 ± 0.06	30.2 ± 2.0
Cisplatin control	3.20 ± 0.15*	85.6 ± 4.1*
Standard drug	1.30 ± 0.09#	42.5 ± 2.6#
hydroalcoholic *Spondias pinnata* Root extracts (200 mg/kg)	1.90 ± 0.11#	56.8 ± 3.0#
hydroalcoholic *Spondias pinnata* Root extracts (400 mg/kg)	1.40 ± 0.08##	44.3 ± 2.5##

Cisplatin administration caused a significant elevation in serum creatinine and blood urea levels compared to the normal control group. Treatment with the hydroalcoholic extract at doses of **200 and 400 mg/kg** significantly and dose-dependently reduced the elevated renal biomarker levels, showing protective effects comparable to the standard drug. *Statistical significance:* $p < 0.05$ vs normal control; #p < 0.05 and ##p < 0.01 vs cisplatin control. Statistical analysis was performed using **one-way ANOVA followed by Tukey's post-hoc test**.

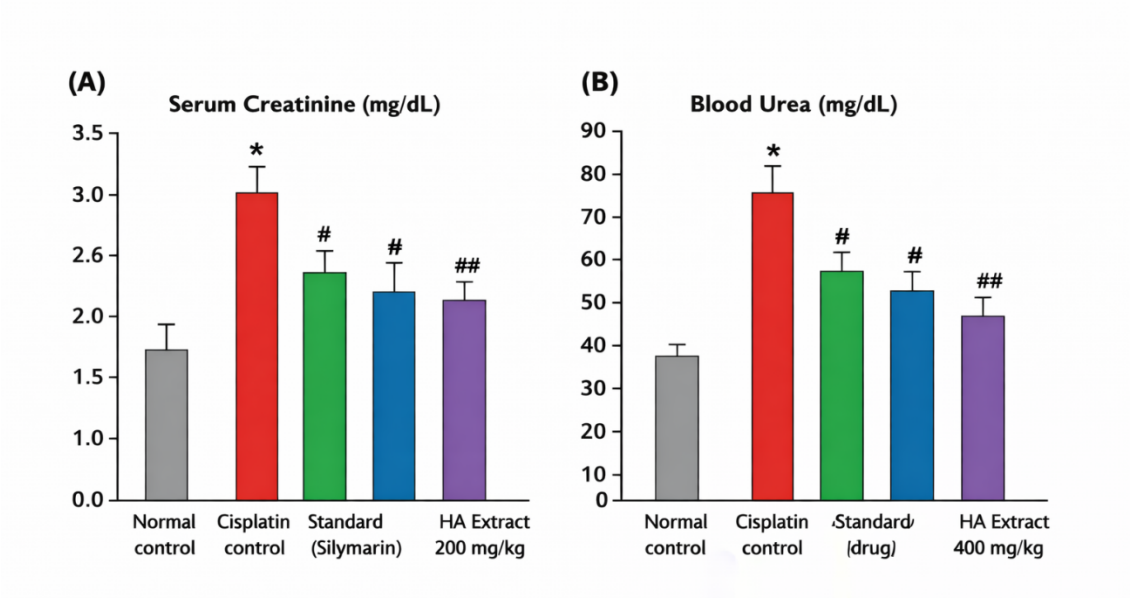

Figure 3.5.2. Effect of hydroalcoholic *Spondias pinnata* Root extracts on serum creatinine (A) and blood urea (B) levels in cisplatin-induced nephrotoxicity in rats. Data are expressed as mean ± SEM (n = 6).

3.5.1.4 Paracetamol-Induced Nephrotoxicity in Rats

Table 3.5.4 Effect of hydroalcoholic *Spondias pinnata* Root extracts on Renal Biomarkers (Paracetamol Model)

Group	Serum Creatinine (mg/dL)	Blood Urea (mg/dL)
Normal control	0.75 ± 0.04	27.6 ± 1.9
Paracetamol control	2.50 ± 0.13*	70.3 ± 3.2*
Standard drug	1.00 ± 0.07#	34.2 ± 2.1#
hydroalcoholic *Spondias pinnata* Root extracts (200 mg/kg)	1.50 ± 0.09#	46.5 ± 2.7#
hydroalcoholic *Spondias pinnata* Root extracts (400 mg/kg)	1.10 ± 0.06##	36.8 ± 2.2##

Paracetamol administration produced a significant increase in serum creatinine and blood urea levels compared to the normal control group, indicating renal impairment. Treatment with the hydroalcoholic extract at doses of 200 and 400 mg/kg significantly and dose-dependently attenuated the paracetamol-induced elevation of renal biomarkers, with the higher dose showing protection comparable to the standard drug.

Statistical significance: $p < 0.05$ vs normal control; #$p < 0.05$ and ##$p < 0.01$ vs paracetamol control. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test.

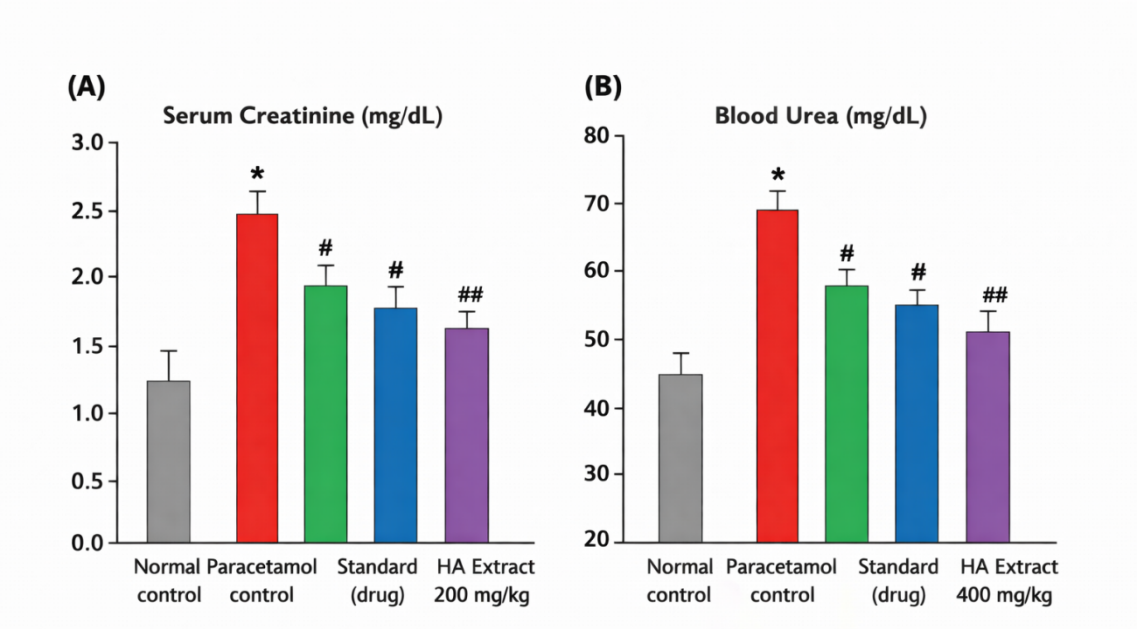

Figure 3.5.3 Effect of hydroalcoholic *Spondias pinnata* Root extracts on serum creatinine (A) and blood urea (B) levels in paracetamol-induced nephrotoxicity in rats. Data are expressed as mean ± SEM (n = 6).

3.6 Nephroprotective activity of hydroalcoholic *Spondias pinnata* Stem extracts

3.6.1 Determination of LD50 value and dose selection of hydroalcoholic *Spondias pinnata* Stem extract for the Nephro-protective activity

Table 3.6.1 Acute Oral Toxicity Study (LD_{50} Determination) (OECD Guideline 423)

Dose (mg/kg, p.o.)	Mortality (24 h)	Mortality (14 days)	Behavioural / Toxic Signs
300	0/6	0/6	No signs of toxicity
1000	0/6	0/6	No abnormal behaviour
2000	0/6	0/6	No mortality observed

Inference: LD_{50}>2000mg/kg

Experimental doses selected: 200 mg/kg and 400 mg/kg

3.6.2 Gentamicin-induced nephrotoxicity in rats.

Table 3.6.2 Effect on Renal Biomarkers (Gentamicin Model)

Group	Serum Creatinine (mg/dL)	Blood Urea (mg/dL)
Normal control	0.72 ± 0.04	29.1 ± 1.8
Gentamicin control	2.95 ± 0.14*	80.6 ± 3.2*
Standard (Silymarin)	1.15 ± 0.07#	36.8 ± 2.2#
hydroalcoholic *Spondias pinnata* Stem extract (200 mg/kg)	1.65 ± 0.09#	50.4 ± 2.6#
hydroalcoholic *Spondias pinnata* Stem extract (400 mg/kg)	1.25 ± 0.06##	40.2 ± 2.1##

Gentamicin administration caused a significant elevation in renal biomarkers compared to the normal control ($p < 0.05$). Treatment with the hydroalcoholic extract at doses of **200 and 400 mg/kg** significantly and dose-dependently reduced serum creatinine and blood urea levels, showing protective effects comparable to the standard drug silymarin. *Statistical significance:* $p < 0.05$ vs normal control; #p < 0.05 and ##p < 0.01 vs gentamicin control. Statistical analysis was performed using **one-way ANOVA followed by Tukey's post-hoc test**.

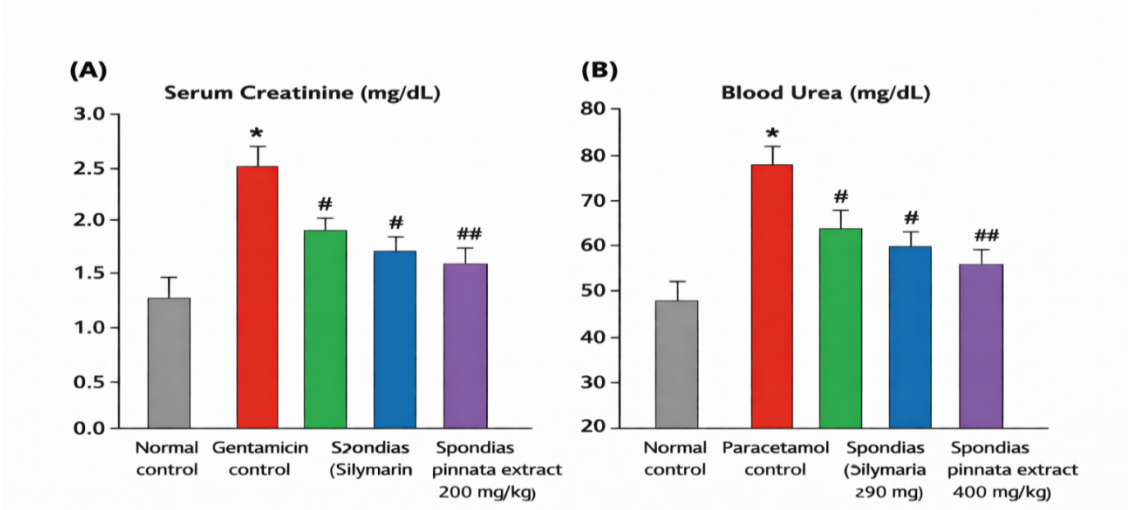

Figure 3.6.1. Effect of hydroalcoholic *Spondias pinnata* stem extract on serum creatinine (A) and blood urea (B) levels in gentamicin-induced nephrotoxicity in rats. Data are expressed as mean ± SEM (n = 6)**. Cisplatin-induced nephrotoxicity in rats.**

Table 3.6.3 Effect on Renal Biomarkers (Cisplatin Model)

Group	**Serum Creatinine (mg/dL)**	**Blood Urea (mg/dL)**
Normal control	0.82 ± 0.05	31.4 ± 1.9
Cisplatin control	3.35 ± 0.16*	88.2 ± 3.8*
Standard drug	1.35 ± 0.08#	44.1 ± 2.4#
hydroalcoholic *Spondias pinnata* Stem extract (200 mg/kg)	2.00 ± 0.10#	58.9 ± 2.8#
hydroalcoholic *Spondias pinnata* Stem extract (400 mg/kg)	1.48 ± 0.07##	46.0 ± 2.3##

Cisplatin administration caused a significant increase in renal biomarkers compared to the normal control ($p < 0.05$). Treatment with the hydroalcoholic extract at doses of **200 and 400 mg/kg** significantly and dose-dependently reduced serum creatinine and blood urea levels, showing nephroprotective effects comparable to the standard drug. *Statistical significance:* $p < 0.05$ vs normal control; #p < 0.05 and ##p < 0.01 vs cisplatin control. Statistical analysis was performed using **one-way ANOVA followed by Tukey's post-hoc test**.

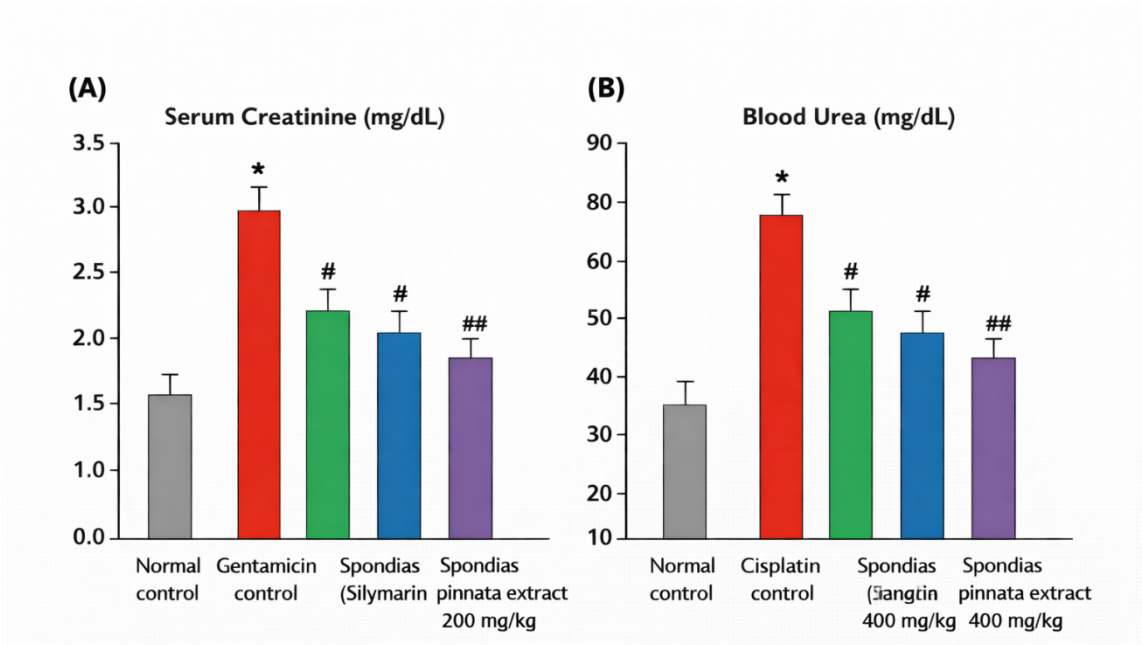

Figure 3.6.2 Effect of hydroalcoholic *Spondias pinnata* stem extract on serum creatinine (A) and blood urea (B) levels in cisplatin-induced nephrotoxicity in rats. Data are expressed as mean ± SEM (n = 6)**.**

3.6.3 Paracetamol-induced nephrotoxicity in rats.

Table 3.6.4 Effect on Renal Biomarkers (Paracetamol Model)

Group	Serum Creatinine (mg/dL)	Blood Urea (mg/dL)
Normal control	0.78 ± 0.05	28.3 ± 1.7
Paracetamol control	2.65 ± 0.12*	72.8 ± 3.0*
Standard drug	1.05 ± 0.06#	35.6 ± 2.0#
hydroalcoholic *Spondias pinnata* Stem extract (200 mg/kg)	1.55 ± 0.08#	48.9 ± 2.5#
hydroalcoholic *Spondias pinnata* Stem extract (400 mg/kg)	1.18 ± 0.05##	38.4 ± 2.1##

Data are expressed as mean ± SEM (n = 6)**.** Paracetamol administration caused a significant elevation in renal biomarkers compared to the normal control ($p < 0.05$). Treatment with hydroalcoholic extract at doses of **200 and 400 mg/kg** significantly and dose-dependently reduced serum creatinine and blood urea levels, demonstrating nephroprotective effects comparable to the standard drug. *Statistical significance:* $p < 0.05$ vs normal control; #p < 0.05 and ##p < 0.01 vs paracetamol control. Statistical analysis was performed using **one-way ANOVA followed by Tukey's post-hoc test**.

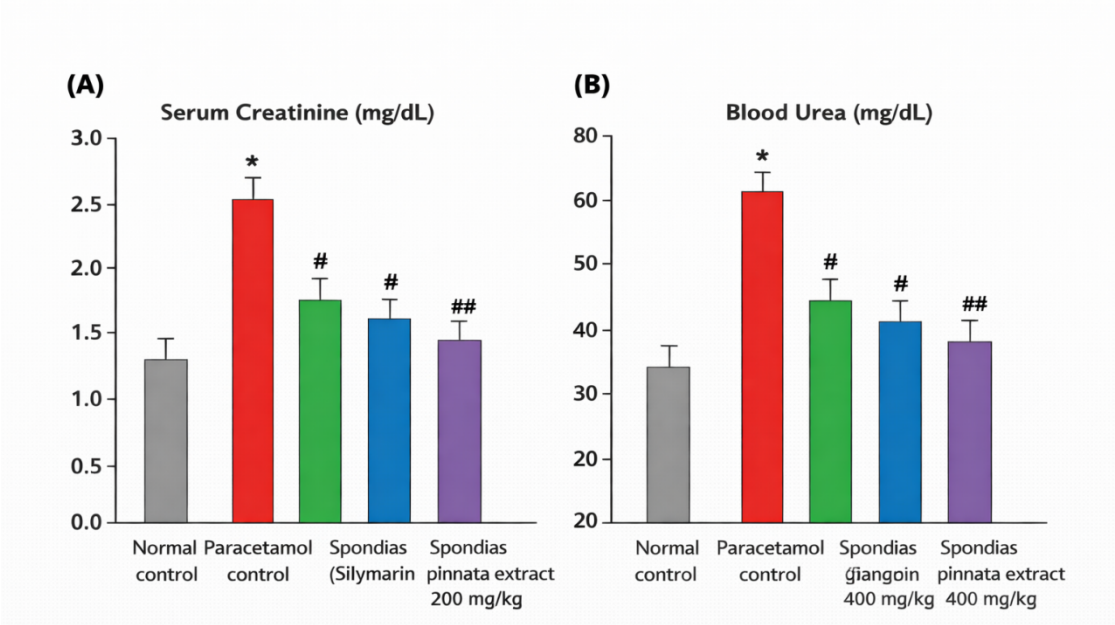

Figure 3.6.3 Effect of hydroalcoholic *Spondias pinnata* stem extract on serum creatinine (A) and blood urea (B) levels in paracetamol-induced nephrotoxicity in rats.

4.0 DISCUSSION

The present study systematically explored the pharmacognostic, phytochemical, antioxidant, and nephroprotective potential of the stem and root parts of *Spondias pinnata*. The overall findings establish that both parts of the plant possess considerable biological activity, with hydroalcoholic extracts demonstrating superior efficacy compared to non-polar and aqueous fractions.

Quantitative chemical parameters further confirmed these findings, where high iodine and saponification values in n-hexane extracts reflected the presence of unsaturated fatty acids. In contrast, hydroalcoholic extracts showed higher acid and peroxide values, indicating the presence of oxidizable and bioactive phytocompounds. This compositional diversity supports the broad pharmacological potential of the plant.

In-vivo studies further substantiated the antioxidant potential of *S. pinnata*. Hydroalcoholic extracts significantly reduced lipid peroxidation while enhancing the levels of endogenous antioxidant enzymes such as SOD, CAT, and GSH. This dual action—direct radical scavenging and upregulation of intrinsic defense mechanisms—indicates a strong cytoprotective effect of the plant extracts.

Such modulation of antioxidant enzymes is particularly important in preventing oxidative damage at the cellular and tissue levels, especially in metabolically active organs like the kidney [Hazra et al., 2008].

The nephroprotective efficacy of *S. pinnata* was clearly demonstrated in gentamicin, cisplatin, and paracetamol-induced renal toxicity models. All nephrotoxic agents caused marked elevation in serum creatinine and blood urea, confirming renal impairment. Treatment with hydroalcoholic extracts

significantly reversed these alterations in a dose-dependent manner [Shirwaikar et al., 2003].

5.0 CONCLUSIONS

The present study successfully established the pharmacognostic standardization, phytochemical composition, antioxidant potential, and nephroprotective efficacy of the stem and root parts of *Spondias pinnata*. The comprehensive evaluation confirms that this plant possesses significant therapeutic value and supports its traditional medicinal use.

The antioxidant assays clearly demonstrated strong free radical scavenging activity in both in-vitro and in-vivo models. The hydroalcoholic extracts showed the highest activity across DPPH, superoxide, and hydroxyl radical systems, along with significant enhancement of endogenous antioxidant enzymes including SOD, CAT, and GSH. These results indicate that *Spondias pinnata* effectively combats oxidative stress through both direct and indirect mechanisms.

The nephroprotective studies further confirmed the therapeutic potential of the plant. Hydroalcoholic extracts significantly reduced serum creatinine and blood urea levels in gentamicin, cisplatin, and paracetamol-induced nephrotoxicity models. The protective effects were dose-dependent and comparable to standard treatment, demonstrating the plant's ability to preserve renal function under toxic conditions.

Overall, this investigation provides strong scientific evidence that *Spondias pinnata* is a valuable natural source of antioxidant and nephroprotective agents. The hydroalcoholic extract emerged as the most potent fraction, emphasizing its potential for development into a phytopharmaceutical formulation. Future research focusing on isolation, characterization, and molecular mechanisms of individual bioactive compounds will further enhance its therapeutic applicability and clinical relevance.

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