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### Research Article

## Antioxidant and Nephroprotective Activity of Flavonoid Rich Fraction of *Alphonsea Sclerocarpa* Thw.

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### ABSTRACT

The kidney is one of the crucial organs to maintain proper homeostasis in the body, and develop nephroprotective agents to retain kidney health and an absolute requisite. Since plant-based medicine gained importance due to fewer side effects and more safety, a herbal approach to develop nephroprotective agents is still necessary. Our present investigation is aimed to screen the antioxidant and nephroprotective activity of *Alphonsea sclerocarpa* leaves. Wistar rats were used in the current paracetamol-induced nephrotoxicity model through standard experimental procedures. N-acetyl cysteine (NAC) is taken as standard, the flavonoid-rich fraction of *A. sclerocarpa* was given to the animals in two doses (200 and 400 mg/kg body weight) for seven days. After the treatment, blood samples were collected from all the animals and sacrificed for histopathological studies. To support this investigation, phytochemical screening, total phenolic, flavonoid, tannin contents were also determined. The antioxidant activity studies by DPPH assay, NO free radical scavenging assay, and total antioxidant assay were also screened using standard protocols. Results unveil that the plant is rich in polyphenolic contents, and the flavonoid-rich fraction has statistically significant ( $P < 0.001$ ) nephroprotective activity in a dose-dependent manner compared to the standard N-acetyl cysteine. The biochemical and histological studies were also conducted in connection with the titled activity and found significant to improve the abnormal blood parameters and enzymatic and non-enzymatic antioxidants in the kidney tissue. To wrap up, all the biochemical, behavioral, and histological data declared that the flavonoid-rich fraction of *A. sclerocarpa* is more potent among all the extracts to protect against oxidative stress and subsequent improvement of the physiological parameters of the kidney. The antioxidant property of the phytochemicals present in the extract may be responsible for the affirmed activity.

### INTRODUCTION

*Alphonsea sclerocarpa* belongs to the family Annonaceae is a rarely distributed tree in tropical Asian countries.<sup>[1]</sup> Traditionally, *Alphonsea* plants are famous for treating infections, urinary problems, kidney stones, etc.<sup>[2]</sup> Phytochemical investigation of *A. sclerocarpa* leaves reported crotosparine sparsiflorine, laurotetanine, isoboldine, lirioidenine and petalinemethine. The bark contains lirioidenine, anonaine, norushinsunine, ushinsunine, stepharine, stepholidine, candicine, phenethyl-trimethylonium, and magnoflorine.<sup>[3]</sup> The plant is also reported to possess polyphenolic components such

as tannins, flavonoids. Pharmacologically *A. sclerocarpa* is proved to exhibit antioxidant,<sup>[4]</sup> antibacterial, antifungal,<sup>[5]</sup> anticancer,<sup>[6]</sup> anti-urolithiatic activity<sup>[7]</sup> in various experimental models.

Oxidative stress is causation for a myriad of chronic diseases, and foraging free radicals at the cellular level can help to contrive the stress-related physiological challenges<sup>[8]</sup> such as aging, Alzheimer's disease, Parkinsonism, diabetes, liver, kidney and heart diseases.<sup>[9]</sup> Atypical food and food habits, exposure to radiation, chemicals & toxic pollutants can increase the oxidative burden on the cell and disturb the normal physiology of the body.<sup>[10,11]</sup> Polyphenols are the metabolic end

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products of the plant and are considered to possess diverse health-promoting properties<sup>[12]</sup> and led to the extensive utilization of flavonoid and phenolic-rich food in the nutrient diet regime.<sup>[13]</sup>

The kidney is a vital organ that maintains electrolyte balance in the body. The nephritic tissue adjusts the volume of the water in the body through the formation of urine. The majority of the drug substances are metabolized to polar compounds and intended to excrete through the renal system.<sup>[14]</sup> Any endogenous or exogenous toxic compounds can damage the nephrons' normal physiology and lead to kidney failure. The filtration will be affected, and biomarkers such as serum creatinine and blood urea levels will indicate renal health.

Attributable to the paucity of proper nephroprotective drugs in contemporary medication, exploring plants for their protective effects, especially for nephroprotective activity, gained importance.<sup>[15]</sup> Few herbs such as turmeric, coriander, garlic, and black cumin were found successful in reassuring kidney health, and systematic endeavors are still essential to conquest to revive nephrotoxicity.<sup>[16]</sup>

In our current study, we have planned bioassay-guided fractionation to evaluate *In vitro* antioxidant and *In-vivo* nephroprotective activities of *A. sclerocarpa* leaves in paracetamol induced nephrotoxicity in rats.

## MATERIALS AND METHODS

### Plant Material

*A. sclerocarpa* was collected from the forest areas of Tirupati in February and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India and voucher specimen was (Pt 0332) preserved in the herbarium.

### Extraction and Fractionation

Five kilograms of the fresh leaves were shade dried at temperatures 25–30°C for 7 days. The dried leaves were powdered in a grinder. The methanolic extract of the leaves was prepared by subjecting to exhaustive extraction using Soxhlet apparatus for 72 hours with methanol. Then each of the extracts was filtered using cotton plugs followed by Whatmann No. 1 filter paper. The filtrates were then concentrated, dried under reduced pressure in the rotary evaporator, and lyophilized to get in powder form. The percentage of yield was calculated.

The methanolic extract was suspended in distilled water and then partitioned between various solvents such as n-hexane, Ethylacetate, Chloroform, n-butanol, methanol, and water successively to get respective fractions.<sup>[17]</sup>

### Experimental Animals

In this study, male Wistar rats were selected and were randomly grouped in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2°C and relative humidity of 30–70%.

A 12:12 light: day cycle was followed. All the animals were allowed free access to water and fed with a standard commercial pelleted rat diet.<sup>[18]</sup> All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional animal ethics committee (IAEC) before experimental studies (1292/ac/09/CPCSEA).

### Phytochemical Screening

The preliminary phytochemical screening of all the leaf extracts of *A. sclerocarpa* was performed according to the standard procedures.<sup>[19]</sup>

### Total Phenolic Content Estimation

#### *Preparation of Standard Gallic Acid for Calibration Curve:*

Concentrations ranging from 25–100 µg/mL of standard gallic acid solution was prepared by dissolving pure gallic acid in methanol; 5 mL of 10% Folin–Ciocalteu reagent and 4 mL of 7% Sodium carbonate were also added to make a final volume of 10 mL followed by the 30 minutes incubation at 40°C. The colored solution thus obtained was measured at 760 nm using a UV-visible spectrophotometer, and a calibration curve was plotted for the average values of the results obtained in triplicates.

#### *Estimation of Total Phenolic Content*

The total phenolic content of all the extracts of *A. sclerocarpa* was estimated by the Folin Ciocalteu method described by Singleton *et al.* (1965) with slight modifications. Various extracts ranging from 25–100 µg/mL concentration were prepared, and the total phenolic content was estimated as described above. The total phenolic content of the extracts was expressed as mg of gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g).<sup>[20]</sup>

### Total Flavonoid Content Estimation

The flavonoids content for *A. sclerocarpa* leaves was determined by the AlCl<sub>3</sub> method using quercetin as standard. 125 µL of the extract solution is added to 75 µL of a 5% NaNO<sub>2</sub> solution. After 6 minutes, 150 µL of 10% AlCl<sub>3</sub> was added, followed by 5 minutes of incubation. 750 µL of 1M NaOH solution was added to this mixture, diluted the final volume to 2500 µL with distilled water, and incubated for 15 minutes to get the pink color. The absorbance was measured at 510 nm. Using the calibration curve, the total flavonoid content was expressed as µg of quercetin equivalents per mg dry matter (µg QE/ mg dry weight). All the experiments were run in triplicate. The mean values and standard deviations were calculated.<sup>[21]</sup>

### Total Tannin Content Estimation

The tannin content for *A. sclerocarpa* leaves was determined by Broadhurst *et al.* (1978) with slight modification, taking tannic acid as a standard. 400 µL of the extract solution is added to 3 mL of vanillin (4% in methanol) solution and 1.5 mL of concentrated hydrochloric acid followed by 15 minutes of incubation. The absorbance was read at 500 nm, and the total condensed tannin content was

expressed as  $\mu\text{g}$  of tannic acid equivalents per mg dry matter ( $\mu\text{g TAE}/\text{mg dry weight}$ ). All the experiments were run in triplicate. The mean values and standard deviations were calculated.<sup>[22]</sup>

### In-vitro Antioxidant Activity

#### 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay

In DPPH assay, 1 ml of different concentrations (25–200  $\mu\text{g}/\text{mL}$ ) of *A. sclerocarpa* extracts were added to the reference solution (0.004% in methanol) in test tubes. The tubes were incubated in the dark for 30 minutes at room temperature; absorbance of the reaction mixture was measured in UV-visible spectrophotometer (Labindia 3000+) at 517 nm. Ascorbic acid was used as standard. Methanol replacing the extract/ascorbic acid served as control (i.e., 1-mL of methanol + 3 mL of DPPH radical solution). Inhibition of DPPH radicals (%) was calculated, and  $\text{IC}_{50}$  value was determined.<sup>[23]</sup>

$$\% \text{ scavenging activity} = \frac{(A_{\text{control}} - A_{\text{test}})}{A_{\text{control}}} \times 100$$

#### NO Free Radical Scavenging Assay

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which the use of Griess Ilosvoy reaction can determine. 2 mL of 10 mM sodium nitroprusside in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of *A. sclerocarpa* extracts at various concentrations, and the mixture was incubated at 25°C for 150 minutes. 0.5 mL was taken out from the incubated mixture and added into 1.0 mL sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 minutes. Finally, 1.0 mL naphthethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 minutes.<sup>[24]</sup> Standard Ascorbic acid is taken for the comparison, and the absorbance at 540nm was measured with a spectrophotometer and percentage inhibition was calculated as

$$\% \text{ NO radical scavenging activity} = \frac{(A_{\text{control}} - A_{\text{test}})}{A_{\text{control}}} \times 100$$

#### Total Antioxidant Activity

Total antioxidant activity for *A. sclerocarpa* leaves was determined by the phosphomolybdenum method. 0.1 mL of various concentrations of plant extracts (25–200  $\mu\text{g}/\text{mL}$ ) were added to 1ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). After an incubation of 90 minutes at 95°C, samples were cooled to room temperature, and the absorbance of the mixture was measured at 695 nm using a UV Visible spectrophotometer (Labindia 3000+). Ascorbic acid was used as standard. 0.1 mL of methanol was used as blank.<sup>[25]</sup>

### Acute Toxicity Studies

Acute oral toxicity study of a flavonoid-rich fraction of *A. sclerocarpa* was carried according to OECD-425 guidelines. A dose of 2000 mg/kg, body weight was administered orally to the first rat, and this rat was observed for mortality and clinical signs like aggressiveness, restlessness, sedation tremor, ataxia, paralysis, convulsion, prostration, unusual locomotion, etc. for the first hour, then hourly for 3 hours and, finally periodically until 48 hours (short term toxicity). The animal was survived, and then four additional rats were orally administered at dose 2000 mg/kg, sequentially at 48-hours intervals. All the experimental animals were maintained under close observation for 14 days (long-term outcomes), and the number of rats that died within the study period was noted. The 50% lethal dose ( $\text{LD}_{50}$ ) value was calculated.<sup>[26]</sup>

### Nephroprotective Assay

The animals were divided into five groups of six animals in each group with overnight fasting (Table 1). Group, I served as control and fed orally with normal saline daily for 7 days. Group II rats were pre-treated orally with normal saline for 7 days followed by paracetamol (2gm/kg) orally on the sixth day. Group III animals were treated orally with 140 mg/kg of N-Acetyl cysteine (NAC) prepared in normal saline solution for 7 days followed by paracetamol (2 gm/kg) orally on the sixth day. Group IV and V received test doses in low (200 mg/kg) and high (400 mg/kg) concentrations of flavonoid-rich fraction of *A. sclerocarpa* leaf for 7 consecutive days followed by paracetamol (2 gm/kg) orally on the sixth day. After 48 hours of paracetamol treatment, blood was collected by cardiac puncture after anesthetization for biochemical parameters and removed both the kidneys for histopathological studies.<sup>[27,28]</sup>

### Biochemical Analysis

The serum was separated from the blood by centrifuging at 4000 rpm and frozen, followed by the biochemical analysis for the levels of SGPT, SGOT, alkaline phosphatase (ALP), total protein, and total total protein bilirubin and triglycerides using an autoanalyzer.

**Table 1:** Grouping the animals to screen nephroprotective assay

Treatment Group	Pre-treatment	Nephrotoxic inducer
Group-I Normal control	Normal saline solution	Normal saline solution
Group-II Disease control	Normal saline solution	2 gm/kg Paracetamol
Group-III Standard	NAC (140 mg/kg)	2 gm/kg Paracetamol
Group-IV Test dose (low)	<i>A. sclerocarpa</i> (200 mg/kg)	2 gm/kg Paracetamol
Group-V Test dose (high)	<i>A. sclerocarpa</i> (400 mg/kg)	2 gm/kg Paracetamol



### Determination of Antioxidant Enzyme Levels

A kidney homogenate solution was prepared using 50 mM tris-HCl (pH 7.4) to a final concentration of 10% (w/v), followed by centrifuging at high rpm (5000) for 10 minutes at 4°C. Total protein content, Malondialdehyde (MDA) level, superoxide dismutase (SOD), catalase (CAT), and free glutathione (GSH) levels were estimated using commercially available kits and standard protocols.<sup>[29]</sup>

### Histopathological Studies of Kidney

10% neutral-buffered formalin solution was used for preserving the kidney for one day at room temperature. The paraffin-fixed kidney tissue was dissected to 5 µm size and stained with hematoxylin and eosin and observed under light microscopy for histopathological examination.

### Statistical Studies

All the values are expressed mean ± SEM; n=6 and studied using one-way ANOVA followed by Dunnett's test, P<0.01 is considered as statistically significant.

## RESULTS

### Percentage Yield

The percentage yield was calculated and depicted in Table 2.

### Phytochemical Screening

Preliminary phytochemical screening of the extracts revealed that the plants are rich in various phytochemicals listed in Table 3.

### Total Phenolic Content Estimation

A calibration curve was plotted by the absorbance and concentrations (mg/mL) using prepared dilutions. The

regression analysis was performed, and the resulting equation was  $Abs = y = 6.827x + 0.2228$ . The coefficient of determination for standard curves was greater than 0.99 ( $R^2 = 0.9918$ ). Thus, the calculated straight line could explain more than 99% of the experimental data (Fig. 1). The total phenolic content of the extracts was calculated and expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). Where y is the absorbance at 760 nm and x is the total phenolic content in the extracts of *A. sclerocarpa*. The values vary from  $52.3 \pm 1.35$  mg GAE/g for the n-hexane extract to  $115.18 \pm 1.87$  mg GAE/g for the methanol extract (Table 4 & Fig. 2) lower and highest content values.

### Total Flavonoid Content Estimation

The total flavonoid content profile of the plant extracts was established through the colorimetric method using  $AlCl_3$ . A calibration curve ( $y = 7.6262x + 0.0831$ ,  $R^2 = 0.9953$ ) was plotted using various concentrations of standard quercetin (0–100 µg/mL) and expressed in quercetin equivalents (QE) per gram (Fig. 3). The total flavonoid content of the extracts was calculated and expressed as mg quercetin equivalents (QE) per gram of sample in dry weight (mg/g). Where y is the absorbance at 760 nm and x is the total flavonoid content in the extracts of *A. sclerocarpa* were depicted in Table 5 and Fig. 4. The results show that methanolic fraction is found to possess high flavonoid content  $28.37 \pm 2.05$  mg QE/mg dry weight followed by the n-butanol extract.

### Total Condensed Tannin Content

The total tannin content of the plant extracts was determined using the Vanillin-HCl colorimetric method.

Table 2. Percentage yield of *A. sclerocarpa*

Extract	n-hexane extract	Ethyl acetate extract	Chloroform extract	n-butanol extract	Methanol extract	Water extract
Percentage yield	2.42	5.76	4.17	9.32	12.39	3.45

Table 3: Preliminary phytochemical screening results of prepared extracts

Phytochemicals	n-hexane extract	Ethyl acetate extract	Chloroform extract	n-butanol extract	Methanol extract	Water extract
Alkaloids	+	+	+	+	+	+
Flavonoids	-	+	+	+	+	+
Terpenoids	+	+	+	-	-	-
steroids	+	+	-	-	-	-
Saponins	-	-	-	-	+	+
Tannins	-	-	-	+	+	+
Proteins	+	-	-	-	-	+
Carbohydrates	-	-	-	-	-	+

(+) present, (-) absent

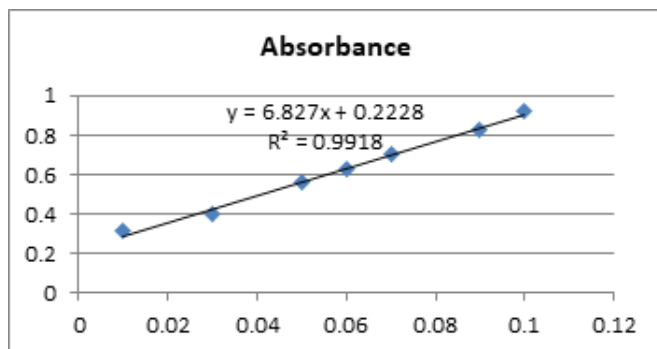


Fig. 1: Calibration curve for Ascorbic acid

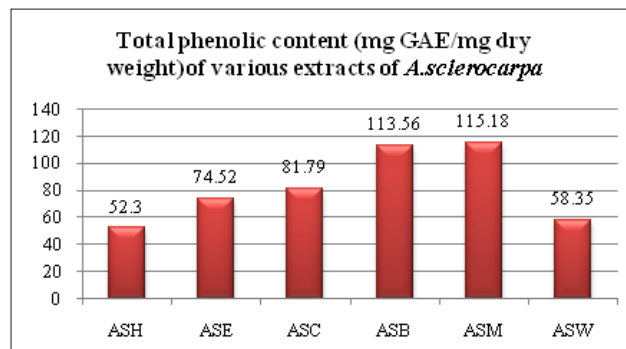
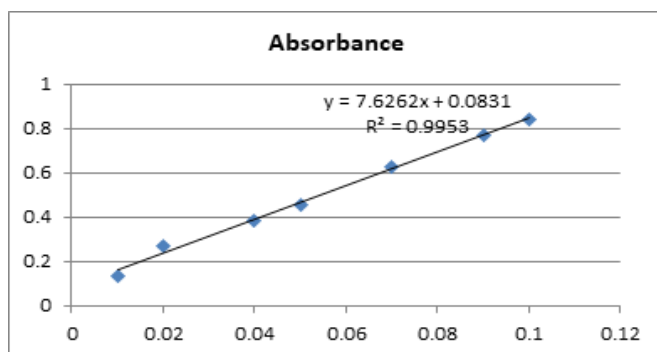
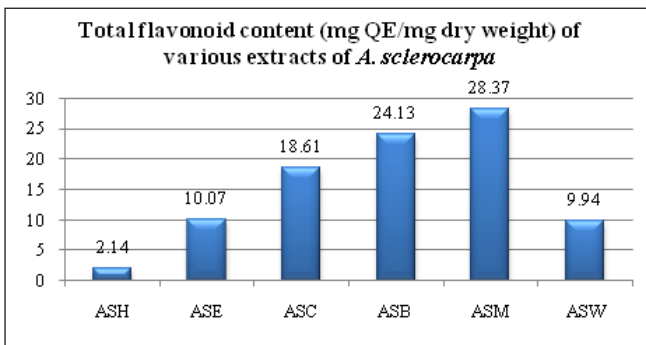
Fig. 2: Total phenolic content of various extracts of *A. sclerocarpa*

Fig. 3: Calibration curve for quercetin

Fig. 4: Total flavonoid content of various extracts of *A. sclerocarpa*Table 4: Total Phenolic content of *A. sclerocarpa* extracts

Extracts	mg GAE/mg dry weight
<i>A. sclerocarpa</i> n-hexane extract (ASH)	52.3 ± 1.35
<i>A. sclerocarpa</i> Ethyl acetate extract (ASE)	74.52 ± 0.65
<i>A. sclerocarpa</i> Chloroform extract (ASC)	81.79 ± 2.34
<i>A. sclerocarpa</i> n-butanol extract (ASB)	113.56 ± 1.95
<i>A. sclerocarpa</i> methanol extract (ASM)	115.18 ± 1.87
<i>A. sclerocarpa</i> water extract (ASW)	58.35 ± 2.04

Table 5: Total flavonoid content of *A. sclerocarpa* extracts

Extracts	mg QE/mg dry weight
<i>A. sclerocarpa</i> n-hexane extract (ASH)	2.14 ± 1.04
<i>A. sclerocarpa</i> Ethyl acetate extract (ASE)	10.07 ± 1.25
<i>A. sclerocarpa</i> Chloroform extract (ASC)	18.61 ± 1.67
<i>A. sclerocarpa</i> n-butanol extract (ASB)	24.13 ± 1.63
<i>A. sclerocarpa</i> methanol extract (ASM)	28.37 ± 2.05
<i>A. sclerocarpa</i> water extract (ASW)	9.94 ± 2.07

A calibration curve ( $y = 6.9973x + 0.1014$ ,  $R^2 = 0.9953$ ) was plotted using various concentrations of standard Tannic acid (0–100 µg/mL) and expressed in Tannic equivalents (TAE) per gram (Fig. 5). The total tannin content of the extracts was calculated and expressed as mg Tannic acid equivalents (TAE) per gram of sample in dry weight (mg/g). Where y is the absorbance at 760 nm and x is the total tannin content in the extracts of *A. sclerocarpa* in µg TAE per mg dry weight of the extract. Results disclosed that the methanolic fraction is lavished with high tannin content  $198.65 \pm 1.87$  mg TAE/mg dry weight followed by water extract  $124.57 \pm 1.05$  mg TAE/mg dry weight (Table 6 & Fig. 6).

### In-vitro Antioxidant Activity

#### DPPH Assay

Antioxidant potential of all the extracts of *A. sclerocarpa* leaves was evaluated using DPPH assay and observed in a dose-dependent manner compared to standard Ascorbic acid. A calibration curve ( $y = 6.7859x + 0.2233$ ,  $R^2 = 0.9928$ ) was plotted using various concentrations of standard Ascorbic acid (0–75 µg/mL) and expressed in percentage (Fig. 7). When compared to the standard Ascorbic acid ( $88.27 \pm 2.76$ ), Methanol ( $79.32 \pm 1.88$ ), and n-butanol ( $56.36 \pm 1.42$ ) fractions were found comparatively better inhibitors than other fractions (Table 7 & Fig. 8).



IC<sub>50</sub> values 30.45 and 55.32 µg/mL of methanol and n-butanol fractions were also comparable with the standard (22.67 µg/mL).

### NO Free Radical Assay

All the leaf fractions of *A. sclerocarpa* were evaluated for nitrite-free radical scavenging property. Percentage-free radical scavenging was plotted against the concentration of the extracts as shown in Fig. 9. The plants exhibited antioxidant activity by competing with oxygen to scavenge for the nitrite radical generated from sodium nitroprusside at physiological pH in an aqueous environment. The antioxidant activity increased with an increased polarity up to methanol in a dose-dependent manner. The maximum free radical scavenging activity was interpolated to give results, as shown in Table 8. *A. sclerocarpa* methanol fraction (77.34%) inhibited the nitrite radical efficiently as compared to the other fractions compared with standard ascorbic acid (81.73%) (Table 8). *A. sclerocarpa* had a maximal scavenging activity with a potent IC<sub>50</sub> value of 75 µg/mL followed by the n-butanol fraction (61.04 µg/mL) ascorbic acid is having 28.52 µg/mL.

### Total Antioxidant Activity

Phosphomolybdenum assay was used to determine the total antioxidant activity of the *A. sclerocarpa* leaf fractions. Ascorbic acid being as a reference standard for comparison (83.12 ± 1.54 mg TE/g), methanolic

fraction showed comparatively better total antioxidant activity than other fractions; methanolic leaf fraction of *A. sclerocarpa* (78.38 ± 1.77 mg TE/g), exhibited higher antioxidant activity followed by methanolic leaf fraction of *A. sclerocarpa* (Table 9 & Fig. 10). IC<sub>50</sub> values of the fractions were comparable with the standard 29.78 µg/mL and calculated as 38.39 & 59.22 µg/mL, respectively.

### Methanolic Leaf Fraction

The results show that the administration of flavonoid-rich fraction of *A. sclerocarpa* leaf was safe up to a dose of 2000 mg/kg. No aforementioned toxic symptoms or mortality were observed at this dose. Hence, the present study selected 1/10th and 1/5th of 2000 mg/kg, i.e., 200 mg/kg and 400 mg/kg as working doses.

### Nephroprotective Activity

The flavonoid-rich fraction of *A. sclerocarpa* leaves was assessed to nephroprotective activity in a paracetamol-induced nephrotoxicity rat model. Two doses (200 & 400 mg/kg) were studied for blood parameters such as urea, creatinine, and BUN levels in the serum and compared with the toxic and standard groups. From the results, it is found that the plant imparted significant improvement in the abnormal serum parameters. Paracetamol doubled the elevated levels of urea, creatinine, and BUN. The standard and treatment with the flavonoid-rich fraction of *A. sclerocarpa*, significantly (\*P<0.01) restored these levels (Table 10 and Fig. 11).

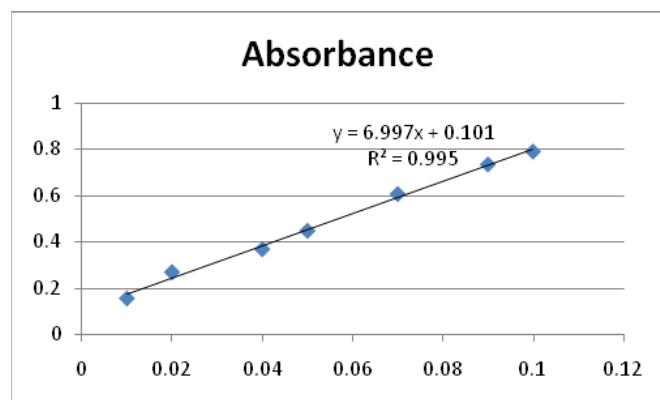


Fig. 5: Calibration curve for Tannic acid

Table 6: Total condensed tannin content of *A. sclerocarpa* extracts

Extracts	mg TAE/mg dry weight
<i>A. sclerocarpa</i> n-hexane extract (ASH)	1.02 ± 2.31
<i>A. sclerocarpa</i> Ethyl acetate extract (ASE)	24.24 ± 2.09
<i>A. sclerocarpa</i> Chloroform extract (ASC)	31.02 ± 0.38
<i>A. sclerocarpa</i> n-butanol extract (ASB)	35.43 ± 1.64
<i>A. sclerocarpa</i> methanol extract (ASM)	198.65 ± 1.87
<i>A. sclerocarpa</i> water extract (ASW)	124.57 ± 1.05

Table 7: DPPH Scavenging activity of *A. sclerocarpa* extracts with IC<sub>50</sub> values

Conc µg/ml	Percentage of inhibition						
	AA	ASH	ASE	ASC	ASB	ASM	ASW
10	44.18 ± 1.45	8.21 ± 1.34	12.87 ± 2.58	6.74 ± 0.47	32.75 ± 1.11	36.79 ± 1.29	15.11 ± 1.07
15	57.3 ± 0.13	9.98 ± 1.87	18.13 ± 1.46	8.02 ± 2.47	35.67 ± 2.74	54.43 ± 1.07	21.17 ± 0.37
25	65.47 ± 1.14	14.03 ± 1.91	22.57 ± 4.31	9.22 ± 0.42	40.12 ± 1.61	57.33 ± 1.64	24.39 ± 1.54
50	71.38 ± 1.22	16.14 ± 0.57	29.64 ± 1.74	11.71 ± 1.48	46.27 ± 1.49	65.71 ± 1.39	32.74 ± 1.39
75	88.27 ± 2.76	18.11 ± 1.65	39.41 ± 1.43	15.73 ± 0.62	56.36 ± 1.42	79.32 ± 1.88	47.21 ± 1.22
IC <sub>50</sub> µg/ml	22.67	220.14	130.09	174.57	55.32	30.45	78.12

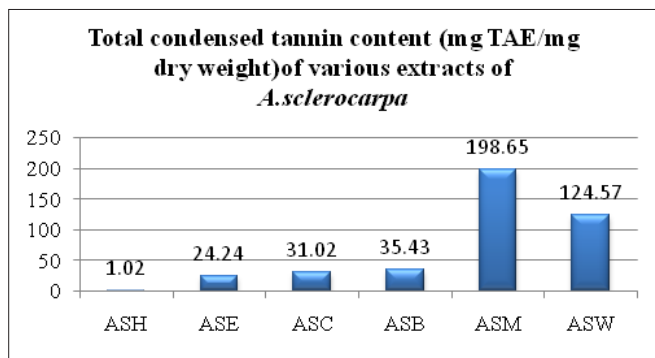


Fig. 6: Total condensed tannin content of various extracts of *A. sclerocarpa*

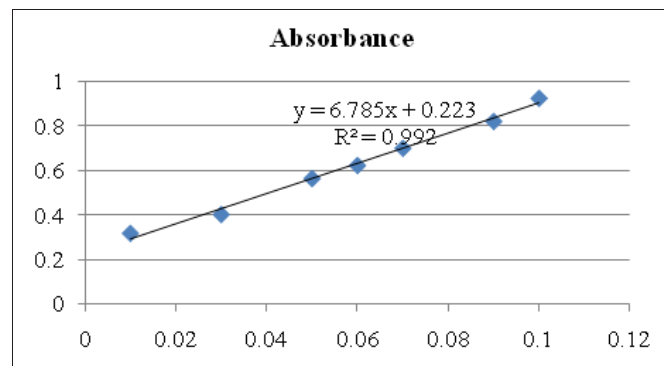


Fig. 7: Calibration curve for Ascorbic acid for DPPH assay

Table 8: NO free radical Scavenging activity of *A. sclerocarpa* extracts

Conc $\mu\text{g/ml}$	AA	ASH	ASE	ASC	ASB	ASM	ASW
10	34.28 $\pm$ 0.91	7.12 $\pm$ 1.26	8.2 $\pm$ 0.55	7.74 $\pm$ 1.43	31.3 $\pm$ 0.49	27.17 $\pm$ 0.33	14.38 $\pm$ 1.27
15	48.42 $\pm$ 0.25	8.21 $\pm$ 1.83	9.32 $\pm$ 1.08	8.89 $\pm$ 1.28	31.78 $\pm$ 0.45	42.97 $\pm$ 1.38	16.47 $\pm$ 1.58
25	44.48 $\pm$ 1.87	10.39 $\pm$ 1.22	10.67 $\pm$ 1.07	10.07 $\pm$ 1.17	38.76 $\pm$ 1.27	38.34 $\pm$ 1.26	19.94 $\pm$ 1.53
50	62.32 $\pm$ 1.24	12.01 $\pm$ 1.51	16.74 $\pm$ 0.44	14.35 $\pm$ 1.53	41.87 $\pm$ 1.39	54.04 $\pm$ 1.67	27.47 $\pm$ 1.35
75	81.73 $\pm$ 1.03	15.12 $\pm$ 1.33	21.17 $\pm$ 0.89	19.15 $\pm$ 1.59	57.23 $\pm$ 1.46	77.34 $\pm$ 1.28	36.23 $\pm$ 1.49
IC <sub>50</sub> $\mu\text{g/ml}$	28.52	378.15	215.91	254.93	61.04	38.01	117.17

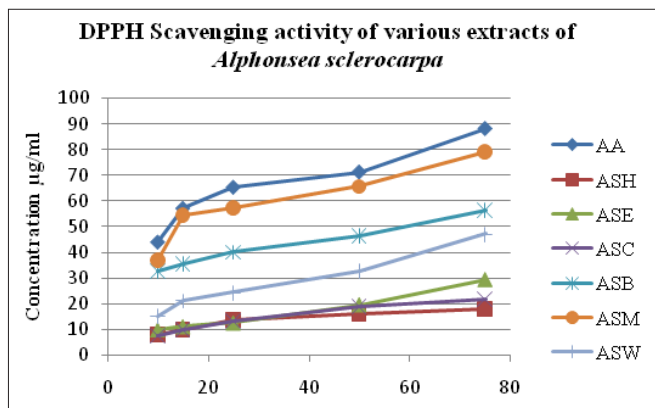


Fig. 8: DPPH scavenging activity of *A. sclerocarpa* extracts

Paracetamol treatment produced a significant ( $P < 0.01$ ) reduction in the enzymatic (superoxide dismutase, catalase, and Glutathione peroxidase) and non-enzymatic antioxidants (glutathione) as well as increased MDA levels in the kidney homogenate when compared to normal control indicating the possible oxidative damage to the renal system. The flavonoid-rich fraction of *A. sclerocarpa* reduced the elevated MDA levels caused by the paracetamol administration. Treatment with *A. sclerocarpa* upregulated the levels of both enzymatic and non-enzymatic antioxidant enzymes, which are statistically significant ( $P < 0.01$ ) to the control group Table 11 and Fig. 12. The 400 mg/kg dose exhibited better comparable activity with the N-Acetyl cysteine among the tested groups.

## Histopathological Studies

The nephroprotective activity of *A. sclerocarpa* leaves was supported by histopathological studies. Paracetamol treatment distorted the accustomed anatomy of the kidney tissue with random tubular degeneration. The appearance of protein matter and RBC in bowman's capsule, glomerular capillaries, and granular cast, along with inflammatory cell infiltration, indicates nephrotoxicity. The low dose (200 mg/kg) treatment with a flavonoid-rich fraction of *A. sclerocarpa* ameliorated the normal physiology of the tissue, and at higher doses (400 mg/kg), the treatment reversed the original architecture of the kidney. The histological features were articulated in Fig. 13.

## DISCUSSION

Flavonoids and tannins are the major polyphenolic compounds produced as metabolic end products of plants that help detoxify. They are considered powerful agents to neutralize the free radicals due to their ability to react with them and regarded as antioxidants, supporting the scavenging mechanism of the human body. Ample literature is available to support the protective effect of these polyphenolics to address various chronic and lifestyle disorders associated with stress. Since synthetic agents are associated with fewer side effects, naturally occurring antioxidants are preferred in nutraceuticals for promoting health.<sup>[30]</sup>

It is important to determine the antioxidant potential of the plants to understand their efficiency in



protecting against cellular stress. Numerous procedures were adopted to screen the antioxidant (*In vitro* & *In vivo*), such as DPPH assay, nitric oxide free radical assay, etc.<sup>[31]</sup>

The enzymes shape metabolites of xenobiotics with improved polarity and reduced therapeutic activity to facilitate excretion. Few of the metabolic end products of synthetic medicine (ex: N-acetyl-p-benzoquinone imine for

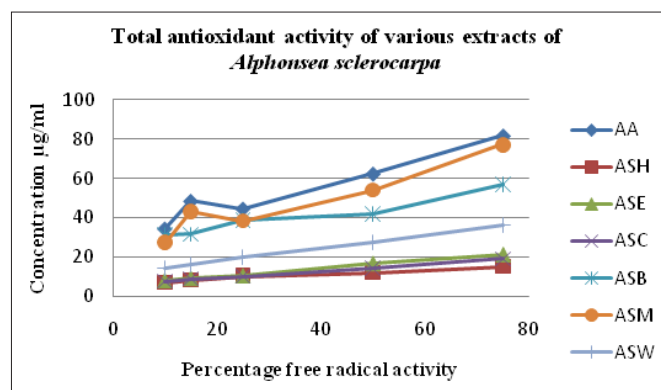


Fig. 9: NO free radical Scavenging activity of *A. sclerocarpa* extracts

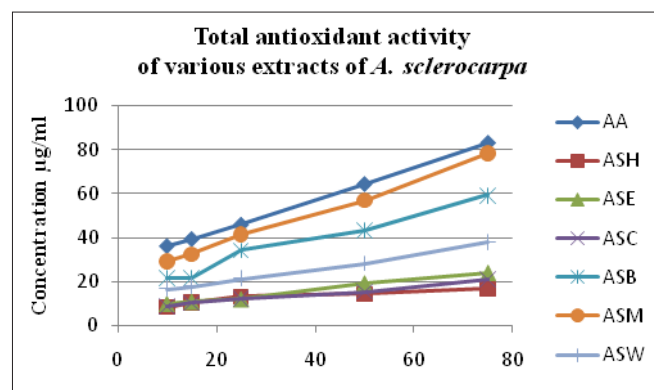
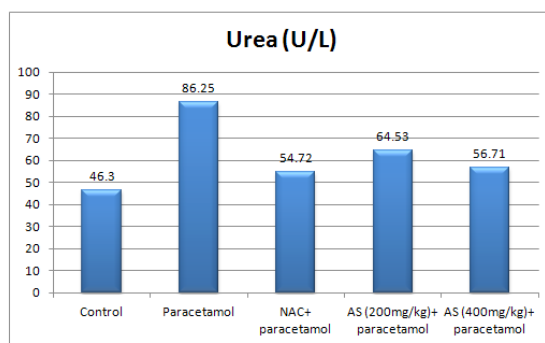


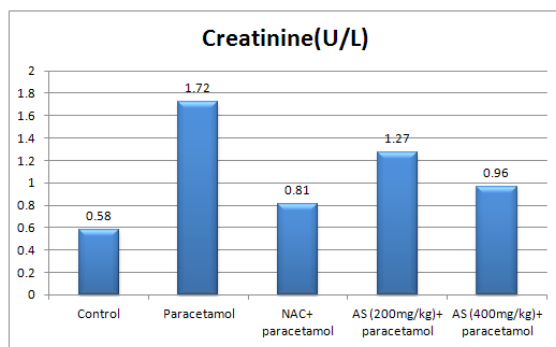
Fig. 10: Total antioxidant activity of *A. sclerocarpa* extracts

Table 9: Total antioxidant activity of *A. sclerocarpa* extracts

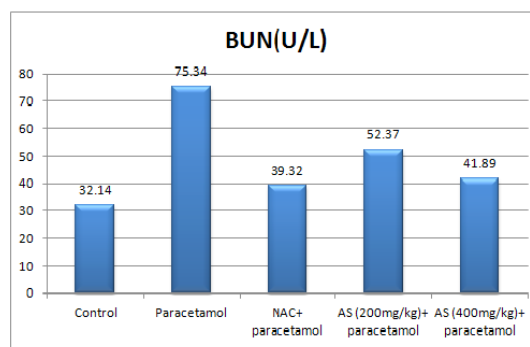
Conc µg/ml	AA	ASH	ASE	ASC	ASB	ASM	ASW
10	36.17 ± 0.84	8.22 ± 1.34	9.91 ± 1.28	8.47 ± 1.63	21.37 ± 1.96	28.84 ± 1.04	16.44 ± 1.08
15	39.39 ± 1.22	10.18 ± 1.39	10.87 ± 1.39	10.08 ± 1.27	21.48 ± 1.73	32.14 ± 0.54	17.89 ± 0.23
25	45.87 ± 1.36	13.08 ± 1.26	11.98 ± 1.27	12.09 ± 1.83	34.12 ± 1.65	41.17 ± 1.63	21.33 ± 0.74
50	64.38 ± 1.27	14.27 ± 1.38	19.07 ± 1.23	15.28 ± 1.97	43.17 ± 1.85	56.74 ± 1.32	28.14 ± 1.39
75	83.12 ± 1.54	16.54 ± 1.32	23.74 ± 1.65	21.08 ± 2.36	59.36 ± 1.72	78.38 ± 1.77	38.12 ± 1.07
IC <sub>50</sub> µg/ml	29.78	364.89	193.13	235.66	59.22	38.39	113.3



A



B

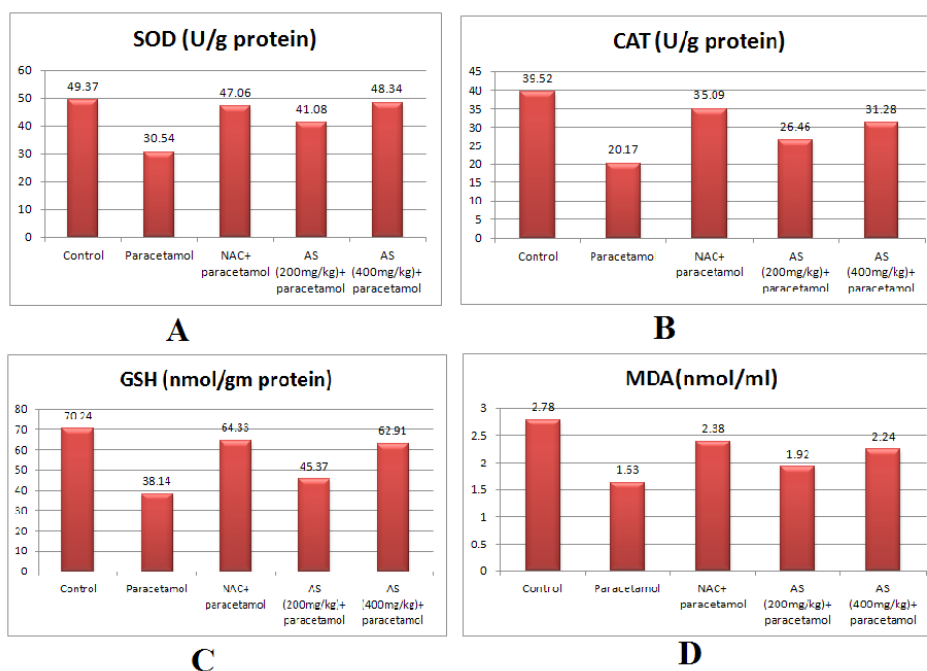


C

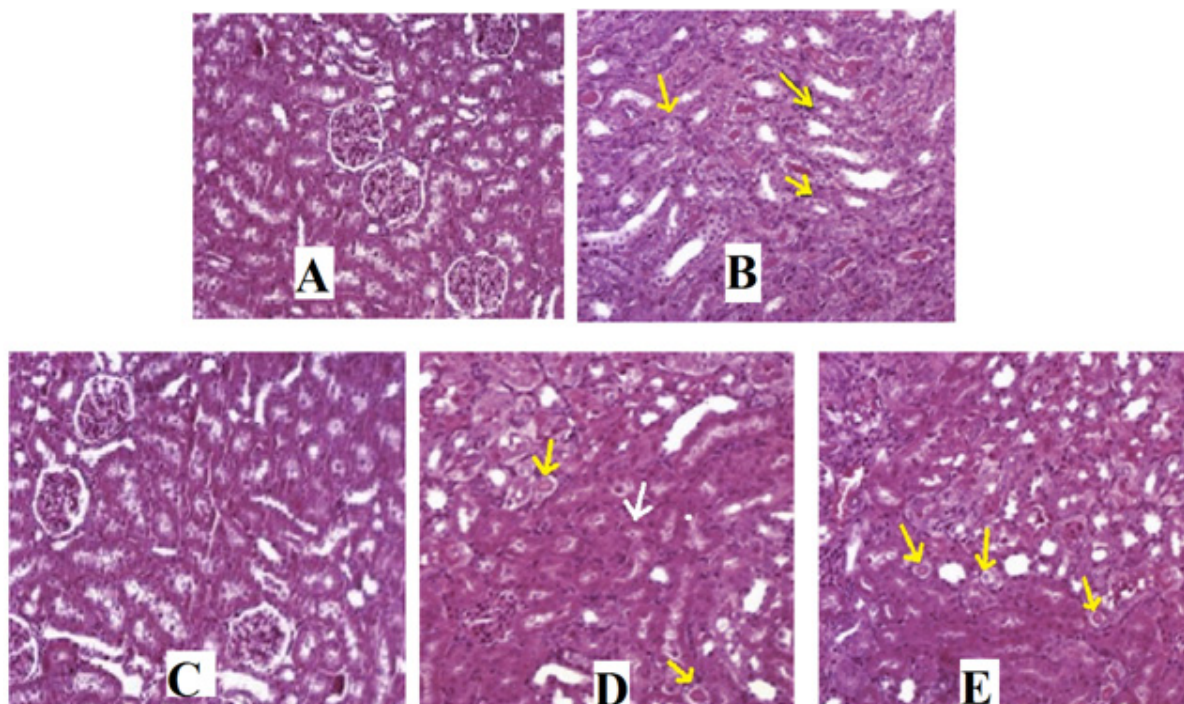
Fig. 11: Effect of *A. sclerocarpa* on serum biochemical parameters

paracetamol) are toxic to crucial organs such as the liver and kidney. The free radical load aggravates the condition further and causes nephrotoxicity. The diminished antioxidant enzymes in the treatment groups are due to the inability of the inbuilt scavengers of the body to defuse the elevated levels of reactive oxygen species.

*A. sclerocarpa* methanolic leaf extract demonstrated the highest antioxidant activity of other extracts. The antioxidant activity was assessed through DPPH assay, NO free radical scavenging assay, and total antioxidant assay. Phytochemical screening has revealed alkaloids, glycosides, flavonoids, tannins, terpenoids,



**Fig. 12:** Effect of *A. sclerocarpa* on antioxidant enzymes of kidney; A: SOD, B: CAT, C: GSH and D: MDA



**Fig. 13:** Histopathological figures showing the effect of *A. sclerocarpa* on kidney anatomy in paracetamol induced nephrotoxicity; A: Normal control, B: Paracetamol control, C: Standard, D: Low dose of AS, E: High dose of AS



**Table 10:** Biochemical parameters on paracetamol induced nephrotoxicity studies

S.No.	Treatment	Urea (Mg/dl)	Creatinine (Mg/dl)	BUN (Mg/dl)
1.	Control	46.3 ± 0.65	0.58 ± 0.33	32.14 ± 0.37
2.	Paracetamol	86.25 ± 0.62*	1.72 ± 1.04*	75.34 ± 1.14*
3.	NAC+ paracetamol	54.72 ± 1.04***	0.81 ± 0.97***	39.32 ± 1.61***
4.	AS (200 mg/kg) + paracetamol	64.53 ± 1.08**	1.27 ± 0.32**	52.37 ± 0.82**
5.	AS (400 mg/kg) + paracetamol	56.71 ± 0.95***	0.96 ± 0.14***	41.89 ± 0.67***

**Table 11:** Effect of on kidney enzymatic and non enzymatic antioxidants

Treatment	SOD (U/gm protein)	CAT (U/gm protein)	GSH (nmol/gm protein)	MDA (nmol/ml)
Control	49.37 ± 1.05	39.52 ± 0.87	70.24 ± 1.12	2.78 ± 1.07
Paracetamol	30.54 ± 0.64*	20.17 ± 1.09*	38.14 ± 0.17*	1.63 ± 0.54*
NAC + paracetamol	47.06 ± 0.72***	35.09 ± 0.98***	64.33 ± 0.08***	2.38 ± 0.02***
AS (200 mg/kg) + paracetamol	41.08 ± 0.08***	26.46 ± 0.11**	45.37 ± 1.33**	1.92 ± 0.65***
AS (400 mg/kg) + paracetamol	48.34 ± 0.67***	31.28 ± 1.44***	62.91 ± 0.93***	2.24 ± 0.74***

Values are mean ± SEM; n=6. \*P<0.01 when compared to control \*\*P<0.05, \*\*\*P<0.01 when compared to paracetamol control (one way ANOVA followed by Dunnett's test)

sterols, etc. The nephro-protective activity of the flavonoid-rich fraction of *A. sclerocarpa* was established *In vivo* using paracetamol-induced nephrotoxicity in a rat model by assessing biochemical parameters of blood, enzymatic and non-enzymatic antioxidants of the kidney followed by histopathological support. The polyphenolic compounds present in the fraction, such as phenols, flavonoids, and tannins, may directly relate to the reported nephroprotective activity. Further research is necessary to isolate nephroprotective molecules from this plant to understand the molecular mechanism involved in the titled activity.

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