

## “Exploring The Traditional Use of *Agave Americana* for Kidney Health: Scientific Validation of Its Nephroprotective Activity”

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Date of Submission: 07-05-2026

Date of Acceptance: 18-05-2026

### Abstract

**Aim:** The study aimed to evaluate the phytochemical composition, antioxidant activity, and nephroprotective potential of the methanolic extract of *Agave americana* against paracetamol-induced nephrotoxicity in Wistar rats. **Materials and Methods:** The dried plant material of *Agave americana* was extracted using methanol by Soxhlet extraction. Preliminary phytochemical screening, total phenolic content (TPC), and total flavonoid content (TFC) estimations were carried out using standard methods. Antioxidant activity was evaluated by DPPH radical scavenging assay. Nephroprotective activity was assessed in paracetamol-induced nephrotoxic rats by estimating serum creatinine, blood urea nitrogen (BUN), urine volume, body weight, and histopathological changes. **Results:** Phytochemical screening revealed the presence of flavonoids, phenolic compounds, glycosides, alkaloids, and saponins in the methanolic extract. The extract exhibited high total phenolic content (64.6 mg GAE/g) and total flavonoid content (55.2 mg RE/g). In the DPPH assay, the extract showed significant antioxidant activity with an IC<sub>50</sub> value of 32.75 µg/ml. In vivo studies demonstrated that the extract significantly reduced serum creatinine and BUN levels, normalized urine volume, and improved renal histopathology in paracetamol-treated rats. The higher dose (500 mg/kg) produced greater nephroprotective activity comparable to silymarin. **Conclusion:** The methanolic extract of *Agave americana* possesses significant antioxidant and nephroprotective activities, which may be attributed to its phenolic and flavonoid constituents. The findings support its traditional medicinal use and suggest its potential as a natural therapeutic agent for the management of nephrotoxicity.

**Keywords:** *Agave americana*, Nephroprotective activity, Paracetamol, DPPH assay and Antioxidant

### I. Introduction

Kidneys are essential for regulating fluid balance, electrolyte homeostasis, acid-base balance, and excreting metabolic waste. Renal damage from drugs, toxins, or oxidative stress can result in nephrotoxicity (Shibata et al., 2011), with paracetamol being a notable culprit when taken in excess, producing severe renal and hepatic toxicity through reactive metabolites and oxidative stress (Robson, 2014). Oxidative stress, characterized by excessive reactive oxygen species production, damages cellular components, leading to renal dysfunction. Antioxidants are crucial in protecting tissues from oxidative damage, promoting interest in medicinal plants rich in antioxidant phytoconstituents for their nephroprotective properties (Halliwell, 1995). *Agave americana* is a medicinal plant known for its therapeutic uses, including anti-inflammatory, antimicrobial, antioxidant (Davis, 2022), and wound-healing properties (Misra & Varma, 2017). It contains bioactive constituents like flavonoids, phenolic compounds, saponins, and glycosides, which have significant pharmacological effects (Leal et al., 2015). Research indicates that these phytochemicals may help prevent tissue injury caused by oxidative stress. This study investigates the phytochemical composition, antioxidant activity, and nephroprotective potential of the methanolic extract of *Agave americana* in rats with paracetamol-induced nephrotoxicity.

### II. Materials and methods

#### 2.1 Chemical used

All chemicals in the study were of analytical grade from standard suppliers. Methanol, Folin–Ciocalteu reagent, gallic acid, aluminum chloride, and rutin were used. DPPH and ascorbic acid were for antioxidant studies. Paracetamol, silymarin, and normal saline was the control.

## 2.2 Procurement of Plant Material

*Agave americana* plant material is obtained from its natural habitat, with rigorous identification and authentication (Erickson & Halford., 2020).

## 2.3 Extraction of Plant Material by Soxhlation Process

The dried and coarsely powdered plant material of *Agave americana* is subjected to extraction using a Soxhlet apparatus, a widely employed method for the efficient recovery of bioactive compounds. A suitable solvent, such as methanol, is selected based on the polarity of the phytochemicals present in the plant (Borodulin et al., 2020).

## 2.4 Phytochemical test of *Agave americana*

In a solubility study, a drug's capacity to dissolve in a particular solvent under predetermined parameters such as temperature, pH, and agitation is evaluated. This process will be repeated with various solvents at room temperature, and the amount of solvent in which the drug appears to dissolve will form the basis for classifying the drug's solubility (such as freely soluble, slightly soluble, very slightly soluble, or practically insoluble) according to standard descriptive solubility terms (Shegute & Wasihun, 2020).

## 2.5 Quantitative Estimation of Phytoconstituents

### 2.5.1 Total phenols content

The total phenolic content of the methanolic extract of *Agave americana* is determined using the Folin-Ciocalteu colorimetric method. The total phenolic content of the extract is calculated from the calibration curve and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g) (Büyüktuncel et al., 2014).

### 2.5.2 Total Flavonoid Content

The total flavonoid content of *Agave americana* extract is determined using the aluminum chloride colorimetric method. The total flavonoid content is calculated from the calibration curve and expressed as milligrams of rutin equivalents per gram of dry extract (mg RE/g). (Sirivibulkovit et al., 2018).

## 2.6 Antioxidant activity by the DPPH test

The DPPH• assay is widely used to evaluate the free radical scavenging potential of plant extracts. It relies on the reduction of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical by hydrogen or electron donors, which results in a decrease in its characteristic deep violet color. This change in absorbance reflects the ability of the test sample to donate electrons or hydrogen atoms, thereby neutralizing free radicals and preventing oxidative damage. (Jadhav et al., 2011).

## 2.7 *In-vivo* Nephroprotective activity in rats

The study is approved by the Institutional Animal Ethics Committee and accordance with its guidelines for the care and use of laboratory animals. 30 mature Wistar albino rats, weighing 150-200 g, are obtained from the Pinnacle Biomedical Research Institute (Sujana et al., 2021).

### Experimental protocol

For the investigation, male Wistar rats weighing between 150 and 200 g were employed. The animals were split up into five groups (n = 6) and the experiment ran for 14 days. Normal Control Normal saline (p.o.) for Group I, Disease Control Group II Paracetamol (750 mg/kg, p.o.), Group III Standard Group Silymarin (40 mg/kg, p.o.) + Paracetamol (750 mg/kg, p.o.), Paracetamol (750 mg/kg, p.o.) Group IV Test Group I + *Agave americana* extract (p.o., 250 mg/kg), Group V Test Group II *Agave americana* extract (500 mg/kg, p.o.) plus paracetamol (750 mg/kg, p.o.) (Shelke et al., 2009).

### 2.7.1 Blood samples for biochemical estimation

Serum biochemical parameters were subsequently measured promptly using commercially available Erba diagnostic kits, following the manufacturer's instructions to ensure accuracy and reliability of the results.

## 2.8 Analysis of general parameters

### 2.8.1 Analysis of urine

The collected urine samples were then analysed for glucose and protein concentrations using standard commercially available diagnostic kits, following the manufacturer's protocols to ensure precise and reliable quantification.

### 2.8.2 Estimation of Body weight

Food and water were removed prior to weighing, and each animal was weighed individually. The recorded body weights were carefully documented for further analysis.

### 2.8.3 Serum Creatinine and blood urea nitrogen (BUN) analysis

Plasma levels of blood urea nitrogen (BUN) and creatinine were measured to assess renal function. Serum creatinine (SCR) is commonly used as a marker of renal function, whereas BUN reflects glomerular filtration rate (GFR) only poorly in cases of mild to moderate renal impairment.

## 1.4 Histopathological Examination

In histopathological tissue, liver tissue was processed by standard histological procedures, embedded in paraffin, and sectioned into thin slices (approximately 5 µm thick) using a microtome. The sections were then stained with hematoxylin and

eosin (H&E) and examined under a light microscope for histopathological changes (Taqi et al., 2018).

### III. Results

#### 3.1 Plant material

**Table 1: Percentage yield of plant material**

S. No	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1.	<i>Agave americana</i>	Methanol	450	26.32	5.84

#### 3.2 Phytochemical Test

**Table 2: Phytochemical study**

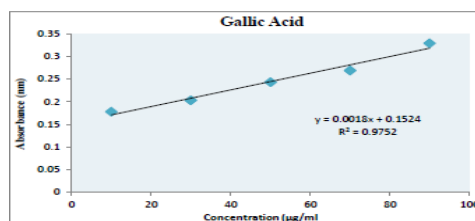
Sr.no	Experiment	Pet. Ether extract
1.	Alkaloids	Present
2.	Glycoside	Present
3.	Carbohydrates	Absent
4.	Flavonoids	Present
5.	Tannin and Phenolic Compounds	Present
6.	Saponin	Present
7.	Test for Triterpenoids and Steroids	Absent

#### 3.3 Quantitative Estimation of Phytoconstituents

##### 3.3.1 Total Phenolic content (TPC) estimation

**Table 3: Standard table for Gallic acid**

S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	10	0.178
2.	30	0.203
3.	50	0.243
4.	70	0.269
5.	90	0.329



**Figure 1: Std curve of gallic acid**

**Table 4: Total Phenolic content of *Agave americana***

S. No	Absorbance	TPC in mg/gm equivalent of Gallic Acid
1	0.185	64.6 mg/gm
2	0.225	
3	0.241	

##### 3.3.2 Total Flavonoids content (TFC) estimation

**Table 5: Standard table for Rutin**

S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	10	0.156
2.	30	0.184
3.	50	0.218
4.	70	0.244
5.	90	0.282

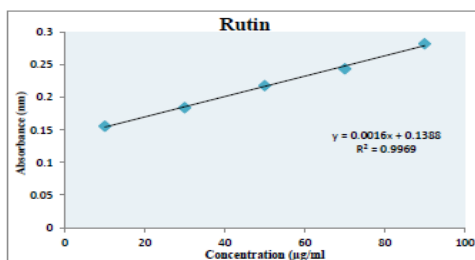


Figure 2: Std curve of rutin

Table 6: Total Flavonoid content in *Agave Americana* extract

S. No	Absorbance	TFC in mg/gm equivalent of Rutin
1	0.163	55.2 mg/gm
2	0.195	
3	0.225	

### 3.4 Anti-Oxidant Activity

#### 3.4.1 DPPH 2, 2- diphenyl-1-picryl hydrazyl Assay

Table 7: DPPH radical scavenging activity of Std. Ascorbic acid

Concentration (µg/ml)	Absorbance	% Inhibition
10	0.499	49.949
30	0.464	53.460
50	0.356	64.292
70	0.292	70.712
90	0.241	75.827
<b>Control</b>	<b>0.997</b>	
<b>IC5012.76</b>		

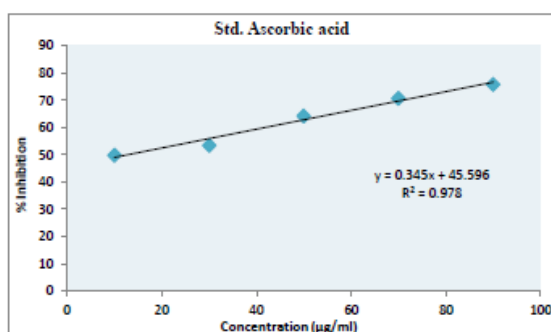


Figure 3: DPPH radical scavenging activity of Std. Ascorbic acid

Table 8: DPPH radical scavenging activity of methanol extract of *Agave americana*

Concentration (µg/ml)	Absorbance	% Inhibition
10	0.545	45.336
30	0.488	51.053
50	0.475	52.357
70	0.439	55.967
90	0.395	60.381
<b>Control</b>	<b>0.997</b>	
<b>IC50</b>	<b>32.75</b>	

### 3.5 Paracetamol induced Nephrotoxicity Model

#### 3.5.1 Estimation of urine volume

Table 9: Urine volume

Groups	Urine Volume (ml)
<b>Group I</b> Normal Control	6.16±1.03
<b>Group II</b> Negative Control Paracetamol (750 mg/kg bwt)	10.98±1.07
<b>Group III</b> Standard Silymarin (40 mg/kg)	7.23±0.54
<b>Group IV</b> Low dose of <i>Agave americana</i> (250 mg/kg)	8.79±1.04
<b>Group V</b> High dose of <i>Agave americana</i> (500 mg/kg)	7.99±0.31

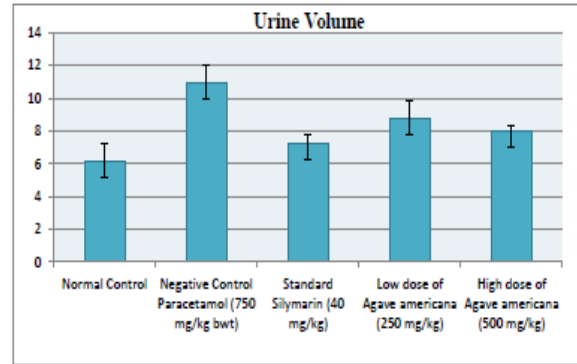


Figure 4: Urine volume

#### 3.5.2 Estimation of Body weight

Table 10: Body weight

Groups	Body Weight
<b>Group I</b> Normal Control	253.13±0.257
<b>Group II</b> Negative Control Paracetamol (750 mg/kg bwt)	263.15±2.34
<b>Group III</b> Standard Silymarin (40 mg/kg)	255.95±0.248
<b>Group IV</b> Low dose of <i>Agave americana</i> (250 mg/kg)	259.90±0.221
<b>Group V</b> High dose of <i>Agave americana</i> (500 mg/kg)	257.20±2.08

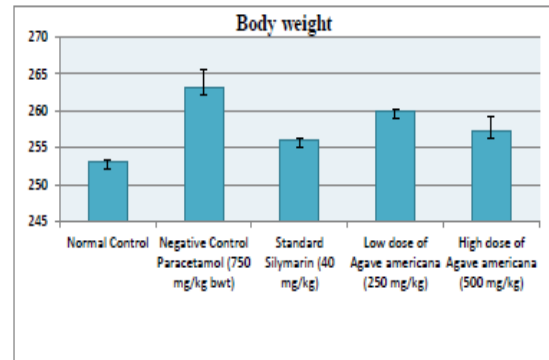


Figure 5: Body weights

#### 3.5.3 Estimation of Serum Creatinine

Table 11: Serum Creatinine

Groups	Serum Creatinine
<b>Group I</b> Normal Control	1.056±0.046
<b>Group II</b> Negative Control Paracetamol (750 mg/kg bwt)	1.85±0.018
<b>Group III</b> Standard Silymarin (40 mg/kg)	1.086±0.022
<b>Group IV</b> Low dose of <i>Agave americana</i> (250 mg/kg)	1.57±0.015
<b>Group V</b> High dose of <i>Agave americana</i> (500 mg/kg)	1.34±0.020

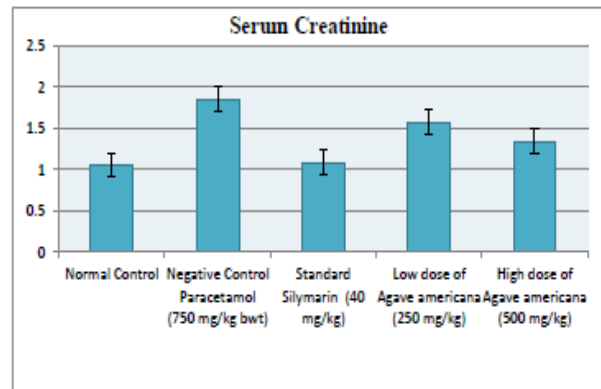


Figure 7: Serum Creatinine

### 3.5.4 Estimation of Serum Blood urea nitrogen (BUN)

Table 12: Serum Blood urea nitrogen

Groups		Serum Blood Urea Nitrogen
Group I	Normal Control	38.73±3.053
Group II	Negative Control Paracetamol (750 mg/kg bwt)	86.51±0.445
Group III	Standard Silymarin (40 mg/kg)	39.25±0.231
Group IV	Low dose of <i>Agave americana</i> (250 mg/kg)	50.15±0.125
Group V	High dose of <i>Agave americana</i> (500 mg/kg)	45.27±0.105

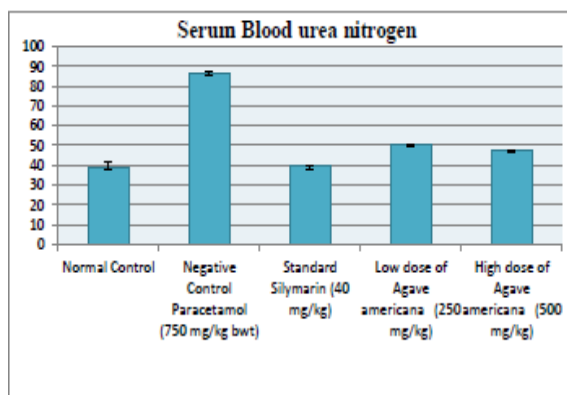
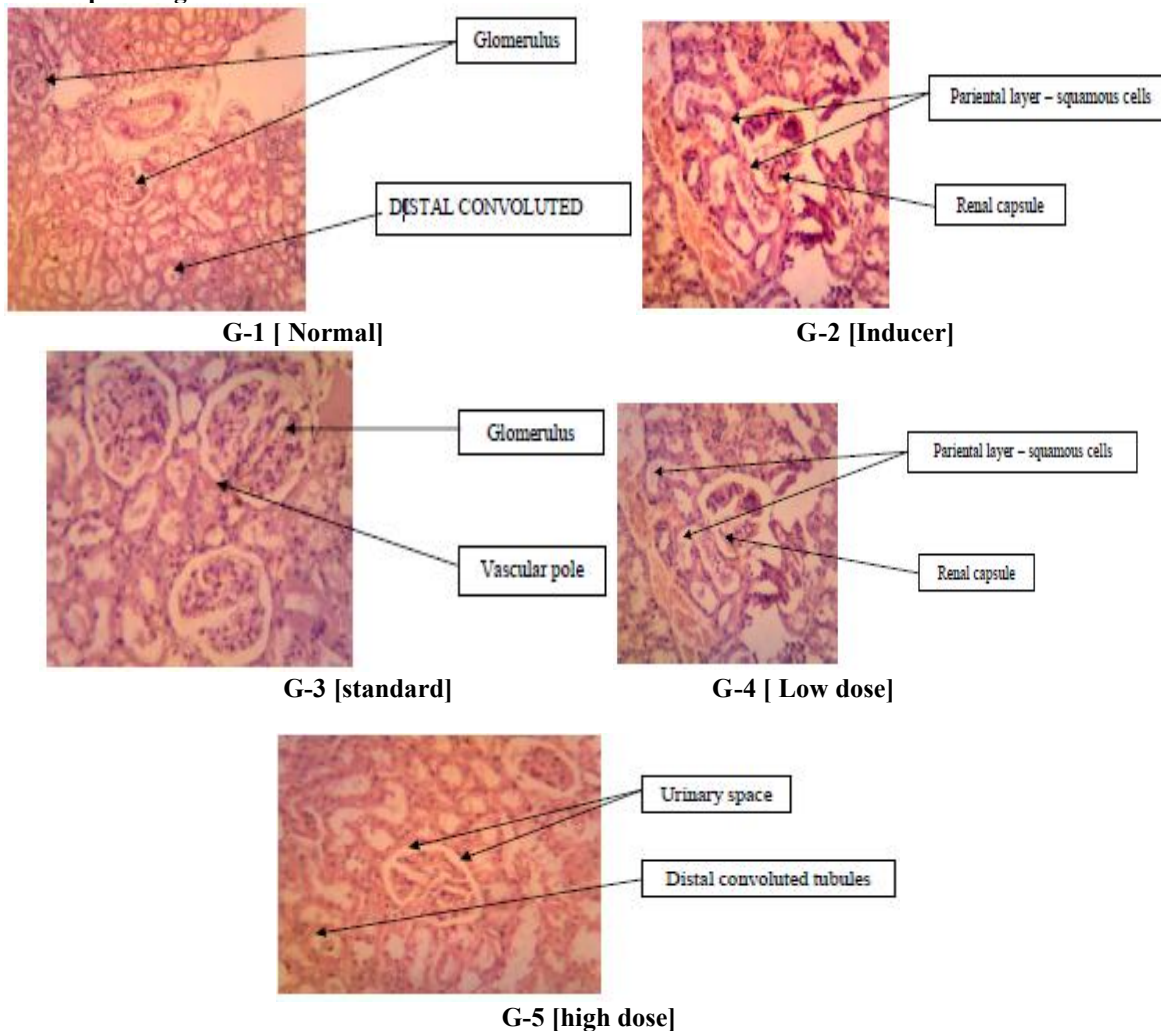


Figure 8: Serum Blood urea nitrogen

### 3.6 Histopathological studies



#### IV. Discussion

The study highlights the nephroprotective and antioxidant properties of *Agave americana*, attributed to its phytochemical composition. Key constituents identified include flavonoids, phenolics, and saponins, with high total phenolic (64.6 mg GAE/g) and flavonoid (55.2 mg RE/g) contents indicating significant antioxidant capacity. The DPPH assay demonstrated a concentration-dependent radical scavenging activity, with an IC<sub>50</sub> of 32.75 µg/ml. In vivo results showed that paracetamol-induced nephrotoxicity was mitigated by *Agave americana* extract, particularly at a higher dose (500 mg/kg), as indicated by reduced serum creatinine and BUN levels, normalization of urine volume, and improved renal architecture. These effects underscore the extract's potential in protecting against oxidative stress-induced renal damage, primarily due to its phenolic and flavonoid content.

#### V. Conclusion

The investigation presents scientific evidence supporting *Agave americana's* traditional use for kidney health. The methanolic extract showed significant nephroprotective activity against cisplatin-induced renal toxicity, with improvements in biochemical markers, urine parameters, and renal architecture. This effect is likely due to the extract's antioxidant properties, linked to its phenolic and flavonoid content. The study highlights *Agave americana* as a potential natural nephroprotective agent and suggests further research to isolate active constituents and understand their mechanisms, enhancing plant-based renal therapies.

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