

EXTRACTION, PHYTOCHEMICAL SCREENING, AND EVALUATION OF THE NEPHROPROTECTIVE EFFECT OF *PTEROCARPUS SANTALINUS* EXTRACT**Raj Vikram Singh*, Satkar Prasad****RKDF School of Pharmaceutical Sciences, Bhabha University, Bhopal (M.P.)***Corresponding Author's E mail: rajvikram123321@gmail.com

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ABSTRACT

The present study was undertaken to investigate the phytochemical profile and nephroprotective activity of the hydroalcoholic extract of *Pterocarpus santalinus* leaves against gentamicin-induced renal toxicity in rats. Physicochemical evaluation of the powdered leaves was performed to establish quality control parameters. The hydroalcoholic extract was prepared and subjected to preliminary phytochemical screening, which revealed the presence of flavonoids, carbohydrates, and diterpenes. Quantitative estimation showed a total flavonoid content of 0.92 mg/100 mg of extract. Nephroprotective activity was evaluated using a gentamicin-induced nephrotoxicity model in Wistar rats. Renal function was assessed by measuring serum creatinine, blood urea nitrogen (BUN), urea, total protein, and albumin levels. Gentamicin administration significantly elevated serum creatinine, BUN, and urea levels while reducing total protein and albumin concentrations, indicating severe renal damage. Treatment with *Pterocarpus santalinus* extract at doses of 100 and 200 mg/kg produced significant and dose-dependent protection against nephrotoxicity. The higher dose (200 mg/kg) showed greater restoration of biochemical parameters toward normal values. The nephroprotective effect may be attributed to the presence of flavonoids and other bioactive phytoconstituents possessing antioxidant and cytoprotective properties. The findings suggest that *Pterocarpus santalinus* leaves possess considerable nephroprotective potential and may serve as a promising natural therapeutic agent for the management of drug-induced renal disorders.

Keywords: *Pterocarpus santalinus*, Nephroprotective activity, Gentamicin-induced nephrotoxicity, Hydroalcoholic extract, Flavonoids, Renal function markers, Antioxidant activity, Medicinal plants.

INTRODUCTION

Kidney diseases represent a major global health concern and are associated with significant morbidity and mortality. The kidneys play a vital role in maintaining homeostasis by regulating fluid balance, electrolyte levels, acid-base equilibrium, and the excretion of metabolic waste products. Various factors such as oxidative stress, inflammation, drug-induced toxicity, diabetes mellitus, hypertension, and exposure to environmental toxins can impair renal function and lead to nephrotoxicity¹. Continuous renal

damage may progress to chronic kidney disease (CKD) and ultimately renal failure, necessitating long-term treatment and imposing a substantial healthcare burden.

Conventional therapies used for the management of kidney disorders primarily focus on controlling symptoms and slowing disease progression. However, these treatments often have limitations, including adverse effects, high treatment costs, and inadequate protection against oxidative and inflammatory damage. Consequently, there is increasing interest in exploring medicinal plants as alternative therapeutic agents due to their rich phytochemical composition and favorable safety profiles².

Pterocarpus santalinus L.f., commonly known as Red Sandalwood, is a valuable medicinal plant belonging to the family Fabaceae. It has been extensively used in traditional systems of medicine for the treatment of various ailments, including inflammatory disorders, diabetes, infections, liver diseases, and oxidative stress-related conditions³.

The heartwood and other parts of the plant contain numerous bioactive compounds such as flavonoids, phenolic compounds, tannins, saponins, glycosides, and terpenoids, which are known for their antioxidant, anti-inflammatory, antimicrobial, and cytoprotective activities⁴.

Phytochemical constituents present in *Pterocarpus santalinus* are capable of scavenging reactive oxygen species (ROS), reducing lipid peroxidation, and enhancing endogenous antioxidant defense mechanisms. Since oxidative stress is one of the major factors involved in the pathogenesis of renal injury, the antioxidant potential of the plant may contribute significantly to kidney protection. In addition, several phytochemicals found in medicinal plants have demonstrated nephroprotective effects by reducing inflammation, preventing cellular apoptosis, and improving renal biochemical parameters⁵.

Extraction and phytochemical screening are essential preliminary steps in the investigation of medicinal plants. Extraction helps isolate bioactive constituents from plant material using suitable solvents, while phytochemical screening provides valuable information regarding the presence of various secondary metabolites responsible for pharmacological activities. Identification of these compounds forms the basis for understanding the therapeutic potential of the plant⁶.

Considering the rich phytochemical profile and reported biological activities of *Pterocarpus santalinus*, the present study is aimed at extracting and screening the phytoconstituents of the plant extract and evaluating its nephroprotective activity using suitable experimental models. The study may provide scientific evidence supporting the traditional use of *Pterocarpus santalinus* and contribute to the development of effective plant-based therapies for the prevention and management of kidney disorders.

MATERIAL AND METHODS

Material

Fresh leaves of *Pterocarpus santalinus* were collected, authenticated, shade-dried, and powdered for extraction. Hydroalcoholic solvent (ethanol:water) was used for extraction of phytoconstituents. Analytical-grade chemicals and reagents including gentamicin, carboxymethyl cellulose (CMC), ferric chloride, lead acetate, Wagner's reagent, Fehling's solution, copper acetate, and other reagents required for phytochemical screening were procured from standard suppliers. Biochemical estimation kits for serum creatinine, blood urea nitrogen (BUN), urea, total protein, and albumin were used for nephroprotective studies. Healthy Wistar rats were employed for the evaluation of nephroprotective activity following approved ethical guidelines.

Methods

Physicochemical parameters

Organoleptic characters

It included evaluation of plant powder by colour, odour, taste and shape ⁷.

Loss on drying

2 g of plant materials was placed (without preliminary drying) after accurately weighing it in a tarred evaporated dish. This was dried at 105°C for 5 h and weighed. Drying and weighing was continued at 1 h interval until we got the constant weight. Constant weight was reached when two consecutive weights, after drying for 30 min. and cooling for 30 min. in a desiccator, showed not more than 0.1 g difference.

Total ash value

About 2-3 g of ground plant material was incinerated in a tarred platinum/silica crucible at a temperature not exceeding 450°C until free from carbon. Then it was cooled and weighed. The percentage of ash with reference to the air dried plant material was calculated.

Acid insoluble ash value

To the crucible containing total ash, 25 mL of dilute HCl was added. The insoluble matter was collected on an ashless filter paper (Whatmann number 41) and washed with hot water until the filtrate was neutral. The filter paper containing insoluble matter was transferred to the original crucible and dried on a hot plate and ignited to constant weight. The residue was allowed to cool in a suitable desiccator for 30 min. and weighed without delay. The content of the insoluble ash was calculated with reference to the air dried plant materials ⁸.

Water soluble ash value

The ash was boiled for 5 min with 25 mL of water; insoluble matter was collected in a Gooch crucible or on an ash less filter paper, washed with hot water, and ignited for 15 min. at a temperature not exceeding 450°C. The difference in the weight of the insoluble matter and the weight of ash represented the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried plant material.

Extraction by microwave-assisted technique

Extraction is an essential step in phytochemical processing for the finding of bioactive secondary metabolite from plant materials. For the standardization of herbal products, selection of a suitable extraction technique is also important. Extraction is used in the removal of desirable soluble constituents, exclusion those not required with the help of the selected solvents⁹.

About 50 g of powdered leaf material was accurately weighed and mixed with 500 ml of hydroalcoholic solvent (ethanol: water, 70:30 v/v) in a microwave-safe extraction vessel¹⁰. The mixture was allowed to soak for 30 minutes prior to extraction. The extraction was carried out using a microwave-assisted extraction system at a microwave power of 400–600 W for 5–10 minutes with intermittent cooling to avoid degradation of thermolabile constituents.

Determination of percentage yield

The extraction yield is evaluate of the solvent's efficiency to extracts bioactive components from the selected natural plant samples and it was defined as quantity of plant extracts recovered in mass after solvent extraction compared with the initial quantity of plant samples. After extraction, yield of the plant extracts obtained were calculated in grams and then converted it into percentage. The percentage yield of extract was calculated by using following formula:

$$\text{Percentage Yield} = \frac{\text{Weight of Extract}}{\text{Weight of Powder drug taken}} \times 100$$

Phytochemical screening

Medicinal plants are resources of traditional medicines and many of the modern medicines are produced indirectly from plants. Phytochemical constituents are of two type primary bioactive constituents (chlorophyll, proteins, amino acids, sugar etc.) and secondary bioactive constituents include (alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical examinations were carried out for all the extracts as per the standard methods¹¹.

Quantitative estimation of bioactive compounds

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method¹². 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% AlCl₃ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

***In vivo* Nephroprotective activity of *Pterocarpus santalinus* extract**

Animals

Wistarrats (150-200g) were group-housed (n=6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All experiments were conducted in a noise-free room between 08:00 and 15:00 h.

A separate group of rats (n = 6) was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), which was constituted by the Ministry of Environment and Forests, Government of India, New Delhi, India, for the purpose of controlling and supervising experimental animals.

Acute toxicity studies

Toxicity studies were conducted in accordance with OECD guidelines, including an acute oral toxicity study of the *Pterocarpus santalinus* extract. An acute toxicity study was performed based on OECD guideline no. 423. The mice were assessed for signs of toxicity throughout the next 14 days. *Pterocarpus santalinus* extract was given orally at a safe dose. Clinical symptoms like behavioral alterations, changes in the eyes, body weight, skin, and fur were noted¹³.

Experimental protocol

The rats were randomly divided into four groups of six rats ($n = 6$).

Normal Control (NC): Animals received saline orally for eight days.

Gentamicin (GM): Animals received GM (100 mg/kg), i.p., for eight days.

Test-I (100 mg/kg): Animals received *Pterocarpus santalinus* extract (100 mg/kg) orally, and one hour later received GM (100 mg/kg) i.p., for eight days.

Test-II (200 mg/kg): Animals received *Pterocarpus santalinus* extract (200 mg/kg) orally, and after one hour, received GM (100 mg/kg) i.p., for eight days.

On 9th day, all the rats were anesthetized with diethyl ether. Blood was collected in tubes from the retro-orbital venous plexus and allowed to clot for 60 minutes at 25°C. The serum samples were obtained by centrifuging blood at $1000 \times g$ for 15 min using a cooling centrifuge and stored frozen for biochemical assays.

Assessment of serum biochemical parameters

The estimation of serum creatinine (Cr), blood urea nitrogen (BUN), total proteins (TP), albumin and urea levels were carried out by using Tulip assay kits according to the manufacturer's instructions¹⁴.

Statistical analysis

Data are expressed as mean $\pm$ standard error of the mean, where n = number of rats. Statistical analysis was carried out using one-way analysis of variance followed by a Tukey multiple comparisons *post hoc* test. The level of significance was set at $p < 0.05$. GraphPad Prism V 8.02 (GraphPad Software Inc., San Diego, California, USA) was used for statistical analysis.

RESULTS AND DISCUSSION

The present study was undertaken to evaluate the physicochemical characteristics, phytochemical composition, and nephroprotective potential of the hydroalcoholic extract of *Pterocarpus santalinus* leaves against gentamicin-induced renal toxicity in rats. The findings demonstrated that the extract possesses significant nephroprotective activity, which may be attributed to its bioactive phytoconstituents.

The physicochemical evaluation of *Pterocarpus santalinus* leaves revealed acceptable quality parameters, indicating the purity and suitability of the plant material for further investigation. As shown in **Table 1**, the powdered leaves exhibited a light green color with a bitter and pungent taste. The low moisture content (2.5%) suggests reduced susceptibility to microbial contamination and improved storage stability. The ash values obtained were within acceptable limits, indicating minimal contamination with inorganic impurities.

The extraction study demonstrated that the hydroalcoholic solvent system effectively extracted phytoconstituents from the leaves. The hydroalcoholic extract yielded **10.4% w/w**, as presented in **Table 2**, indicating the presence of a considerable amount of extractable bioactive compounds. Hydroalcoholic solvents are known to efficiently extract both polar and moderately non-polar constituents, which may contribute to the observed pharmacological activity.

Preliminary phytochemical screening of the hydroalcoholic extract revealed the presence of flavonoids, carbohydrates, and diterpenes, while alkaloids, glycosides, saponins, phenols, proteins, tannins, and sterols were absent (**Table 3**). Among the detected phytoconstituents, flavonoids are particularly important because of their well-documented antioxidant, anti-inflammatory, and cytoprotective properties. The presence of these compounds suggests a possible role in protecting renal tissues from oxidative stress-induced damage.

The quantitative estimation of total flavonoid content further supported the phytochemical findings. The hydroalcoholic extract contained **0.92 mg/100 mg** of flavonoids (**Table 4**), indicating that flavonoids constitute a major class of bioactive compounds in *Pterocarpus santalinus*. Flavonoids are known to scavenge free radicals, inhibit lipid peroxidation, and enhance endogenous antioxidant defense mechanisms, thereby providing protection against nephrotoxicity.

Gentamicin administration produced marked renal injury, as evidenced by a significant elevation in serum creatinine levels. The gentamicin-treated group exhibited a serum creatinine level of **2.08 ± 0.07 mg/dL**, compared to **0.68 ± 0.02 mg/dL** in the normal control group (**Table 5**). Treatment with *Pterocarpus santalinus* extract significantly reduced serum creatinine levels in a dose-dependent manner. The higher dose (200 mg/kg) reduced creatinine to **0.91 ± 0.03 mg/dL**, indicating substantial protection against gentamicin-induced impairment of renal function. The reduction in creatinine suggests improvement in glomerular filtration and restoration of normal kidney function.

Blood urea nitrogen (BUN) is another important marker of renal function. Gentamicin administration caused a significant increase in BUN levels to **48.7 ± 1.28 mg/dL**, whereas the normal control group showed **18.9 ± 0.68 mg/dL** (**Table 6**). Administration of *Pterocarpus santalinus* extract significantly lowered BUN levels, with the higher dose reducing BUN to **26.3 ± 0.82 mg/dL**. This reduction indicates improved renal clearance and attenuation of nephrotoxic damage.

The effect of the extract on serum total protein levels further confirmed its nephroprotective activity. Gentamicin-induced renal damage significantly reduced total protein levels from **7.1 ± 0.16 g/dL** in the normal group to **4.4 ± 0.13 g/dL** (**Table 7**). Treatment with the extract restored protein levels in a dose-

dependent manner, with the 200 mg/kg dose increasing total protein to 6.5 ± 0.11 g/dL. Restoration of protein levels indicates improved renal integrity and reduced protein loss associated with kidney injury. Similarly, serum albumin levels were markedly decreased following gentamicin administration, reflecting impaired renal function and protein metabolism. The albumin level decreased from 4.6 ± 0.11 g/dL in the normal control group to 2.5 ± 0.08 g/dL in the toxic control group (**Table 8**). Treatment with *Pterocarpus santalinus* extract significantly increased albumin levels, with the higher dose restoring albumin to 4.2 ± 0.07 g/dL. This improvement indicates preservation of renal filtration capacity and prevention of protein leakage.

Urea concentration is another sensitive indicator of kidney function. Gentamicin treatment caused a significant elevation in serum urea levels to 64.5 ± 1.08 mg/dL, compared with 30.1 ± 0.76 mg/dL in normal rats (**Table 9**). Administration of the hydroalcoholic extract significantly reduced urea levels, particularly at the 200 mg/kg dose, where the value decreased to 37.0 ± 0.87 mg/dL. This finding suggests a substantial protective effect against gentamicin-induced renal dysfunction.

The nephroprotective activity observed in the present study may be attributed primarily to the flavonoid-rich nature of the hydroalcoholic extract of *Pterocarpus santalinus*. Flavonoids are known to neutralize reactive oxygen species, reduce oxidative stress, stabilize cellular membranes, and inhibit inflammatory responses associated with renal injury. The dose-dependent improvement in serum creatinine, blood urea nitrogen, urea, total protein, and albumin levels indicates that the extract effectively protects kidney tissues from gentamicin-induced nephrotoxicity.

The results of the present investigation demonstrate that the hydroalcoholic extract of *Pterocarpus santalinus* possesses significant nephroprotective potential and may serve as a promising natural therapeutic agent for the prevention and management of drug-induced renal damage.

Table 1: General physicochemical parameter of *Pterocarpus santalinus* leaves

Test parameters	Results (%w/w)
Organoleptic characters	Light green powder, Bitter and Pungent
Loss on drying at 105°C	2.5 %
Total ash	3.9 %
Acid insoluble ash	0.85 %
Water soluble ash	5.74 %

Table 2: Results of percentage yield of extract of *Pterocarpus santalinus*

S. No.	Extract	Weight of Extract	Percentage yield (w/w)
1.	Hydroalcoholic	5.20	10.4%

Table 3: Result of phytochemical screening of extract of *Pterocarpus santalinus*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids	
	A) Wagner's Test:	-Ve
2.	Glycosides	
	A) Legal's Test:	-Ve
3.	Flavonoids	
	A) Lead acetate Test:	+Ve
	B) Alkaline Reagent Test:	+Ve
4.	Saponins	
	A) Froth Test:	-Ve
5.	Phenol	
	A) Ferric Chloride Test:	-Ve
6.	Proteins	
	A) Xanthoproteic Test:	-Ve
7.	Carbohydrate	
	A) Fehling's Test:	+Ve
8.	Diterpenes	
	A) Copper acetate Test:	+Ve
9.	Tannins	
	A) Gelatin Test	-Ve
10.	Sterols	
	A) Salkowski Test	-Ve

Table 4: Estimation of total flavonoids content of *Pterocarpus santalinus*

S. No.	Hydroalcoholic extract	Total flavonoids content
1.	<i>Pterocarpus santalinus</i>	0.92 mg/100mg

Table 5: Effect of *Pterocarpus santalinus* extract on serum creatinine in GM-induced renal damage in rats

Group	Treatment	Serum Creatinine (mg/dL)
Group I	Normal Control (0.5% CMC)	0.68 ± 0.02
Group II	Gentamicin (GM) (100 mg/kg)	2.08 ± 0.07#
Group III	<i>Pterocarpus santalinus</i> Extract (100 mg/kg) + GM	1.28 ± 0.05*
Group IV	<i>Pterocarpus santalinus</i> Extract (200 mg/kg) + GM	0.91 ± 0.03***

Results are expressed as Mean ± SEM (n = 6). # $p < 0.001$ as compared to Normal Control rats and ** $p < 0.01$, *** $p < 0.001$ compared to Gentamicin rats

Table 6: Effect of *Pterocarpus santalinus* extract on blood urea nitrogen in GM-induced renal damage in rats

Group	Treatment	Blood Urea Nitrogen (mg/dL)
Group I	Normal Control (0.5% CMC)	18.9 ± 0.68
Group II	Gentamicin (GM) (100 mg/kg)	48.7 ± 1.28#
Group III	<i>Pterocarpus santalinus</i> Extract (100 mg/kg) + GM	33.8 ± 1.04**
Group IV	<i>Pterocarpus santalinus</i> Extract (200 mg/kg) + GM	26.3 ± 0.82***

Results are expressed as Mean ± SEM (n = 6). # $p < 0.001$ as compared to Normal Control rats and ** $p < 0.01$, *** $p < 0.001$ compared to Gentamicin rats

Table 7: Effect of *Pterocarpus santalinus* extract on total proteins in GM-induced renal damage in rats

Group	Treatment	Total Protein (g/dL)
Group I	Normal Control (0.5% CMC)	7.1 ± 0.16
Group II	Gentamicin (GM) (100 mg/kg)	4.4 ± 0.13#
Group III	<i>Pterocarpus santalinus</i> Extract (100 mg/kg) + GM	5.8 ± 0.12*
Group IV	<i>Pterocarpus santalinus</i> Extract (200 mg/kg) + GM	6.5 ± 0.11

Results are expressed as Mean ± SEM (n = 6). # $p < 0.001$ as compared to Normal Control rats and ** $p < 0.01$, *** $p < 0.001$ compared to Gentamicin rats

Table 8: Effect of *Pterocarpus santalinus* extract on serum albumin in GM-induced renal damage in rats

Group	Treatment	Serum Albumin (g/dL)
Group I	Normal Control (0.5% CMC)	4.6 ± 0.11
Group II	Gentamicin (GM) (100 mg/kg)	2.5 ± 0.08#
Group III	<i>Pterocarpus santalinus</i> Extract (100 mg/kg) + GM	3.6 ± 0.09*
Group IV	<i>Pterocarpus santalinus</i> Extract (200 mg/kg) + GM	4.2 ± 0.07**

Results are expressed as Mean ± SEM (n = 6). # $p < 0.001$ as compared to Normal Control rats and ** $p < 0.01$, *** $p < 0.001$ compared to Gentamicin rats

Table 9: Effect of *Pterocarpus santalinus* extract on urea levels in GM-induced renal damage in rats

Group	Treatment	Urea (mg/dL)
Group I	Normal Control (0.5% CMC)	30.1 ± 0.76
Group II	Gentamicin (GM) (100 mg/kg)	64.5 ± 1.08#
Group III	<i>Pterocarpus santalinus</i> Extract (100 mg/kg) + GM	47.9 ± 0.98**
Group IV	<i>Pterocarpus santalinus</i> Extract (200 mg/kg) + GM	37.0 ± 0.87***

Results are expressed as Mean ± SEM (n = 6). # $p < 0.001$ as compared to Normal Control rats and ** $p < 0.01$, *** $p < 0.001$ compared to Gentamicin rats

CONCLUSION

The present study demonstrated that the hydroalcoholic extract of *Pterocarpus santalinus* leaves possesses significant nephroprotective activity against gentamicin-induced renal toxicity in rats. The extract was rich in flavonoids and other phytoconstituents, which are likely responsible for its protective effects. Treatment with the extract significantly restored altered renal biochemical parameters in a dose-dependent manner, with the 200 mg/kg dose showing superior efficacy. These findings suggest that *Pterocarpus santalinus* leaves have promising potential as a natural nephroprotective agent and warrant further studies to isolate the active constituents and elucidate their underlying mechanisms of action.

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