EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Coden USA: EJBPBO
Volume: 13
Issue: 1
Year: 2026

AN EXPERIMENTAL PHYTOCHEMICAL INVESTIGATION AND ASSESSMENT OF NEPHRO-PROTECTIVE ACTIVITY OF VARIEGATED CROTON PLANT EXTRACT ON EXPERIMENTAL RATS

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DOI: https://doi.org/10.5281/zenodo.18150707

How to cite this Article: Muskan Kumari Pandey[1]*, Dr. Abhishek Sharma[2], Mr. Ritesh Malvi[3], Dr. Surendra Jain[4]. (2026). AN EXPERIMENTAL PHYTOCHEMICAL INVESTIGATION AND ASSESSMENT OF NEPHRO-PROTECTIVE ACTIVITY OF VARIEGATED CROTON PLANT EXTRACT ON EXPERIMENTAL RATS. European Journal of Biomedical and Pharmaceutical Sciences, 13(1), 211–219.

Article Received on 05/12/2025 **Article Revised on 25/12/2025** **Article Published on 05/01/2026**

ABSTRACT

The search for effective nephroprotective agents from natural sources has become crucial in combating the increasing prevalence of kidney diseases. This study investigates the nephroprotective effects of *Variegated Croton*, a plant with a long history of traditional use in treating kidney-related disorders. Nephrotoxicity was generated in Wistar rats with nephrotoxic drugs, and the potential protective benefits of *Variegated Croton* extract were comprehensively investigated. The extract is high bioactive chemicals including phenolics and flavonoids, revealed strong antioxidant activity in the DPPH assay, implying that it can reduce oxidative stress, which is a primary cause of kidney injury. The results showed that the extract considerably improved renal function, as seen by lower serum creatinine and blood urea nitrogen (BUN) levels and a noticeable rise in urine output. These findings demonstrate the extract's capacity to promote renal filtration and prevent malfunction. Histopathological investigation validated the nephroprotective effects by revealing a decrease in kidney damage, including tubular necrosis, glomerular damage, and inflammation. These findings collectively support the concept that *Variegated Croton* has strong nephroprotective qualities, which are most likely mediated by antioxidant and anti-inflammatory pathways, supporting its traditional use and indicating its potential as a therapeutic agent for kidney protection.

KEYWORDS: Nephroprotective, *Variegated Croton*, Plant extract, Antioxidant, DPPH assay, Nephrotoxicity, Renal function, Serum creatinine, Blood urea nitrogen (BUN), Histopathology, Wistar rats.

1. INTRODUCTION

Medicinal plants have been extensively used in traditional systems of medicine for the prevention and treatment of various diseases, owing to their rich content of bioactive phytochemicals **(Ogbuagu *et al.*, 2022).** In recent years, scientific interest in plant-derived therapies has increased significantly due to their therapeutic efficacy, cost-effectiveness, and relatively fewer adverse effects compared to synthetic drugs. Phytochemical investigations play a vital role in identifying biologically active compounds present in medicinal plants and provide a scientific basis for their pharmacological applications (**Süntar, 2020).**

Renal disorders remain a major global health concern, with nephrotoxicity being a common complication associated with exposure to chemicals, drugs, metabolic imbalances, and environmental toxins. Kidney damage is often characterized by elevated serum creatinine, blood urea nitrogen (BUN), altered urine output, and structural damage to renal tissues **(Barnett and Cummings 2018).** Oxidative stress is a key factor in the development and progression of nephrotoxicity, as excessive generation of reactive oxygen species (ROS) leads to lipid peroxidation, protein oxidation, inflammation, and cellular injury in renal tissues. Therefore, agents with antioxidant and nephroprotective properties are of significant therapeutic interest **(Tejchman *et al.*, 2021).**

Herbal medicines rich in phenolics, flavonoids, alkaloids, and glycosides have demonstrated protective effects against oxidative stress–induced renal damage. These compounds exert their nephroprotective actions by scavenging free radicals, enhancing endogenous antioxidant defense systems, reducing inflammation, and preserving normal renal architecture **(Mohany *et al.*, 2021).** Experimental animal models, particularly rats, are widely employed to evaluate nephroprotective activity and to assess biochemical, physiological, and histopathological changes associated with kidney injury and recovery **(Casanova *et al.*, 2017).**

Variegated Croton (*Codiaeum variegatum*), belonging to the family Euphorbiaceae, is an ornamental plant that has also been used traditionally for various medicinal purposes. Different parts of the plant have been reported to possess antioxidant, anti-inflammatory, antimicrobial, and cytoprotective properties, which are attributed to its diverse phytochemical composition **(Dinakaran *et al.*, 2024).** However, despite these traditional claims, systematic scientific studies exploring the phytochemical profile and nephroprotective potential of Variegated Croton remain limited **(Pandey and Singh 2023).**

The present study was therefore designed to conduct an experimental phytochemical investigation of Variegated Croton plant extracts using different solvents and to evaluate their nephroprotective activity in experimental rats. The study aims to identify key phytoconstituents responsible for biological activity and to assess the protective effects of the extract on renal function through biochemical parameters and histopathological examination. This research seeks to provide scientific validation for the traditional use of Variegated Croton and to explore its potential as a natural therapeutic agent for the prevention and management of nephrotoxicity.

2. METHODS AND MATERIAL

2.1 Chemicals

Ethanol were obtained from Shree Renuka Sugars, a reputable supplier of analytical reagents. Millipore Sigma provided the Chloroform. Merck provided the Ammonia. 95% Alcohol were obtained from Clorofiltind. Magnesium were obtained from Sagar Steel Corporation while Grasim Industries provided the Sodium Hydroxide. Hindustan Zinc Merck obtained from Conc. H_2SO_4. Vinipul Inorganics provided the Glacial Acetic Acid while Ferry Chem Industries provided the Conc. HCl.

2.2 Procurement of plant material

Plant material of *Variegated Croton* was collected from regions traditionally known for the medicinal use of the species. The collection site was carefully selected based on its ethnobotanical relevance and natural habitat. The plant specimen was taxonomically identified and authenticated by a qualified botanist from a recognized institution. A voucher specimen was prepared, labelled, and deposited in the institutional herbarium for future reference and documentation.

Following authentication, the plant material was thoroughly washed with clean water to remove dust, soil, and other surface contaminants. It was then shade-dried under controlled environmental conditions to protect its phytochemical integrity and prevent degradation. Once fully dried, the material was coarsely ground using a mechanical grinder. The powdered plant material was stored in airtight containers at room temperature until further use in extraction and phytochemical analysis **(Griesbach *et al.*, 2012).**

2.3 Extraction of Plant Material by Soxhlation Process

The dried, coarsely powdered leaves of *Variegated Croton* were subjected to solvent extraction using a Soxhlet apparatus, an established technique for the effective isolation of bioactive constituents. Solvents such as petroleum ether and methanol were chosen based on the polarity profile of the target phytochemicals. The plant powder was loaded into a thimble and inserted into the Soxhlet extractor, while the solvent was placed in a round-bottom flask and gently heated.

As the solvent vaporized, it ascended, condensed in the cooling unit, and percolated repeatedly through the plant material, ensuring continuous extraction. This cycle was maintained for 6 to 8 hours or until the solvent in the siphon became transparent, indicating that the plant matrix had been thoroughly exhausted. Upon completion of the extraction, the solvent extract was filtered to remove residual plant debris and concentrated under reduced pressure using a rotary evaporator to eliminate excess solvent **(López and Castro 2020).**

The resulting semi-solid crude extract was further dried in a desiccator and stored in a sealed, airtight container at low temperature for future phytochemical screening and pharmacological evaluation. This approach ensured optimal extraction efficiency and preserved the integrity of the bioactive components for subsequent nephroprotective studies. The percentage yield of the extract was calculated using the following formula:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

2.4 Quantitative Estimation of Phytoconstituents

Preliminary phytochemical studies confirmed the presence of phenols, alkaloids, flavonoids, saponins, and tannins, thus the plant material was obtained for quantitative estimate. **(Sarumathy *et al.*, 2011).**

2.4.1 Determination of total phenols content by spectrophotometric method

The total phenolic content of *Variegated Croton* was determined using the Folin-Ciocalteu colorimetric technique. In a clean test tube, combine 40 µL of the plant extract (1 mg/mL in methanol) or standard gallic acid solution with 3.16 mL of distilled water and 200 µL of Folin-Ciocalteu reagent. The mixture was gently shaken and left to sit for 8 minutes at room temperature.

After that, add 600 µL of sodium carbonate solution to the reaction mixture and thoroughly mix it.

The solution was incubated at 40°C for 30 minutes to allow color development. Following incubation, the mixture's absorbance at 760 nm was measured using a UV-visible spectrophotometer against a reagent blank. A calibration curve was produced using gallic acid standard solutions at values of 20, 40, 60, 80, and 100 µg/mL. The plant extract's total phenolic content was quantified as gallic acid equivalents (GAE) **(Stratil *et al.*, 2006).**

2.4.2 Determination of total flavonoid content by spectrophotometric method

The total flavonoid content of *Variegated Croton* was determined using the aluminium chloride colorimetric technique. In this step, 0.2 g of plant extract was dissolved in 1 ml of deionized water. 0.5 ml of the stock solution was combined with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium chloride hexahydrate ($AlCl_3$), 0.1 ml of 1 M potassium acetate (CH_3COOK), and 2.8 ml of deionized water.

To allow color to develop, the resulting combination was incubated at room temperature for 40 minutes. After incubation, the solution's absorbance at 510 nm was measured using a UV-visible spectrophotometer, with deionized water serving as a blank. Calibration was performed using rutin as the standard. Standard solutions were produced at concentrations of 20, 40, 60, 80, and 100 µg/ml The total flavonoid content was estimated from the rutin calibration curve and given as milligrams of rutin equivalents (RE) per gram of dry extract **(da *et al.*, 2015).**

2.5 Determination of antioxidant activity by the DPPH test

The antioxidant activity of *Variegated Croton* extract was assessed using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, a widely accepted spectrophotometric method. This assay is based on antioxidants' ability to contribute hydrogen atoms or electrons to the stable DPPH radical, causing a detectable drop in absorbance. The purple-colored DPPH radical exhibits a strong absorption peak in the UV-visible range, which diminishes upon reduction by an antioxidant (AH) or another radical species (R•), according to the reactions:

$DPPH• + AH \rightarrow DPPH\text{-}H + A•$

$DPPH• + R• \rightarrow DPPH\text{-}R$

The approach involved adding 50 µl of a methanolic extract of Variegated Croton at different doses (range from 20 to 100 µg/ml) to 5 mL of a 0.004% DPPH solution produced in methanol. The mixture was then kept at room temperature for 30 minutes in the dark to allow the reaction to take place. Following incubation, the absorbance at 517 nm was measured with a spectrophotometer, with methanol serving as a blank. The % of DPPH radical scavenging activity was estimated using the following equation:

% Scavenging Activity = 100[(Ac – As)/Ac]

Where, Ac and As are absorbances of negative control and sample, respectively **(Sirivibulkovit *et al.*, 2018).**

2.6 Acute Toxicity Study

An acute oral toxicity study of the methanolic extract of *Variegated Croton* was conducted in compliance with OECD Guideline 423, using a stepwise dosing technique with three rats per dose level. The Institutional Animal Ethics Committee had previously provided ethical clearance.

The extract was given orally at dosages of 5, 50, 300, and 2000 mg/kg body weight. Throughout the 14-day observation period, none of the tested doses resulted in mortality or treatment-related adverse consequences. The treated animals showed normal behavior and physiological functions, with no symptoms of toxicity or discomfort. Furthermore, body weight and food intake remained similar, with no statistically significant alterations compared to the control group **(Singh *et al.*, 2016).**

2.7 *In vivo* study of Nephroprotective activity in rats

2.7.1 Experimental work

The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) and was conducted in full compliance with established ethical guidelines for animal research. A total of 30 healthy adult Wistar albino rats, each weighing approximately 200 ± 20 g, were procured from Pinnacle Biomedical Research Institute (PBRI), Bhopal. The animals were housed under standard laboratory conditions in accordance with institutional guidelines, including controlled temperature (22 ± 2 °C), relative humidity (55 ± 5%), and a 12-hour light/dark cycle. All rats had free access to standard pellet diet and water ad libitum throughout the experimental period **(Neelima *et al.*, 2020).**

2.7.1.1 Experimental modelling

During acclimation, rats were fed a diet of Golden Feed, Bhopal, and fresh water ad libitum and kept under constant circumstances (temperature: 22 ± 1 C, humidity: 40-60%, and controlled illumination utilizing a 14 h:10 h light-dark cycle for a week prior to the studies). Sixteen hours before the experiment, the animals were only given water. All surgical procedures were carried out under sterile settings **(Abitbol *et al.*, 1994).**

2.7.1.2 Drugs

- **Paracetamol**

A paracetamol suspension was produced in 1% carboxymethylcellulose in saline phosphate buffer, and animals were fed 750 mg/kg of this suspension orally via gastric tube (1 ml of suspension contains 750 mg/kg of paracetamol).

➢ **N-Acetylcysteine (NAC)**

NAC (50 mg) was provided and a solution was prepared in 0.9% saline, pH 7.0. Animals were administered 50 mg/kg of this NAC solution orally by gastric tube.

2.7.1.3 Experimental protocol

The animals were randomly divided into five groups; and six rats in each group

Group I: Acted as the untreated control group.

Group II: Was given paracetamol orally at a dose of 750 mg/kg bwt.

Group III: Received a daily dose of 50 mg/kg bwt of N-acetylcysteine by oral route.

Group IV: Received a daily oral administration of 250 mg/kg bwt *Variegated croton* extracts.

Group V: Received a daily oral administration of 500 mg/kg bwt *Variegated croton* extracts.

All therapies were administered for 30 days, beginning 4 days before the oral delivery of N-acetyl cysteine. Throughout the experiment, every animal's body weight was measured weekly. **(Canayakin *et al.*, 2016).**

2.7.2 Blood samples for biochemical estimation

Blood samples were collected from retro orbital venous plexus in non-heparinized tubes, centrifuged at 3000 rpm for 20 minutes, and blood sera were collected and stored at 4 ºC prior immediate determination of serum biochemical constituents which was performed by using ready-made kits from Erba.

2.8 Analysis of general parameters

2.8.1 Analysis of urine

After the last treatment animals were shifted to metabolic cages for 24 h in order to collect their urine individually and to estimate urine volume. Urine samples were assayed for glucose and protein by using standard diagnostic kits.

2.8.2 Estimation of Body weight

Upon completion of the trial, each set of animals was kept separate within their cage. Remove the food and water, weigh each animal individually, and record the results.

2.8.3 Serum Creatinine and blood urea nitrogen (BUN) analysis

Plasma samples were collected for the analysis of creatinine and blood urea nitrogen (BUN) levels. To assess these concentrations, samples were either spiked with 10 µL of a creatinine standard solution prepared in 0.2 N HCl or with 10 µL of 0.2 N HCl alone as a control. Serum creatinine (SCR) and BUN are commonly used to evaluate kidney function; however, BUN is a poor indicator of glomerular filtration rate (GFR) in cases of mild to moderate renal impairment. BUN was measured using a biochemical analyzer. In this assay, urea is enzymatically converted to ammonia and carbon dioxide by urease. Subsequently, glutamate dehydrogenase catalyzes the reaction of ammonia with alpha-ketoglutarate to produce glutamate and NAD. The oxidation of NADH to NAD is monitored by the reduction in absorbance at 340 nm **(Rojas *et al.*, 2002).**

3. RESULT AND DISCUSION

3.1 Procurement of plant material

Table 1: Percentage Yield of plant material.

Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
Variegated Croton	Methanol	512	28.69	5.60
	Pet. Ether	486	22.71	4.67

3.2 Phytochemical Test

Table 2: Phytochemical test of extract.

Experiment	Presence or absence of phytochemical test
	Petroleum ether extract
Alkaloids	
Dragendroff's test	- ve
Mayer's reagent test	- ve
Wagner's reagent test	- ve
Hager's reagent test	- ve
Glycoside	
Borntrager test	- ve
Legal's test	- ve
Killer-Killiani test	- ve
Carbohydrates	
Molish's test	+ ve
Fehling's test	+ ve
Benedict's test	+ ve

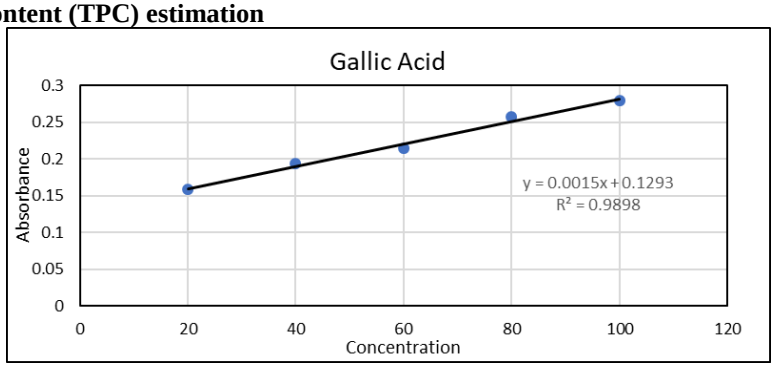
Barfoed's test	+ ve
Proteins and Amino Acids	
Biuret test	+ ve
Ninhydrin test	+ ve
Flavonoids	
Alkaline reagent test	+ ve
Lead Acetate test	+ ve
Tannin and Phenolic Compounds	
Ferric Chloride test	+ ve
Saponin	
Foam test	+ ve
Test for Triterpenoids and Steroids	
Salkowski's test	- ve
Libbermann-Burchard's test	- ve

Table 3: Phytochemical test of *Variegated Croton* extract of Methanolic extract.

1.	Alkaloids	
1.1	Dragendroff's test	+ ve
1.2	Mayer's reagent test	+ ve
1.3	Wagner's reagent test	+ ve
1.3	Hager's reagent test	+ ve
2.	Glycoside	
2.1	Borntrager test	+ ve
2.2	Legal's test	+ ve
2.3	Killer-Killiani test	+ ve
3.	Carbohydrates	
3.1	Molish's test	- ve
3.2	Fehling's test	- ve
3.3	Benedict's test	- ve
3.4	Barfoed's test	- ve
4.	Proteins and Amino Acids	
4.1	Biuret test	- ve
4.2	Ninhydrin test	- ve
5.	Flavonoids	
5.1	Alkaline reagent test	+ ve
5.2	Lead Acetate test	+ ve
6.	Tannin and Phenolic Compounds	
6.1	Ferric Chloride test	+ ve
7.	Saponin	
7.1	Foam test	+ ve
8.	Test for Triterpenoids and Steroids	
8.1	Salkowski's test	- ve
8.2	Libbermann-Burchard's test	- ve

3.3 Quantitative Estimation of Phytoconstituents

3.3.1 Total Phenolic content (TPC) estimation



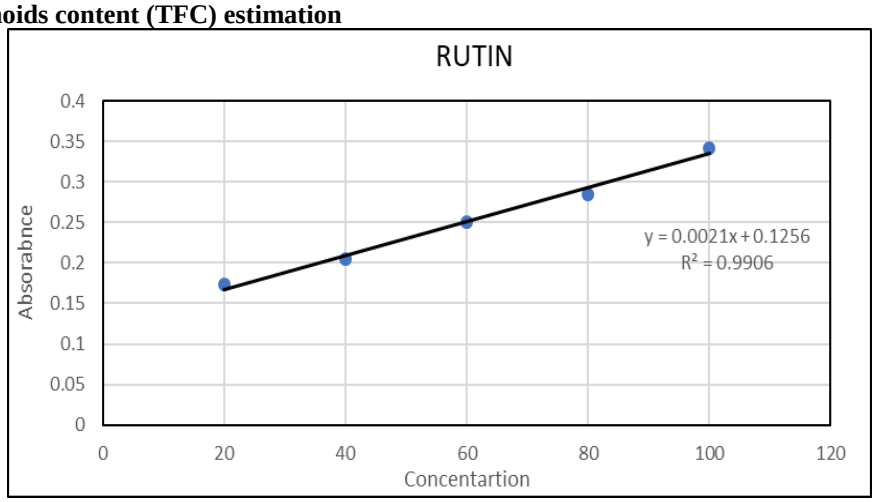
Graph 1: Represent standard curve of Gallic acid.

3.3.1.1 Total Phenolic Content

Table 4: Total Phenolic Content in* Variegated Croton *extract.

Absorbance	TPC in mg/gm equivalent of Gallic Acid
0.161	85.7 mg/gm
0.210	
0.275	

3.3.2 Total Flavonoids content (TFC) estimation



Graph 2: Represent standard curve of Rutin.

3.3.2.1 Total Flavonoid Content

Table 5: Total Flavonoid Content in* Variegated Croton *extract.

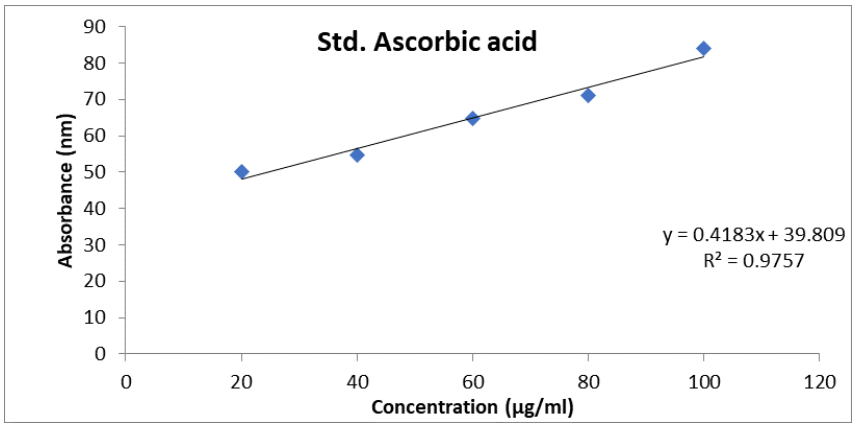
Absorbance	*TFC in mg/gm equivalent of Rutin*
0.200	60.7 mg/gm
0.242	
0.301	

3.4 Anti-Oxidant Activity

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

Table 6: DPPH radical scavenging activity of Std. Ascorbic acid.

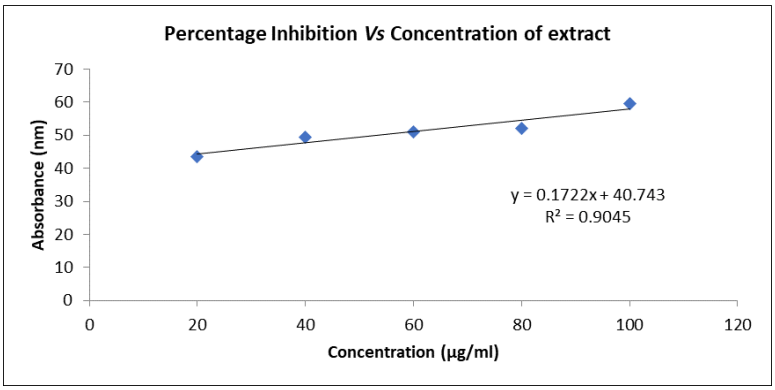
Concentration (µg/ml)	Absorbance	% Inhibition
20	0.491	50.200
40	0.452	54.709
60	0.352	64.729
80	0.289	71.042
100	0.161	83.867
Control	**0.998**	
IC50	24.38	



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

***Table 7: DPPH radical scavenging activity of methanol extract of* Variegated Croton.**

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.531	43.570
40	0.476	49.415
60	0.462	50.903
80	0.425	51.965
100	0.381	59.511
Control		0.941
IC50		53.81



Graph 4: Represents the Percentage Inhibition* Vs *Concentration of* Variegated Croton *extract

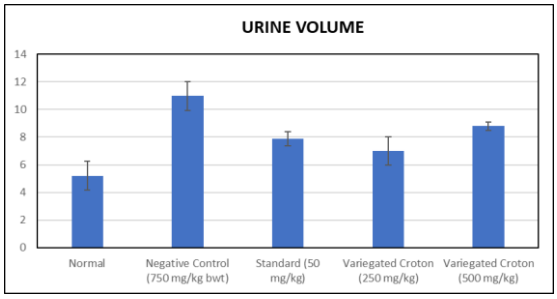
3.6 Paracetamol induced Nephrotoxicity Model

Figure 1: Paracetamol induced Nephrotoxicity Model.

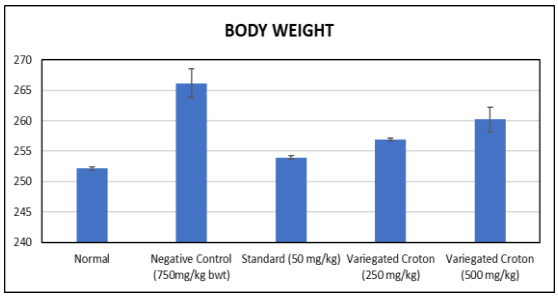
3.7 Analysis of general parameters

Table 8: Analysis of general parameters.

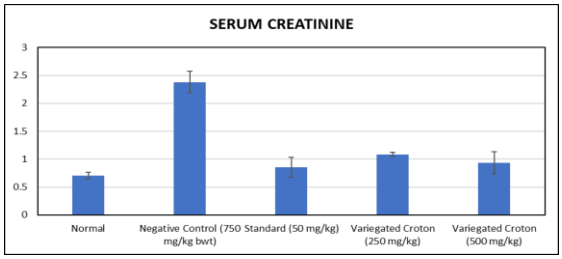
Groups	Urine Volume (ml)	*Body Weight*	*Serum Creatinine*	*Serum Blood Urea Nitrogen*
Normal	5.21±1.03	252.13±0.260	0.70±0.06	25.08±0.53
Negative Control (750 mg/kg bwt)	10.98±1.05	266.15±2.35	2.38±0.19	41.11±0.85
Standard (50 mg/kg)	7.89±0.52	253.95±0.251	0.85±0.18	27.09±0.56
Variegated Croton (250 mg/kg)	6.99±1.03	256.90±0.224	1.08±0.04	33.85±0.99
Variegated Croton (500 mg/kg)	8.79±0.31	260.20±2.05	0.93±0.20	30.76±0.56



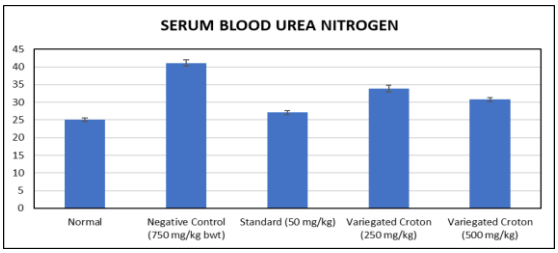
Graph 5: Urine volume.



Graph 6: Body weight.



Graph 7: Serum Creatinine.



Graph 8: Serum Blood urea nitrogen.

3.8 Histopathological studies

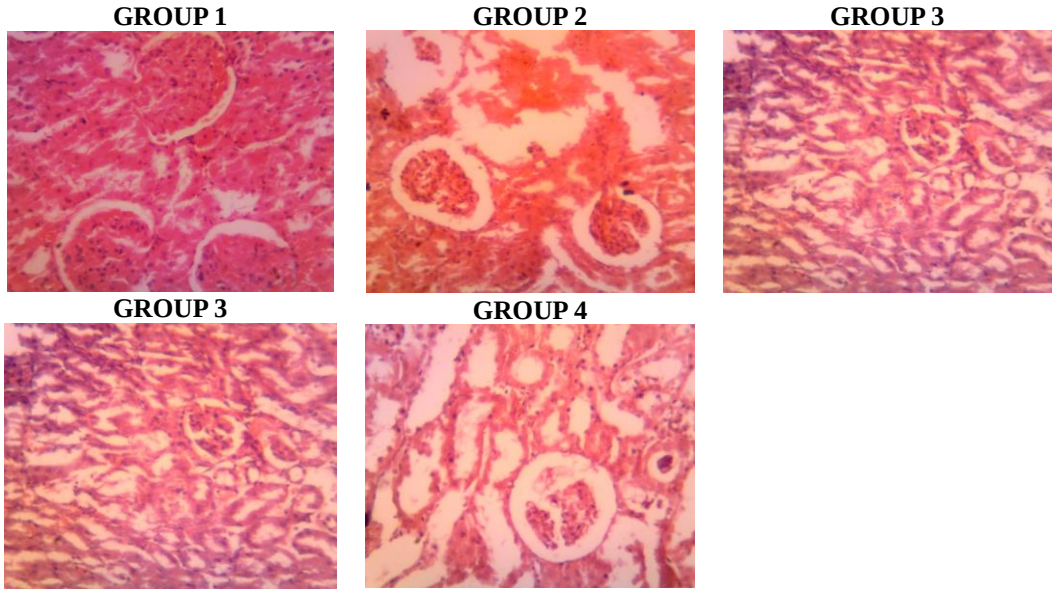


Figure 2: Histopathological studies.

DISCUSSION

The extraction yield of Variegated Croton was slightly higher with methanol (5.60%) compared to petroleum ether (4.67%), indicating that methanol is more efficient in extracting plant constituents. Phytochemical screening revealed clear solvent-dependent differences: the methanolic extract contained alkaloids, glycosides, flavonoids, tannins, and saponins, whereas the petroleum ether extract mainly contained carbohydrates, proteins, flavonoids, tannins, and saponins. This highlights the importance of solvent selection, as methanol proved more effective in extracting pharmacologically important compounds such as alkaloids and glycosides.

Quantitative analysis showed appreciable levels of total phenolics (85.7 mg/g) and total flavonoids (60.7 mg/g), suggesting strong antioxidant potential. In the DPPH assay, the methanolic extract exhibited moderate antioxidant activity with an IC_{50} value of 53.81 µg/mL, which was lower than that of ascorbic acid (24.38 µg/mL) but still indicative of substantial radical scavenging ability.

In vivo studies demonstrated that Variegated Croton extract significantly improved renal function parameters in treated groups. Dose-dependent reductions in serum creatinine, blood urea nitrogen, and urine volume were observed, with the 500 mg/kg dose showing effects comparable to the standard treatment. Histopathological findings further supported these results, as higher doses of the extract restored near-normal renal architecture and reduced nephrotoxic damage. Overall, the findings suggest that Variegated Croton possesses notable antioxidant and nephroprotective properties, supporting its potential therapeutic application in renal disorders.

4. CONCLUSION

This study provides compelling scientific evidence for the nephroprotective activity of *Variegated Croton* extract. The extract demonstrated significant protective effects against nephrotoxicity by restoring kidney function, reducing oxidative stress, and preserving renal histoarchitecture. The findings suggest that *Variegated Croton* exerts its nephroprotective effects through anti-inflammatory, antioxidant, and possibly diuretic mechanisms, helping to counteract oxidative damage and maintain renal homeostasis. The absence of toxicity in acute studies further supports the plant's safety profile, making it a promising natural alternative for kidney protection. However, to fully establish its therapeutic potential, research is necessary, including characterization of active compounds, mechanistic studies, and clinical trials in human subjects. Future investigations were help to determine optimal dosage, long-term safety, and clinical efficacy of *Variegated Croton* in managing kidney-related disorders.

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