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"An Experimental Evaluation, Phytochemical Estimation and Anti-Urolithic Activity of Herbal Plant Extract on Wistar Rats"

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ABSTRACT
This study evaluated the phytochemical profile and anti-urolithic activity of Sinapisarvensis leaf extracts in Wistar rats. Phytochemical screening confirmed that the methanolic extract contained flavonoids, phenolics, alkaloids, tannins, glycosides, and carbohydrates, whereas the petroleum ether extract showed limited constituents such as saponins and terpenoids. Extraction using petroleum ether and methanol revealed significant differences in yield and extract characteristics, with methanol yielding 5.49% compared to 1.85% for petroleum ether, indicating a higher abundance of polar phytochemicals. The methanolic extract showed notable levels of bioactive compounds, with total phenolic content (TPC) of 84.1 mg/g GAE and total flavonoid content (TFC) of 45.46 mg/g RE. Acute oral toxicity studies demonstrated no mortality or adverse effects in rats up to 2000 mg/kg, supporting extract safety. Doses of 200 mg/kg and 400 mg/kg were selected for further evaluation. In an in vivo ethylene glycol-induced urolithiasis model, the extract significantly reduced urine volume abnormalities, moderated body weight gain, and decreased elevated urinary calcium, oxalate, and phosphate levels. Furthermore, it lowered serum BUN, creatinine, and urea, indicating improved renal function. Overall, Sinapisarvensismethanolic extract, particularly at 400 mg/kg, exhibited strong anti-urolithic and nephroprotective effects comparable to the standard drug Cystone, highlighting its therapeutic potential.The results highlight the therapeutic promise of Sinapisarvensismethanolic leaf extract as a natural, safe, and effective agent for the prevention and management of urolithiasis. Its rich phytochemical content contributes to antioxidant and nephroprotective effects, warranting further studies to elucidate its mechanisms and validate its clinical potential.

Keywords: Sinapisarvensis, phytochemical screening, anti-urolithic activity, Wistar rats, phenolics, flavonoids, nephroprotection

I. INTRODUCTION

Urolithiasis, commonly known as kidney stone disease, is a widespread and recurrent disorder of the urinary tract characterized by the formation of crystalline deposits such as calcium oxalate, uric acid, and phosphate within the kidneys. It affects a significant portion of the global population and is associated with severe pain, urinary obstruction, renal dysfunction, and a high recurrence rate **(Alelign and Petros 2018).**Conventional treatment approaches, including surgical removal, lithotripsy, and pharmacotherapy, provide relief but are often costly, invasive, and associated with side effects or limited efficacy in preventing recurrence. These limitations have encouraged the search for safer, more effective, and affordable alternatives **(Geraghty et al., 2018).**

Medicinal plants have been widely explored in traditional systems of medicine for managing urinary calculi due to their diuretic, antioxidant, anti-inflammatory, and crystal-inhibiting properties **(Fitriet al., 2020).**Phytochemicals such as flavonoids, phenolics, tannins, and saponins present in herbal remedies are believed to reduce supersaturation of stone-forming constituents, enhance urinary flow, and protect renal tissues from oxidative damage. As a result, plant-based therapies hold promise as natural anti-urolithic agents with fewer adverse effects compared to synthetic drugs **(Allam and Sabra 2024).**

Sinapisarvensis L., commonly referred to as wild mustard, is an annual herb belonging to the family Brassicaceae. It is widely distributed in temperate regions and is often considered a weed in agricultural lands. Despite its weedy nature, this plant has been traditionally used in folk medicine to treat various ailments, including respiratory disorders, inflammation, and microbial infections. The presence of glucosinolates and their hydrolysis products in Sinapis species is well-documented, and these compounds are known to possess antimicrobial, anticancer, and antioxidant properties **(Ayadiet al., 2022).**Phytochemical evaluation of S. arvensis is crucial, as it not only helps in identifying the classes of bioactive compounds present but also provides a scientific basis for its medicinal applications. Preliminary screening of phytochemicals serves as a foundation for further isolation, characterization, and pharmacological testing of individual compounds. Moreover, such investigations are important for validating traditional claims, promoting the safe use of herbal remedies, and exploring their potential as sources of novel therapeutic agents **(Anand et al., 2019).**

The present study was therefore designed to carry out a systematic phytochemical evaluation of Sinapisarvensis leaf extracts and to assess their anti-urolithic activity using Wistar rats as an experimental model. Urolithiasis was induced experimentally using ethylene glycol, and the effect of the extract was compared against a standard anti-urolithic drug.

II. MAERIAL AND METHODS

2.1 Chemicals

Glacial Acetic Acid, Nitroprusside, Sodium Hydroxide and Ammonia was procured from Merck. Ethyl acetate was received from Research lab. 95% Alcohol, Conc. HCl and Chloroform was acquired from Clorofiltind, while Normal saline was obtained from JoinHubPharma.Ethylene glycolwas received from Orion Chem. All other solvents, Chemicals and reagents used were of analytical (AR) grade and purchased from Molychem, Fizmerck, Rankem and Himedia.

2.2 Plant Collection and Authentication

Leaves of Sinapisarvensis, a commonly cultivated species in private home gardens and agricultural fields, were harvested with care. The freshly collected plant materials were rinsed thoroughly with running water to remove surface impurities, air-dried at room temperature, and subsequently ground into a coarse powder using a mechanical grinder. The powdered material was stored in airtight containers to preserve its phytochemical integrity for further analysis.

The plant specimen was identified and authenticated by a qualified botanist to ensure taxonomic accuracy, and a voucher specimen was deposited for future reference.

2.3 Preparation of Plant Extract

The powdered leaf material of Sinapisarvensis was subjected to successive solvent extraction using a Soxhlet apparatus. Initially, approximately [150gm] of the powdered sample was loaded into a thimble and defatted using petroleum ether (boiling point 40–60°C) to remove lipophilic and non-polar constituents. The extraction was performed by maintaining the temperature of the heating mantle within the specified range.

After the defatting step, the plant material was air-dried to remove residual solvent. The dried residue was then reintroduced into the Soxhlet apparatus and extracted with methanol for 72 hours to obtain polar and semi-polar phytoconstituents.

The methanolic extract was concentrated under reduced pressure using a rotary evaporator at 40°C to remove the solvent. The final concentrated extract was collected and stored in airtight containers at 4°C until further use **(Zreenet al., 2022).** Extraction yield of all extracts was calculated using the following equation below:

Formula of Percentage yield =

$$\frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100$$

2.4 Quantitative Tests

- **Spectrophotometric Quantification of Total Phenolic Content: -**

The total phenolic content (TPC) of the plant extract was quantified using the Folin–Ciocalteu colorimetric method, with gallic acid serving as the standard reference compound. Briefly, 0.2 mL of plant extract from the stock solution was mixed with 2.5 mL of Folin–Ciocalteu reagent, followed by the addition of 2 mL of 7.5% sodium carbonate solution. The resulting mixture was diluted with distilled water to a final volume of 7 ml. After incubating the reaction mixture at room temperature for 2 hours in the dark, the absorbance was measured at 760 nm using a UV-Vis spectrophotometer.

A calibration curve was constructed using gallic acid standard solutions at concentrations of 20, 40, 60, 80, and 100 µg/mL. The Folin–Ciocalteu reagent reacts with phenolic compounds and other reducing agents to produce a blue chromophore, which is quantified spectrophotometrically. The total phenolic content of the extract was expressed in terms of milligrams of gallic acid equivalents (GAE) per gram of extract (mg GAE/g) **(Nikolaevaet al., 2022).**

- **Spectrophotometric Quantification of Total Flavonoid Content: -**

The total flavonoid content (TFC) of the plant extract was evaluated using the aluminum chloride colorimetric method with rutin as the standard. In this assay, 0.5 ml of the plant extract was mixed with 2 ml of distilled water, followed by the addition of 0.15 ml of 5% sodium nitrite solution. After allowing the mixture to stand for 6 minutes, 0.15 ml of 10% aluminum chloride solution was added, and the reaction mixture was again left to stand for 6 minutes. Subsequently, 2 ml of 4% sodium hydroxide was added to the mixture, which was then thoroughly mixed.

The absorbance of the resulting solution was measured at 510 nm using a UV–Vis spectrophotometer. A calibration curve was generated using standard solutions of rutin at concentrations of 20, 40, 60, 80, and 100 µg/ml. The total flavonoid content of the extract was calculated from the standard curve and expressed as milligrams of rutin equivalents per gram of dry extract weight (mg RE/g) **(Perk et al., 2016).**

2.5 Acute Toxicity Studies

An acute oral toxicity study was conducted to evaluate the safety profile of the methanol extract of Sinapisarvensis leaves, following the OECD Guideline 423. A total of 15 healthy rats were randomly divided into five groups, with three animals in each group. The groups included a control group, which received normal saline, and four treatment groups administered the extract at doses of 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg body weight, respectively.

Doses were calculated individually based on each animal's body weight. Prior to administration, the animals were fasted overnight. Following treatment, the animals were observed closely for signs of toxicity, behavioural changes, and mortality—initially during the first four hours post-dosing, then at regular intervals over the first 24 hours, and subsequently on a daily basis for a period of 14 days. Throughout the study, food and water intake were recorded daily per cage. Body weight changes, as well as general health and cage-side observations, were monitored continuously to detect any adverse effects. Ethical approval for the experimental protocol was obtained from the Institutional Animal Ethical Committee before the commencement of the study **(Rodrigues-Junior et al., 2017).**

2.6 In vivo study of urolithic activity in rats

2.6.1 Experimental work

The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee. A total of 30 adult Wistar albino rats (weighing 200 ± 20 g) were obtained from the Pinnacle Biomedical Research Institute (PBRI), Bhopal. Upon arrival, the animals were housed in standard laboratory conditions, in accordance with institutional guidelines.

During a one-week acclimatization period, the rats were provided with a standard diet (Golden Feed, Bhopal) and fresh water ad libitum. Environmental conditions were strictly maintained with a controlled temperature of 22 ± 1°C, relative humidity between 40–60%, and a regulated 14-hour light and 10-hour dark cycle.

Sixteen hours prior to the start of the experiment, food was withdrawn, and the animals were allowed access to water only. All experimental procedures, including surgical interventions where applicable, were conducted under aseptic conditions to minimize the risk of infection and ensure animal welfare **(Khan et al., 2021).**

2.6.2 Experimental protocol

In this investigation aiming to assess the effects on kidney stone formation in male Wistar albino rats, a urolithiasis model was established using ethylene glycol (0.75% v/v). The rats were organized into groups, each comprising six individuals.

Group I served as the control, following a standard diet for rats and access to water as per their requirements. Groups II to VII were exposed to ethylene glycol (0.75% v/v) dissolved in their drinking water from the initiation of the study until day 28, deliberately inducing the formation of kidney stones. This induction period lasted from day 1 to day 14. Group II was deliberately induced to develop the disease, representing the condition with kidney stones. In the subsequent phase, spanning from the 14th to the 28th day, Group III

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was administered the reference drug Cystone at a dosage of 750 mg/kg b.w. Meanwhile, Groups IV to V received varying doses of the Sinapisarvensisextract, namely 200 mg/kg b.w, p.o., and 400 mg/kg b.w, p.o., respectively. These administrations were carried out from the 14th to the 28th day, constituting the treatment period. On the crucial 28th day of the study, a blood sample (1 mL) was meticulously obtained from each animal through the retro-orbital plexus under anesthesia. Concurrently, urine samples were collected from all the animals on 28th days. Comprehensive biochemical analyses were then conducted on these samples, focusing on measuring BUN and creatinine levels in the blood serum samples and calcium, oxalate, phosphate in urine samples **(Abo-Ghneimet al., 2024).**

Experimental Design

- Group I served as normal control with standard Diet + Water
- Group II served asDisease Control with ethylene glycol only
- Group III Served as Standard Drug Treated with Cystone (750 mg/kg b.w., p.o.)
- Group IV Served as lower concentration of theSinapisarvensis leaves extract (200 mg/kg b.w., p.o.)
- Group V Served as higher concentration of the Sinapisarvensis leaves extract (400 mg/kg b.w., p.o.)

2.6.3 Biochemical and Urinary Analysis

Urine samples were collected from all experimental animals on Day 28 using individual metabolic cages to confirm the presence of kidney stone formation. To preserve the integrity of the samples, a drop of concentrated hydrochloric acid (HCl) was added, and the samples were stored at 4°C until analysis. Urine output was measured on both Day 14 and Day 28 using polypropylene collection cages to monitor changes in urinary volume across the treatment period. Concurrently, the body weight of each animal was recorded at regular intervals throughout the study to assess any treatment-related effects on general health.

On Day 29, blood samples were collected from the retro-orbital plexus under anesthesia induced by ether. The collected blood was then centrifuged at 15,000 rpm for 10 minutes to separate the serum. The resulting serum samples were carefully handled and analyzed to determine key biochemical parameters, including blood urea nitrogen (BUN), uric acid, and creatinine levels. These markers provided critical insights into renal function and the therapeutic impact of the test substances **(Amarteyet al., 2015).**

- Collect 24-hour urine samples at specific intervals to measure parameters such as calcium, oxalate, phosphate, and creatinine.
- Evaluate serum markers of kidney function, including blood urea nitrogen (BUN), creatinine, and uric acid.

III. RESULTS

3.1 Plant Collection

Table 1: Plant collection

Plant name	Plant part used	Weight (gm)
Sinapisarvensis	Leaves	150

3.2 Percentage yield of extracts

Table 2: Percentage yield of Sinapisarvensisextract

Solvent	Color of extract	Theoretical weight (gm)	Yield in gms	% Yield
Pet. Ether	Yellow	150	2.781	1.854
Methanol	Brownish	150	8.235	5.49

3.3 Solubility determination

Table 3: Solubility Determination of Sinapisarvensisextract

Solvent	Pet. Ether	Methanol
Water	Insoluble	Slightly soluble
Methanol	Insoluble	Soluble
Chloroform	Slightly soluble	Slightly soluble
DMSO	Soluble	Soluble
Petroleum Ether	Soluble	Insoluble

3.4 Phytochemical screening

3.4.1 Qualitative Phytochemical Analysis of extracts

Table 4: Phytochemical analysis of Sinapisarvensisextracts

		Result	
S. No.	**Experiment**	**Pet. Ether extract**	**Methanol extract**
Test for Carbohydrates			
1.	Molisch's Test	-	+
2.	Fehling's Test	-	+
3.	Benedict's Test	-	+
4.	Bareford's Test	-	+
Test for Alkaloids			
1.	Mayer's Test	-	+
2.	Hager's Test	-	+
3.	Wagner's Test	-	+
4.	Dragendroff's Test	-	+
Test for Terpenoids			
1.	Salkowski Test	+	+
2.	Libermann-Burchard's Test	+	+
Test for Flavonoids			
1.	Lead Acetate Test	-	+
2.	Alkaline Reagent Test	-	+
3.	Shinoda Test	-	+
Test for Tannins and Phenolic Compounds			
1.	$FeCl_3$ Test	-	+
2.	Lead Acetate Test	-	+
3.	Gelatine Test	-	+
4.	Dilute Iodine Solution Test	-	+
Test for Saponins			
1.	Froth Test	+	-
Test for Protein and Amino acids			
1.	Ninhydrin Test	-	-
2.	Biuret's Test	-	+
3.	Million's Test	-	+
Test for Glycosides			
1.	Legal's Test	-	+
2.	Keller Killani Test	-	+
3.	Borntrager's Test	-	+

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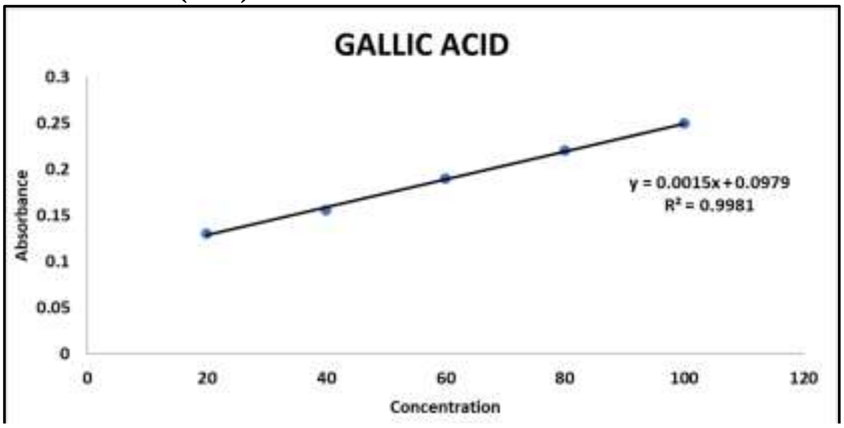
3.4.2 Quantitative phytochemical estimation

- **Total Phenolic Content (TPC) Estimation:**



Graph 1: Graph represent standard curve of Gallic acid

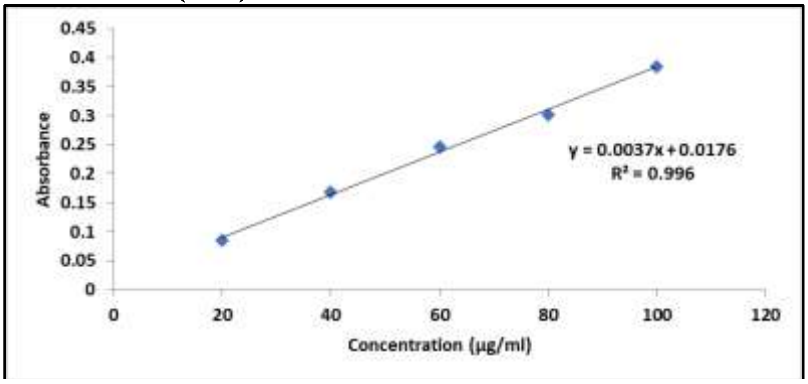
Table 9: Total Phenolic content in Sinapisarvensisextract

S. No	Absorbance
1.	0.141
2.	0.177
3.	0.230

Table 5:Total Phenolic Content of extract Sinapisarvensis

Extracts	Total Phenolic content (mg/gm equivalent of Gallic acid)
Methanol	84.1

- **Total Flavonoid Content (TFC) Estimation:**



Graph 2: Graph represent standard curve of Rutin

Table 6: Total Flavonoid content in Sinapisarvensisextract

S. No	Absorbance
1	0.095
2.	0.177
3.	0.191

Table 7: Total Flavonoid Content of extract Sinapisarvensis

Extracts	Total Phenolic content (mg/gm equivalent of Gallic acid)
Methanol	45.46

Figure 1: Total Phenolic content in Sinapisarvensisextract

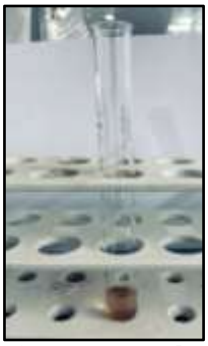


Figure 2: Total Flavonoid Content in Sinapisarvensisextract

3.5 In vivo acute oral toxicity (OECD 423)

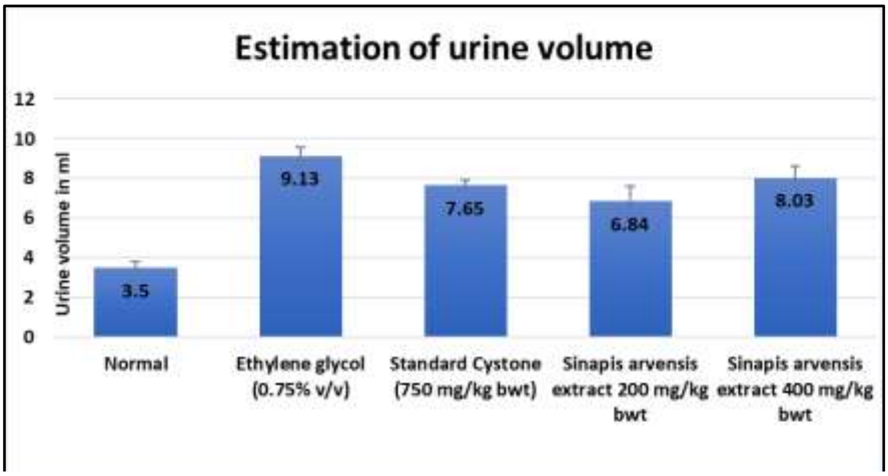
Table 8: General appearance and behavioural observations of acute oral toxicity study for control and treated groups:

Observations	Control	5 mg/kg	50 mg/kg	300 mg/kg	2000 mg/kg
Food intake	Normal	Normal	Normal	Normal	Normal
Body weight	Normal	No change	No change	No change	No change
Temperature	Normal	Normal	Normal	Normal	Normal
Changes in skin and fur	No effect	No effect	No effect	No effect	No effect
Urination	Normal	No effect	No effect	No effect	No effect
Diarrhoea	Not present	Not present	Not present	Not present	Not present
Death	Alive	Alive	Alive	Alive	Alive

3.6 In vivo anti-urolithic activity

Table 9: Estimation of urine volume

Groups	Urine Volume (ml)
Normal	3.50±0.45
Ethylene glycol (0.75% v/v)	9.13±0.30
Standard Cystone (750 mg/kg bwt)	7.65±0.24
Sinapisarvensisextract 200 mg/kg bwt	6.84±0.76
Sinapisarvensisextract 400 mg/kg bwt	8.03±0.61



Graph 3: Estimation of urine volume

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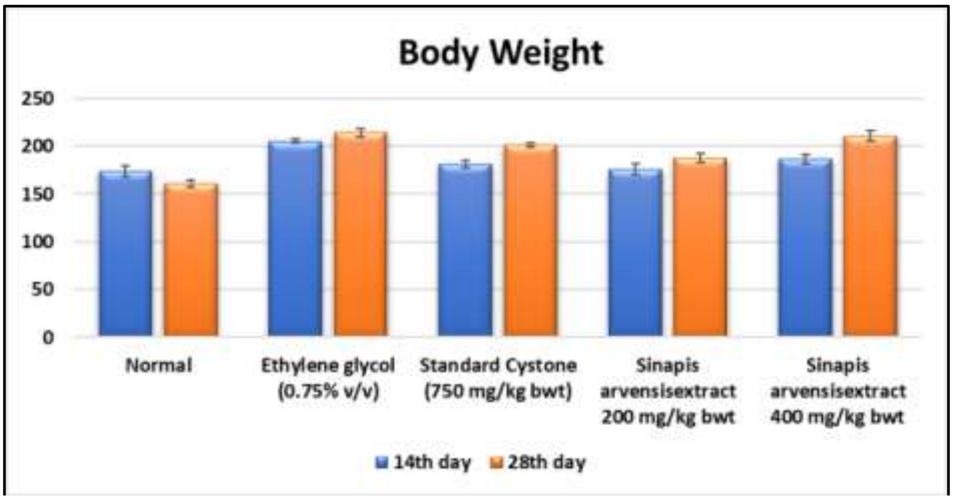
Table 10: Estimation of Body weight

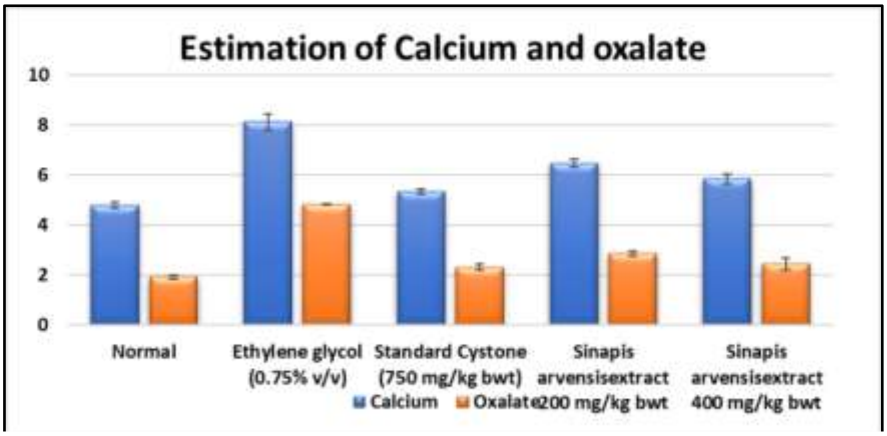
Groups	Body weight	
	14th day	28th
Normal	173.7±6.32	160.8±3.63
Ethylene glycol (0.75% v/v)	205.4±2.45	214.1±4.45
Standard Cystone (750 mg/kg bwt)	181.09±3.37	201.4±2.29
Sinapisarvensisextract 200 mg/kg bwt	175.7±6.23	187.6±4.65
Sinapisarvensisextract 400 mg/kg bwt	186.6±4.46	210.5±5.38



Graph 4: Estimation of body weight

Table 11: Estimation of calcium, oxalate, and phosphate

Groups	Calcium	Oxalate	Phosphate
Normal	4.8±0.12	1.93±0.08	141.87±3.62
Ethylene glycol (0.75% v/v)	8.13±0.32	4.83±0.03	374.54±2.53
Standard Cystone (750 mg/kg bwt)	5.34±0.09	2.32±0.12	164.94±4.92
Sinapisarvensisextract 200 mg/kg bwt	6.48±0.15	2.87±0.09	222.02±6.76
Sinapisarvensisextract 400 mg/kg bwt	5.85±0.21	2.45±0.25	189.54±3.74



Graph 5: Estimation of Calcium and oxalate

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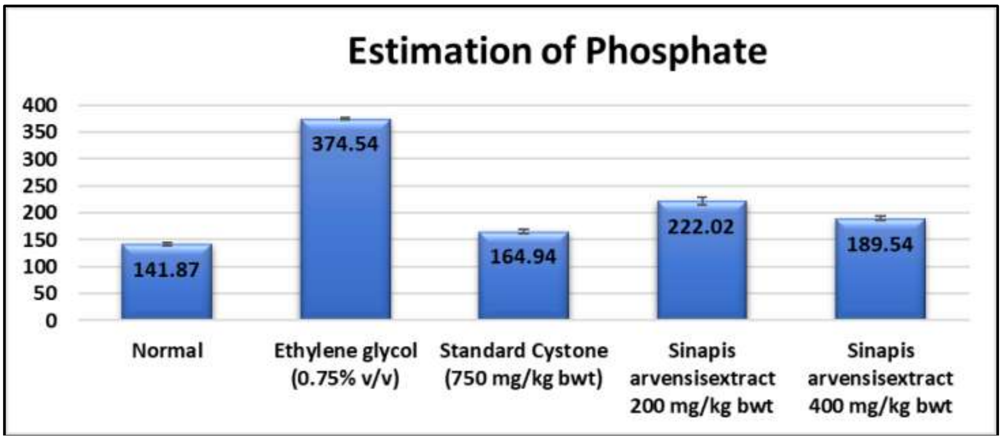
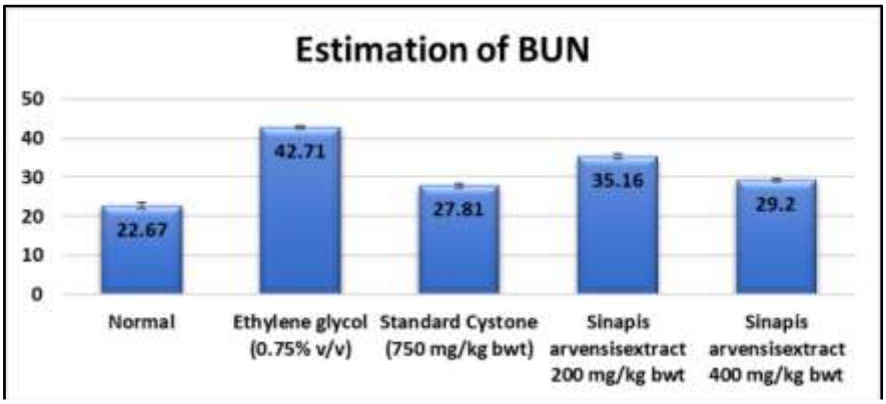


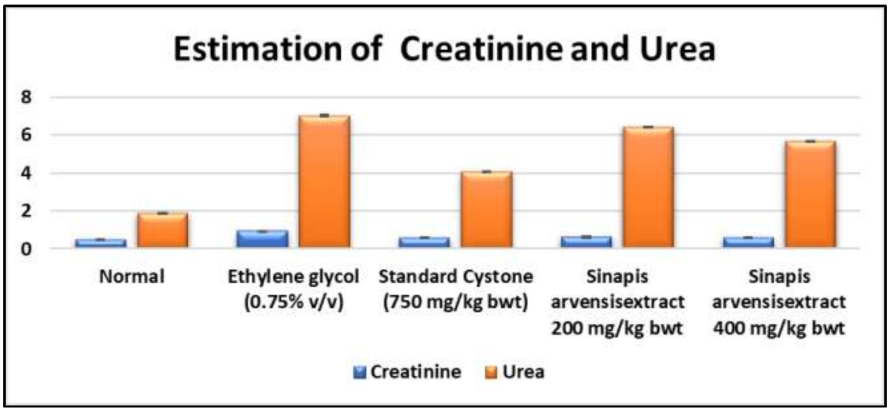
Graph 6: Estimation of Phosphate

Table 12: Estimation of serum BUN, Creatinine and Urea

Groups	BUN	Creatinine	Urea
Normal	22.67±0.74	0.50±0.003	1.88±0.012
Ethylene glycol (0.75% v/v)	42.71±0.34	0.93±0.01	7.02±0.033
Standard Cystone (750 mg/kg bwt)	27.81±0.58	0.57±0.03	4.04±0.023
Sinapisarvensisextract 200 mg/kg bwt	35.16±0.67	0.62±0.04	6.43±0.014
Sinapisarvensisextract 400 mg/kg bwt	29.20±0.45	0.59±0.02	5.67±0.019



Graph 7: Estimation of BUN in blood serum



Graph 8: Estimation Creatinine and Urea in blood serum

IV. CONCLUSION

The findings from this study strongly support the therapeutic potential of Sinapisarvensismethanolic leaf extract in the prevention and management of urolithiasis. Its rich phytochemical content, particularly phenolic and flavonoid compounds, contributes to its antioxidant and nephroprotective effects. The extract was found to be safe for oral administration at high doses, and its significant activity in reducing stone-forming constituents and improving kidney function highlights its possible role as a natural alternative to conventional anti-urolithic treatments. Overall, Sinapisarvensis demonstrates considerable promise as a plant-based therapeutic agent, and further research including mechanism of action studies and clinical trials is warranted to fully validate and harness its medicinal benefits.

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