A Comparative Study of *Plectranthus scutellarioides* and *Phragmites vulgaris* Plant Extracts For Evaluation of Antiurolithiatic Activity

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ABSTRACT

Urolithiasis, the formation of urinary stones, remains a prevalent urological disorder with limited effective preventive options. This study aimed to evaluate the antiurolithiatic activity of *Plectranthus scutellarioides* and *Phragmites vulgaris*, individually and in combination, using primarily *in vitro* calcium oxalate (CaC_2O_4) crystal inhibition assays and *in vivo* assays using *Rattus norvegicus* animal model. The experimental design involved the preparation of aqueous extracts and tea formulations from the plant materials, followed by qualitative phytochemical analysis to identify bioactive constituents. *In vitro* assessments measured the percent inhibition of CaC_2O_4 crystal nucleation, aggregation, and growth, while *in vivo* studies employed a sodium oxalate-induced urolithiatic rat model to assess urinary parameters, biochemical markers, and electrolytes, as well as hepatotoxicity of the formulated tea. Phytochemical screening confirmed the presence of triterpenes, sterols, phenols, alkaloids, and fatty acids in both plants, with *P. vulgaris* also containing flavonoids and tannins. *In vitro* results revealed that *P. vulgaris* had the strongest inhibition across all stages of CaC_2O_4 crystallization. *P. scutellarioides* demonstrated good antiurolithiatic activity, particularly *in vivo*, with improvements in urinary output and normalization of serum creatinine, urea, calcium, magnesium, and phosphorus levels. The combined extract showed moderate but consistent effects *in vivo*, suggesting additive benefits without synergistic enhancement. All treatments showed no signs of hepatotoxicity, as evidenced by stable alanine aminotransferase (ALT) levels. Overall, these findings highlight *P. vulgaris* as the most potent antiurolithiatic agent among the tested treatments, with *P. scutellarioides* also showing therapeutic promise. The study also highlights the therapeutic promise of tea-based formulations for future application.

Keywords: antiurolithiatic tea, calcium oxalate, hepatotoxicity, kidney stones, serum kidney function

INTRODUCTION

Background of the Study

Kidney stones, also known as urolithiasis, are major health concern because of the prevalence they cause. The condition results in hard mineral and salt deposits in the kidneys that are primarily composed of calcium oxalate, uric acid, or other substances. Historically, medications and dietary changes were utilized to treat and prevent kidney stones. However, the research of alternative medicines has grown in popularity, with several plants being investigated for potential antiurolithiatic properties.

Plectranthus scutellarioides, also known as *Mayana*, and *Phragmites vulgaris,* locally known as *Tanubong*, are two plants that have long been used in folk medicine to treat a variety of diseases, including urinary tract disorders. *P. scutellarioides* is traditionally known for its anti-inflammatory and diuretic effects (Mustarichie et al., 2017; Nisa et al., 2021), while *P. vulgaris* is traditionally known for its diuretic and lithotriptic effects (Ahmed et al., 2016). Despite their historical applications, there have been no further investigations on their antiurolithiatic activity. Some studies also suggest further scientific exploration of *P. scutellarioides* plant on its inhibitory activity (Levita et al., 2016). Moreover, there are no comprehensive scientific studies comparing the antiurolithiatic activity of these two plants.

Therefore, this research seeks to fill this gap by comparing the possible antiurolithiatic activities of *P. scutellarioides* and *P. vulgaris* plant extracts as well as their synergistic activity, providing insight into their potential as natural alternatives or supplements to conventional treatments for kidney stone prevention.

Statement of the Objectives

The primary objective of this study was to compare the antiurolithiatic activities of *P. scutellarioides* (leaf) and *P. vulgaris* (shoot), both individually and in combination. The study involved the formulation of herbal tea using the dried leaves of *P. scutellarioides*, dried shoots of *P. vulgaris* and their combination, and the assessment of its hepatoxicity in a laboratory animal model. The research was conducted from January 2025 to May 2025.

Specifically, the study aimed to:

1. Determine the percent yields of crude extracts from *P. scutellarioides* leaf and *P. vulgaris* shoot.

2. Evaluate the *in vitro* antiurolithiatic activities of *P. scutellarioides* leaf extract, *P. vulgaris* shoot extract, and a 1:1 combined extract, by assessing the percent inhibition in the following assays:
    a. Crystal nucleation
    b. Crystal aggregation
    c. Crystal growth

3. Compare the effectiveness of *P. scutellarioides*, *P. vulgaris*, and the combined extracts in inhibiting:
    a. Crystal nucleation
    b. Crystal aggregation
    c. Crystal growth

4. Assess the *in vivo* effect of *P. scutellarioides* leaf extract*, P. vulgaris* shoot extract, and the 1:1 combined extract on calcium oxalate crystal formation in a laboratory animal model, by monitoring:
    a. Urinary parameters
    b. Serum kidney function markers
    c. Electrolytes

5. Formulate an antiurolithiatic herbal tea of *P. scutellarioides, P. vulgaris*, or a combination of both.

6. Assess the hepatotoxicity of the formulated herbal tea in laboratory animal models.

METHODOLOGY

Research Design

The study employed an experimental and developmental research design to evaluate the antiurolithiatic activity of plant extracts, comparing them with *sambong* and *Cystone*® through *in vitro* assays based on absorbance and turbidity, and *in vivo* tests on rats induced with urolithiasis. Tea formulations using *Plectranthus scutellarioides*, *Phragmites vulgaris*, and their combination were developed and administered to animal models for toxicity testing.

Study Site and Sample Collection

Plant samples were gathered from Barangay Bascaran, Solano, Nueva Vizcaya, with laboratory work conducted at Saint Mary's University, in collaboration with a chemist from the University of Santo Tomas and a private diagnostic clinic in Bambang. Plant species were taxonomically certified by Nueva Vizcaya State University, and laboratory rats were sourced from a local pet shop with veterinary certification.

Data Gathering Procedure

Collection, Drying, and Extraction of Plant Materials

P. scutellarioides (leaf) and *P. vulgaris* (shoot) were collected from Bascaran, Solano, Nueva Vizcaya, with *P. scutellarioides* cultivated under controlled conditions prior to harvesting. Both samples were thoroughly washed with distilled water to remove impurities. The leaves of *P. scutellarioides* were air-dried for 1–2 weeks, while the shoots of P. vulgaris were oven-dried at 45 °C for 3 days. The dried materials were ground into powder, stored in airtight containers, and subjected to decoction by boiling at 80 °C for an hour, followed by overnight percolation. The resulting extracts were filtered, concentrated using a rotary evaporator, and refrigerated in sterile bottles for use in antiurolithiatic assays.

Determination of Percent Yield

The percentage yield was computed by weighing the dried extract after evaporation and dividing it by the initial weight of plant material. The formula used was:

$$\% \text{ Yield} = (\text{Weight of Dried Extract} / \text{Weight of Plant Material}) \times 100$$

In vitro Antiurolithiatic Assays

Crystal nucleation was assessed by mixing calcium chloride and sodium oxalate in the presence of extracts or controls, and measuring absorbance at 600 nm, as described by Mosquera et al. (2020) and Zarin et al. (2020), with minor modifications to the measurement protocol. Aggregation was tested using pre-formed calcium oxalate monohydrate crystals, and observing the effect of the extracts on particle clustering following the procedure of Bawari et al. (2018) as cited by Mosquera et al. (2020) with minor modifications in measurements. Crystal growth was analyzed by oxalate depletion rate at 230 nm using a slurry of CaC_2O_4 crystals, with inhibition percentages computed similarly to nucleation and aggregation. All tests were performed in 5 replicates using standard protocols and equipment based on the procedure of Mosquera et al. (2020).

Microscopic Evaluation

Crystal formation and morphology were visually examined under a Leica DM300 microscope at 4x magnification. Differences in size, shape, and number of CaC_2O_4 crystals in the presence and absence of treatments were documented.

In vivo Antiurolithiatic Testing

The antiurolithiatic effect of the plant extracts against sodium oxalate-induced urolithiasis in rats was determined based on the procedure of Sayed, (2023). Rats were divided into prophylactic and curative regimen groups and further subdivided into treatment groups receiving extracts, *Cystone*, or saline. Sodium oxalate was administered intraperitoneally to induce urolithiasis. Extracts were administered either alongside NaOx (prophylactic) or after stone induction (curative). Groupings also included treatments with individual and combined plant extracts to compare efficacy.

Urinalysis

Rats were isolated in metabolic cages for 24-hour urine collection before the experiment's end. Urinary volume, pH, and specific gravity were measured. Crystals were further analyzed microscopically after centrifugation of urine samples.

Biochemical Markers and Electrolytes

Blood was collected via cardiac puncture, and serum was separated for testing. Kidney function markers (creatinine, urea, uric acid) and electrolytes (calcium, phosphorus, magnesium) were analyzed using PROFAME diagnostic kits to assess renal impact and systemic balance post-treatment.

Formulation of Tea

Dried and powdered plant parts were filled into 2-gram tea bags. Moisture content and uniformity were ensured to maintain stability and dosing. Packaging followed clean, room-temperature storage standards, ensuring product quality for future therapeutic application.

Hepatotoxicity Testing of Formulated Tea

Tea formulations were administered to rats via oral gavage for 7 days, followed by ALT level testing to assess liver safety. Blood samples were processed for serum, which was then analyzed at a local diagnostic lab. ALT values were interpreted according to UCLA-DLAM reference ranges, with deviations indicating potential hepatotoxic effects.

Treatment of Data

Mean and standard deviation were used to summarize values for both *in vitro* and *in vivo* testing.

An independent samples t-test was used to compare the crystal aggregation rate and crystal growth of *P. vulgaris* and the combined extract.

One-Way Analysis of Variance (ANOVA) with Tukey HSD as a post hoc test was used to compare the crystal nucleation test of P. scutellarioides, P. vulgaris, and their combination in vitro, as well as to compare levels of kidney function markers and electrolytes in animal models.

The following normal range of values for the different kidney function markers and electrolytes in rats were used as reference in the study: (Houtmeyers et al., 2016); University of California, Los Angeles (UCLA) – Division of Laboratory Animal Medicine (DLAM).

Ethical Consideration

The study was conducted with strict adherence to ethical guidelines, receiving approval from the Saint Mary's University Research Ethics Board (SMUREB) and an animal research permit from the Bureau of Animal Industry through PITAHC-IACUC. Emphasizing the Reduction principle of the 3Rs, the study design minimized animal use, notably by excluding Blumea balsamifera (*Sambong*) as a positive control *in vivo*. Laboratory animals underwent a seven-day acclimatization period with unrestricted access to food and water. Restraint techniques were carefully employed to reduce stress and the risk of injury, and plant extracts were administered via intraperitoneal injection for systemic effects, while oral gavage was used for hepatotoxicity tests to ensure precise dosing.

RESULTS AND DISCUSSIONS

Section 1: Percent Yield

Table 1

Percent Yields of Plant Samples

	Plant Material (g)	Dried Extract (g)	Percent Yield (%)
P. scutellarioides	639.3	92.52	**14.472**
P. vulgaris	415.12	45.42	**10.941**

Plectranthus scutellarioides (14.472%) showed a higher percent yield than *Phragmites vulgaris* (10.941%), suggesting it may be more efficient for extraction. This result supports previous studies, such as those by Ntungwe et al. (2021) and Astuti et al. (2021), which highlight the high extractability and rich secondary metabolite content of P. scutellarioides. The difference in yields may be due to variations in phytochemical composition, moisture content, and plant structure. These factors are critical when choosing plant materials for therapeutic use, as higher yields can enhance extraction efficiency and reduce formulation costs.

Section 2: Phytochemical screening

Table 2

Detected Secondary Metabolites from Plant Samples

Secondary Metabolites	*P. scutellarioides*	*P. vulgaris*
Essential Oil	+	-
Triterpenes	+	+
Sterols	+	+
Phenols	+	+
Fatty Acids	+	+
All carbohydrates except tetroses and trioses	+	-
Anthraquinones	-	+
Coumarins	+	+
Anthrones	+	+
Tannins	-	+
Flavonoids	-	+
Alkaloids	+	+
Steroids	+	-
Amino acid	-	-

Table 2 reveals that both *P. scutellarioides* and *P. vulgaris* are rich in secondary metabolites, including triterpenes, sterols, phenols, fatty acids, and alkaloids, with *P. vulgaris* also containing tannins and flavonoids. These findings align with previous studies of Fajardo et al. (2017), highlighting the antioxidant and antiurolithiatic potential of *P. vulgaris* due to its flavonoid and tannin content. In contrast, *Plectranthus* species exhibit varying phytochemical profiles influenced by species and environmental conditions. The presence of diverse bioactive compounds in both plants suggests their potential value in developing plant-based treatments

for urolithiasis (Farouk et al., 2023). *P. vulgaris*, in particular, stands out as a promising candidate for such formulations.

Section 3: *In vitro* Antiurolithiatic Activity of the Plant Extracts

To evaluate the potential antiurolithiatic properties of the plant extracts, three *in vitro* assays were conducted: crystal nucleation, aggregation, and growth inhibition, supported by microscopic analysis. These tests measured the ability of *P. scutellarioides*, *P.vulgaris*, and their combination to prevent the formation and development of calcium oxalate crystals. The results are presented in Table 3, which shows the mean percent inhibition values, the statistical tests, and their corresponding interpretations.

Table 3

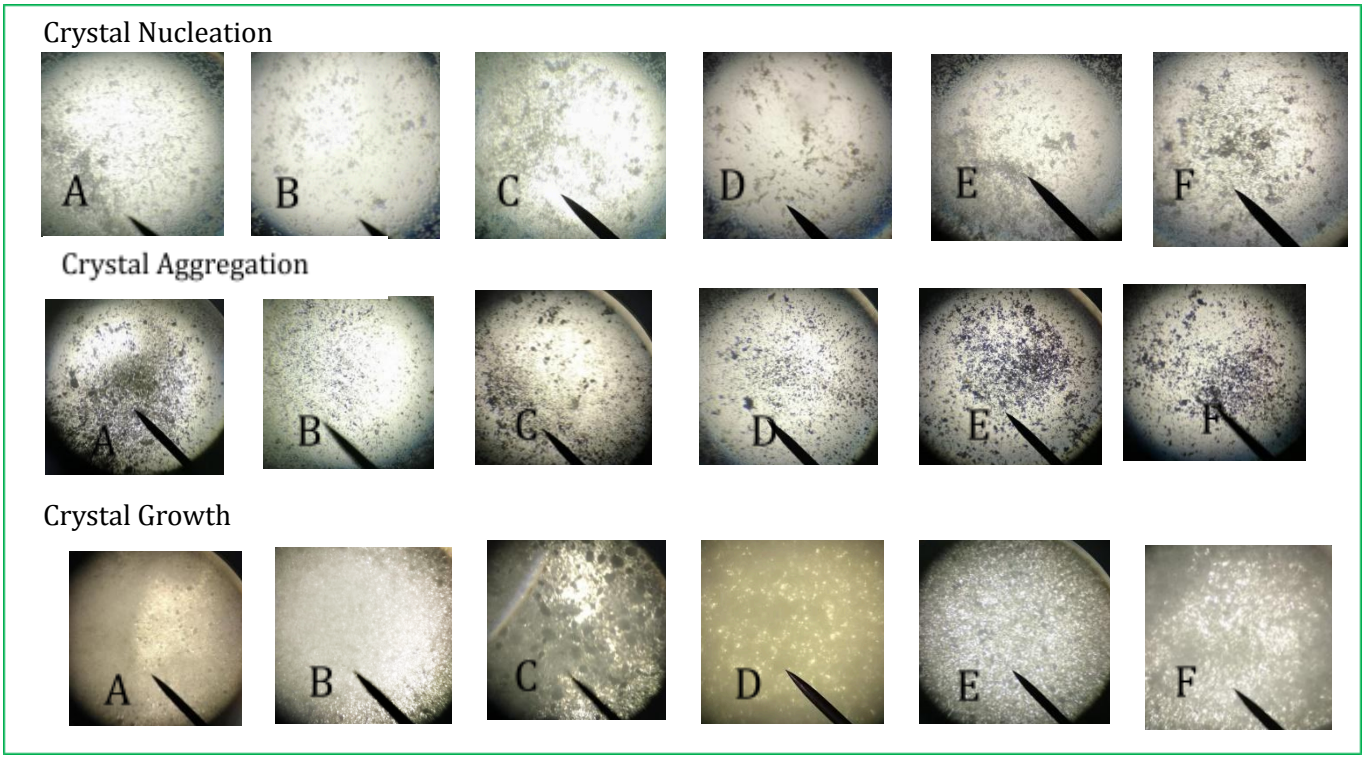
Comparisons of the percent inhibitions of the different extracts in the in vitro tests

***In vitro* Test**	**Extracts**	**n**	**Mean ± SD**	**Statistic**	**p-value**	**Interpretation**
Crystal Nucleation Assay	*P. scutellarioides*	2	17.34 ± 10.15	F=3.069	0.103	Not significant
	P. vulgaris	5	39.97 ± 5.83			
	Combined	4	19.73 ± 21.2			
Crystal Aggregation Assay	*P. vulgaris*	5	36.61 ± 7.01	t=3.212	0.012	Significant
	Combined	5	17.91 ± 10.97			
Crystal Growth Assay	*P. vulgaris*	3	7.79 ± 4.65	t=0.363	0.735	Not Significant
	Combined	3	6.70 ± 2.29			

The results show no significant difference in the percent inhibition of crystal nucleation among the three extract groups (*P. scutellarioides, P. vulgaris*, and their combination), but *P. scutellarioides* exhibited negative values in aggregation and growth assays, limiting further comparison. *P. vulgaris* extract showed significantly higher inhibition of crystal aggregation than the combined extract, although no significant difference was observed in crystal growth between the two. Overall, *P. vulgaris* demonstrated superior antiurolithiatic activity, effectively inhibiting calcium oxalate crystal nucleation, aggregation, and growth. This supports the claim by Farouk et al. (2023) that *P. vulgaris* possesses strong antioxidant and anti-inflammatory properties due to its high flavonoid and tannin content, which are known to interfere with calcium oxalate crystallization (Kumar & Pradhan, 2014; Yuen & Ng, 2015). Microscopic analysis confirmed these findings, showing fewer, smaller, and fragmented crystals in *P. vulgaris*-treated samples, while *P. scutellarioides* and the combined extract produced larger and more structured crystals, consistent with the lower inhibitory effects observed *in vitro*.

Figure 5

Microscopic Evaluation of crystals in in vitro assays. A.) P. scutellarioides, B.) P. vulgaris, C.) Combined Extract, D.) Sambong, E.) Cystone, F.) Distilled Water.



Overall, *P. vulgaris* demonstrated the most promising antiurolithiatic activity, particularly in inhibiting crystal nucleation and aggregation, although only the effect on aggregation was statistically significant. *P. scutellarioides* and the combined extract showed weaker and inconsistent activities. These findings support the potential of *P. vulgaris* as a candidate for further development of antiurolithiatic formulations and suggest that combining the two extracts may not enhance therapeutic efficacy.

Section 4: *In vivo* effects of Plant extracts on Sodium oxalate–induced urolithiasis

This section evaluates the therapeutic and preventive effects of *P. scutellarioides*, *P. vulgaris*, and their combined aqueous extracts on sodium oxalate -induced urolithiatic rats. The assessment includes analysis of urinary parameters (volume, pH, specific gravity), serum biochemical markers (creatinine, urea, uric acid), and electrolyte levels (calcium, phosphorus, and magnesium), which are all essential indicators of renal function and lithogenesis.

Table 4 presents urinary output, pH, and specific gravity for rats across the prophylactic and curative groups. Rats treated with the extracts—particularly the combined formulation—showed increased urine output, which may enhance the elimination of crystals and reduce the risk of stone formation. A significant elevation in urinary pH was also observed, especially with *P. scutellarioides*, potentially contributing to the solubilization of calcium oxalate crystals.

Table 4

Effect of aqueous extracts on urinary output and pH of urolithiatic rats

	Urinary Parameters (Mean ± Standard Deviation)		
Groups	Volume (mL/24hr)	pH	Specific Gravity
Prophylactic			
Group 1: Saline	11.67 ± 2.89	7.33 ± 0.577	1.02 ± 0.012
Group 2: NaOx	11.88 ± 2.02	7.33 ± 0. 577	1.01 ± 0.001
Group 3: Extracts			
3A: *P.scutellarioides*	21.35 ± 7.28	8.50 ± 0.707	0.51 ± 0.714
3B: *P. vulgaris*	17.90 ± 4.10	7.50 ± 0.707	1.02 ± 0.008
3C: Combined	12.88 ± 3.88	8.33 ± 1.16	1.01 ± 0.001
Group 4: *Cystone*	21.75 ± 3.18	7.50 ± 0.707	1.04 ± 0
Curative			
Group 1: Saline	10.00 ± 2.89	8.00 ± 0.577	1.01 ± 0.012
Group 2: NaOx	10.00 ± 2.02	8.00 ± 0.577	1.04 ± 0.001
Group 3: Extracts			
3A: *P.scutellarioides*	16.67 ± 1.44	7.67 ± 1.15	1.02 ± 0.001
3B: *P. vulgaris*	15.25 ± 5.30	8.00 ± 0	1.02 ± 0.001
3C: Combined	23.40 ± 7.21	8.50 ± 0.707	1.01 ± 0.001
Group 4: *Cystone*	12.67 ± 2.52	6.67 ± 0.577	1.02 ± 0.014

In both prophylactic and curative models, *P. vulgaris* and *P. scutellarioides* extracts showed significant diuretic activity, with *P. scutellarioides* and *Cystone*® inducing the highest urine output in prevention, while the combined extract exhibited the greatest diuretic effect in treatment, surpassing *Cystone*® (Mustarichie et al., 2017; Aziz et al., 2021). The extracts also caused urinary alkalinization—particularly with *P. scutellarioides* and the combined formulation—which may reduce calcium oxalate crystallization by increasing urine pH and dilution, although excessive alkalinity could risk phosphate stone formation (Manisha et al., 2023; Mohamed et al., 2024). Overall, these changes in urinary volume, pH, and specific gravity suggest that these plant extracts enhance urine flow and modulate urinary conditions to prevent or treat urolithiasis, positioning them as promising herbal agents for managing kidney stones.

Table 5 presents the effects of aqueous extracts of *P. scutellarioides*, *P. vulgaris*, and their combination on serum biochemical markers—creatinine, urea, and uric acid—in sodium oxalate–induced urolithiatic rats under both prophylactic and curative models.

Table 5

Effect of aqueous extracts on some serum kidney function markers of urolithiatic rats

		Biochemical Markers (Mean ± Standard Deviation)		
Groups	**n**	**Creatinine (mg/dl)**	**Urea (mgs/dl)**	**Uric Acid (mg/dl)**
Prophylactic				
Group 1: Saline	3	0.67 ± 0.115	16.04 ± 2.27	14.34 ± 0.255
Group 2: NaOx	3	0.69 ± 0.308[a]	17.79 ± 1.13[d]	19.78 ± 0.220[d]
Group 3: Extracts				
3A: *P. scutellariodes*	3	0.33 ± 0.133[a]	13.28 ± 0.127[b]	7.87 ± 0.127[a]

3B: *P. vulgaris*	3	0.13 ± 0[a]	12.16 ± 0.127[b]	8.60 ± 0.254[b]
3C: Combined	3	0.56 ± 0.390[a]	15.52 ± 0.127[c]	10.02 ± 0.170[c]
Group 4: *Cystone*	3	0.33 ± 0.177[a]	5.97 ± 0. 127[a]	9.90 ± 0.153[c]
Curative				
Group 1: Saline	3	0.53 ± 0.115	17.24 ± 2.27	14.63 ± 0.255
Group 2: NaOx	3	0.86 ± 0.308[a]	17.99 ± 1.13[c]	19.78 ± 0.220[d]
Group 3: Extracts				
3A: *P. scutellariodes*	3	0.44 ± 0.204[a]	8.21 ± 0.340[b]	10.76 ± 0.112[b]
3B: *P. vulgaris*	3	0.24 ± 0.039[a]	8.96 ± 0[b]	11.18 ± 0.128[c]
3C: Combined	3	0.58 ± 0.234[a]	8.43 ± 0.133[b]	8.82 ± 0.126[a]
Group 4: *Cystone*	3	0.38 ± 0.300b[a]	6.72 ± 0[a]	11.40 ± 0[c]

Legend: Mean and standard deviation were tested with ANOVA and Tukey HSD as post hoc (α=0.05). Values with different column superscript letters of the same regimen are significantly different.

The table shows that sodium oxalate (NaOx) induced significant kidney dysfunction in rats, evidenced by elevated serum creatinine, urea, and uric acid levels compared to controls (Houtmeyers et al., 2016). Treatment with *P. vulgaris*, *P. scutellarioides*, their combined extract, and the standard drug *Cystone* significantly reduced these markers, with *P. vulgaris* exhibiting the strongest nephroprotective effect on creatinine and urea, and *P. scutellarioides* being more effective in lowering uric acid (Arra et al., 2024; Brancalion et al., 2012). The combined extract showed moderate but consistent improvements, supporting the potential of multi-herbal formulations. These findings align with previous studies highlighting the renal benefits of plant phytochemicals and underscore the value of early, prophylactic intervention in managing urolithiasis and renal impairment.

Table 6 summarizes the effects of aqueous plant extracts on serum electrolyte levels—calcium, phosphorus, and magnesium—in sodium oxalate–induced urolithiatic rats, assessed under both prophylactic and curative models. These serum elements are critical markers for evaluating mineral balance and kidney function, as disturbances in their levels often reflect pathological changes associated with urolithiasis. The administration of *P. scutellarioides*, *P. vulgaris*, their combination, and *Cystone*® was examined to determine their potential in restoring electrolyte homeostasis disrupted by sodium oxalate. Significant differences among the treatment groups provide insights into the mineral-modulating effects and therapeutic relevance of these extracts in mitigating renal damage and supporting recovery.

Table 6

Effect of aqueous extracts on some serum elements of urolithiatic rats

		Electrolytes (Mean ± Standrd Deviation)		
Groups	**n**	Calcium (mmol/L)	Phosphorus (mmol/L)	Magnesium (mmol/L)
Prophylactic				
Group 1: Saline	3	9.97 ± 0.248	13.08 ± 0.046	0.67 ± 0
Group 2: NaOx	3	10.31 ± 0[e]	14.34 ± 0.133[e]	0.59 ± 0[a]
Group 3: Extracts				
3A: *P. scutellarioides*	3	9.16 ± 0.021[b]	10.49 ± 0.211[c]	0.69 ± 0.005[b]
3B: *P. vulgaris*	3	8.91 ± 0.064[a]	8.54 ± 0.046[b]	0.74 ± 0.006[c]
3C: Combined	3	9.93 ± 0.025[d]	10.97 ± 0.085[d]	0.66 ± 0[b]
Group 4: *Cystone*	3	9.33 ± 0.025[c]	5.32 ± 0.061[a]	0.82 ± 0.032[d]
Curative				

Group 1: Saline	3	9.86 ± 0.248	13.11 ± 0.046	0.67 ± 0
Group 2: NaOx	3	10.31 ± 0[e]	14.49 ± 0.133[d]	0.59 ± 0[a]
Group 3: Extracts				
3A: *P. scutellarioides*	3	9.23 ± 0.017[b]	9.40 ± 0.051[b]	0.70 ± 0.006[c]
3B: *P. vulgaris*	3	9.02 ± 0.017[a]	8.94 ± 0.133[a]	0.64 ± 0[b]
3C: Combined	3	9.55 ± 0.010[c]	11.40 ± 0.260[c]	0.59 ± 0.006[a]
Group 4: *Cystone*	3	9.63 ± 0.010[d]	8.77 ± 0.098[a]	0.59 ± 0.006[a]

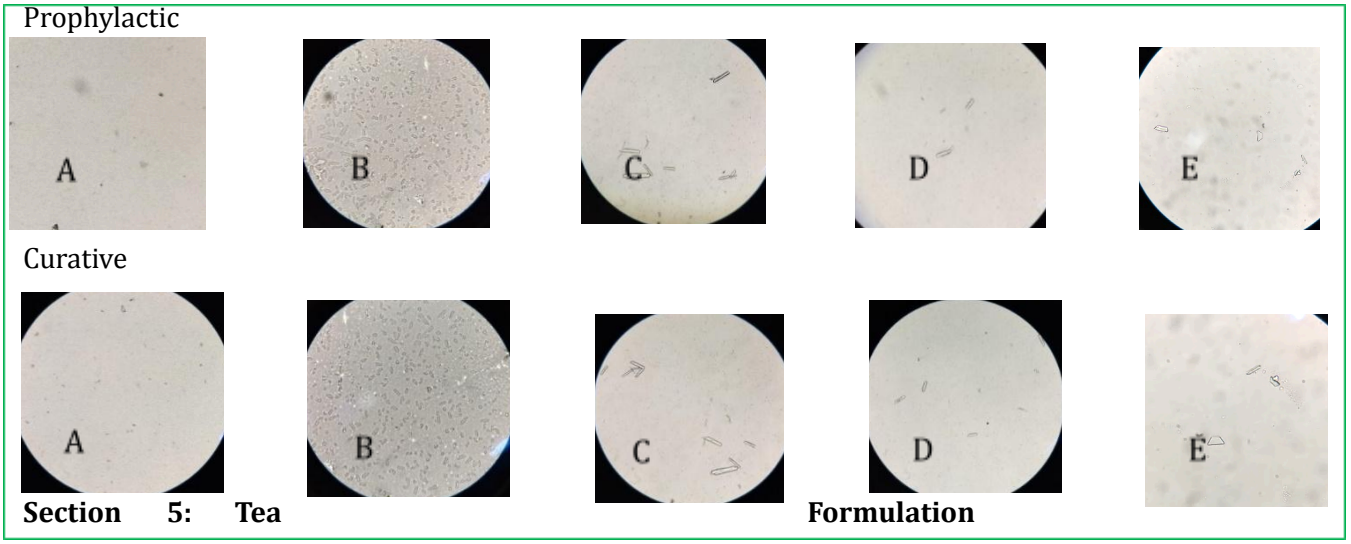
Legend: Mean and standard deviation were tested with ANOVA and Tukey HSD as post hoc (α=0.05). Values with the different column superscript letters of the same regimen are significantly different.

Sodium oxalate (NaOx) induced kidney dysfunction in rats, reflected by significantly elevated serum creatinine, urea, and uric acid levels, which were effectively reduced by treatment with *P. vulgaris*, *P. scutellarioides*, their combined extract, and the standard drug *Cystone* (Houtmeyers et al., 2016). Among these, *P. vulgaris* showed the most potent nephroprotective effects, particularly in lowering creatinine and urea, while *P. scutellarioides* was more effective in reducing uric acid levels, consistent with the known renal benefits of phytochemicals such as flavonoids and sterols (Arra et al., 2024; Brancalion et al., 2012). The combined extract demonstrated moderate but consistent efficacy, supporting the potential of multi-herbal formulations for managing urolithiasis-related kidney impairment. These results emphasize the therapeutic promise of these plants and the importance of early prophylactic treatment to mitigate renal damage.

Figure 6 presents the microscopic evaluation of urinary crystals from experimental rats across different treatment groups. This analysis was conducted to assess the qualitative impact of plant extracts on the formation and presence of calcium oxalate crystals in the urine. Visual comparison of crystal morphology and abundance offers insight into the antiurolithiatic potential of each treatment, especially in relation to the standard drug *Cystone*.

Figure 6

Microscopic Evaluation of crystals in urine. Saline, NaOx, P.scutellarioides, P. vulgaris, Cystone, respectively (A-E).



Section 5: Tea Formulation

The individual teas were labeled as follows:

F1 – *P. scutellarioides* (Scutel Tea)

F2 – *P. vulgaris* (TanuBrew)

F3 – Combination tea (*P. scutellarioides* + *P. vulgaris*, 1:1) – (UroBlend)

Table 7

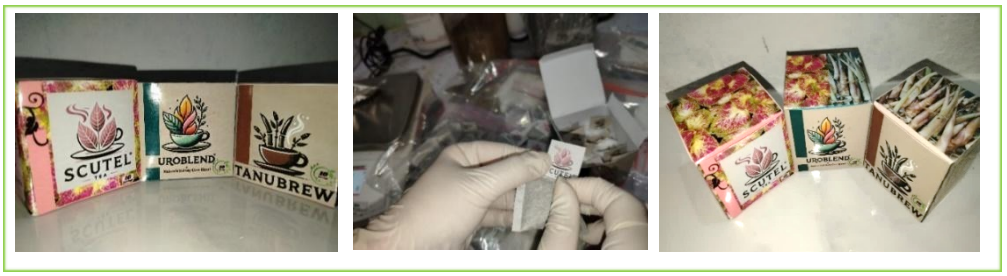
Organoleptic Properties of the Formulated Tea

Formulation	Color	Aroma	Solubility
F1	Deep red-brown	Grassy-sweet	Good
F2	Light yellow-brown	Earthy-minty	Good
F3	Reddish brown	Herbal-sweet	Excellent

The combination tea (F3) exhibited the most acceptable organoleptic properties based on informal sensory evaluation. The successful formulation of herbal teas from *P. scutellarioides*, *P. vulgaris*, and their combination provides a promising avenue for the development of natural antiurolithiatic agents. Furthermore, the use of dried leaf decoctions is a practical, culturally acceptable, and cost-effective approach for traditional medicine integration. Future studies should focus on optimizing dosage, evaluating long-term safety, and conducting *in vivo* toxicity and efficacy trials to validate these findings.

Figure 7

Formulated Tea



Section 6: Hepatotoxicity (ALT)

The evaluation of hepatotoxicity is a crucial aspect of assessing the safety profile of any therapeutic formulation. Alanine aminotransferase (ALT) is a liver enzyme commonly used as a biomarker to detect liver damage or inflammation. Figure 7 illustrates the ALT enzyme activity measured in serum samples.

Figure 8

Levels of alanine transferase liver enzyme (ALT) in rats treated with formulated tea

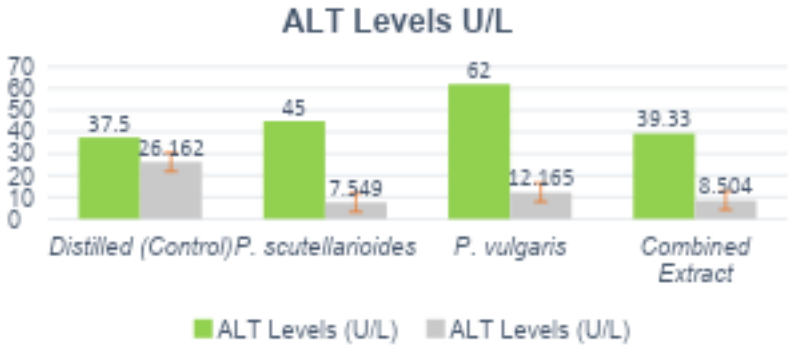


Figure 8 shows that alanine aminotransferase (ALT) levels in rats treated with *P. scutellarioides* and *P. vulgaris* teas were slightly elevated compared to the control, with *P. vulgaris* causing a more noticeable increase, while the combined extract's ALT levels remained close to baseline. These findings suggest mild effects on liver function without severe hepatotoxicity, aligning with previous studies indicating that minor ALT elevations may reflect phytochemical metabolism rather than liver damage (Subramanya et al., 2018; Mandlekar et al., 2006; Houtmeyers et al., 2016). The combination extract's moderation of ALT levels suggests a potential protective effect, supporting the safety of these teas as therapeutic agents for urolithiasis without significant liver toxicity.

Further Discussion

During in vitro assays, varying color intensities of plant extracts at different concentrations affected absorbance readings, making it difficult to distinguish true biological inhibition from optical interference caused by pigmented compounds such as flavonoids and chlorophyll. To address this, the study standardized the analysis at one concentration (100 µg/mL) to minimize color-related variability and improve result accuracy. *In vivo*, individual animal variability and ethical considerations influenced the study design; Sambong was excluded as a positive control to reduce animal use in accordance with the 3Rs principle, since its antiurolithiatic efficacy is well established. These methodological choices balanced scientific rigor with ethical responsibility, enhancing the study's reliability and integrity.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

This study evaluated the antiurolithiatic activity of *Plectranthus scutellarioides* and *Phragmites vulgaris* plant extracts as well as the hepatotoxicity of the formulated herbal tea. The study demonstrated that *P. scutellarioides* had a higher percent yield compared to *P. vulgaris*. *P. vulgaris* exhibited stronger antiurolithiatic activity, particularly in inhibiting crystal nucleation and aggregation, as well as inhibition of crystal growth. Phytochemical screening revealed that both plants were rich in secondary metabolites, with *P. vulgaris* additionally containing tannins and flavonoids, which may have contributed to its superior activity. *In vivo* studies in urolithiatic rats confirmed the efficacy of the extracts. *P. vulgaris* and the combined extract groups showed improved urine output and favorable urinary pH levels. Biochemical and electrolyte analyses indicated protective effects on kidney function, with reduced urea and creatinine levels, regulated calcium and phosphorus concentrations, and enhanced magnesium retention. Formulated tea products showed acceptable hepatotoxicity profiles, with ALT levels remaining within a safe range. Overall, *P. vulgaris* demonstrated promising potential as a main ingredient in antiurolithiatic herbal tea formulations, supported by its strong biological activity and acceptable safety profile.

Recommendations

1. Utilize the result of the study for product upscaling, exploring the incorporation of additional natural ingredients with complementary antiurolithiatic effects.
2. Further studies should isolate and characterize the active compounds in *P. vulgaris* responsible for its high inhibitory effects.
3. Evaluate a broader range of doses and conduct sub-chronic or chronic toxicity studies to establish the long-term safety and optimal dosage of the formulated tea for preventive and therapeutic use.

4. Further toxicological studies, such as histopathological analysis of liver and kidney tissues, should be conducted to fully confirm the non-toxic nature of *P. scutellarioides*, *P. vulgaris*, and their combined extract.
5. Explore and evaluate the palatability of the formulated tea.

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