In Vitro Evaluation of the Anti-Urolithiatic Activity of Combined Leaf Extracts of Sambong (*Blumea balsamifera*) and Sampa-Sampalukan (*Phyllanthus niruri*)

Precious Dianne D. Defenio, Mery Joy M. Dela Cruz, Angeline Rovy J. Frusa, Apple Jane D. Siroy, Dr. Erwin M. Faller

Department of Pharmacy, St. Alexius College, Koronadal City, South Cotabato, Philippines

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ABSTRACT

Urolithiasis, primarily caused by calcium oxalate (CaOx) crystal deposition within the renal system, shows a significant and growing public health concern in the Philippines. Although Sambong (*Blumea balsamifera*) and Sampa-sampalukan (*Phyllanthus niruri*) are widely known within the traditional ethnopharmacology for treating urinary tract disorders, their scientific validation in terms of their combined anti-urolithiatic efficacy remains unexplored. This study evaluated the *in vitro* anti-urolithiatic activity of ethanolic leaf extract combinations (*B. balsamifera* and *P. niruri*) across three formulation ratios (75:25, 50:50, and 25:75) at a fixed working concentration of 400 µg/mL. The inhibitory ability against CaOx crystal growth was determined using a 10-minute static turbidimetric assay, measuring absorbance changes at 620 nm via UV-Vis spectrophotometry, with Potassium citrate as the positive control and distilled water as the baseline. In the Statistical analysis, using Two-Way Analysis of Variance (ANOVA) and Tukey's HSD post hoc test showed that anti-urolithiatic activity was significantly proportion-dependent ($p < 0.0001$). The 25:75 (*B. balsamifera* : *P. niruri*) ratio achieved synergistic inhibition (4.01%), whereas the 75:25 and 50:50 ratios demonstrated pro-crystallization / enhanced turbidity (-1.50% and -0.92%, respectively). Potassium citrate presented the highest overall efficacy (9.72%). Time and the treatment-time interaction were not statistically significant ($p > 0.05$), indicating early, stable, and sustained inhibitory effects against crystal growth. These findings demonstrate that a higher proportion of *P. niruri* is essential to achieve synergistic crystal growth inhibition, likely due to its active site surface coating by its rich polyphenol, lignan, and ellagic acid content. Overall, this study provides experimental support for optimizing ratio-dependent, and standardized polyherbal formulations in the clinical management of urolithiasis.

Keywords: Urolithiasis, *Blumea balsamifera, Phyllanthus niruri,* Calcium oxalate, Crystal growth

INTRODUCTION

Urolithiasis, or kidney stone formation, is a growing global health concern, with millions of new cases reported annually and notable regional and sex-related disparities (Wei et al., 2025). It has also been identified as a significant risk factor for chronic kidney disease (CKD), as individuals with kidney stones are more likely to develop CKD over time (Kim et al., 2024). In 2021, the global burden of urolithiasis reached 106 million new cases, with men accounting for approximately 67% (71.1 million) of all incident cases (GBD 2021 Urolithiasis Collaborators, 2024).

According to Zheng et al. (2025), the incidence of urolithiasis varies considerably across regions. An example of this was China reporting an age-standardized incidence rate of 964.7 cases per 100,000 population, which is lower than the global average of approximately 1,243 cases per 100,000 population. In addition to these regional disparities, the increasing burden of urolithiasis among adolescents and young adults has emerged as a critical public health concern. Wei et al. (2025) reported 6,467,487 cases within this younger population, with males accounting for 57.8% of the cases. The study also revealed a notable increase in incidence (AAPC = 0.29) among individuals aged 20 to 24 years. These alarming figures underscore the urgent need for improved diagnostic tools and effective preventive and therapeutic interventions across affected populations.

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In the Philippines, the burden of urolithiasis remains substantial. The Philippine Council for Health Research and Development (2024) reported that urolithiasis accounts for approximately 25% of all admissions to the Philippine General Hospital Division of Urology. It emphasized that untreated cases may result in serious complications, including edema and complete urinary obstruction. The National Kidney and Transplant Institute (NKTI) recognizes kidney stones as one of the major contributors to chronic kidney disease, a condition that continues to rise nationwide. In 2024, NKTI reported that more than 64,845 Filipinos were undergoing dialysis, representing a 22% increase from the previous year, with urolithiasis identified as a significant contributing factor (Philippine News Agency., 2025).

Locally, the growing burden of kidney disease, including kidney stones, has prompted healthcare initiatives in South Cotabato and Koronadal City. In response to the increasing number of kidney disease cases, the South Cotabato Provincial Hospital (SCPH) established a Chronic Kidney Disease (CKD) Clinic in 2024 (South Cotabato Provincial Hospital, 2024). Hospital records further demonstrate the persistent occurrence of kidney stone cases, with 44 cases recorded in 2020, 36 in 2021, 87 in 2022, 98 in 2023, and a peak of 132 cases in 2024, followed by 107 cases in 2025. This indicates that urolithiasis remains a significant local health concern that warrants further investigation and the development of effective management strategies.

During the increasing prevalence of urolithiasis, scientific investigations have demonstrated the therapeutic potential of Sambong *(Blumea balsamifera*) and Sampa-sampalukan (*Phyllanthus niruri*) in managing kidney stone formation. These medicinal plants act through different mechanisms as *Blumea balsamifera* primarily inhibits the aggregation phase by preventing the adhesion of small crystal particles into larger stones (Widhiantara & Jawi., 2021) while *Phyllanthus niruri* acts during the early stages of stone formation by inhibiting crystal nucleation and growth (Pucci et al., 2018) and by disrupting ion precipitation, *Phyllanthus niruri* prevents the formation of a stable crystal lattice (Nirumand et al., 2018).

The pathogenesis of urolithiasis involves a process of crystal nucleation, growth, and aggregation, with each stage governed by distinct physicochemical mechanisms (Widhiantara & Jawi., 2021). Since individual plant extracts often target only specific stages of this process, combining botanical extracts with complementary mechanisms of action may provide a more comprehensive inhibitory effect against stone formation. Despite the documented anti-urolithiatic properties of *Blumea balsamifera* and *Phyllanthus niruri*, the potential synergistic effect of their combined extracts in inhibiting calcium oxalate crystal formation and growth remains largely unexplored. Therefore, this study seeks to determine their ability to inhibit calcium oxalate crystal growth and provide scientific evidence supporting their traditional use in the management of urolithiasis, thereby contributing to the advancement of Philippine phytomedicine.

METHODOLOGY

Plant Collection and Authentication

Fresh, mature, and healthy leaves of *Blumea balsamifera* and *Phyllanthus niruri* were harvested from Brgy. Panay, Santo Niño, South Cotabato. The plant specimens were submitted to a licensed botanist for taxonomic authentication, and voucher specimens were deposited for future reference (Rathore et al., 2025).

The collected leaves of Sambong (*Blumea balsamifera*) and Sampa-sampalukan (*Phyllanthus niruri*) were cleaned separately by removing foreign matter, rinsed with distilled water, blotted dry, and then shade-dried at 25–30 °C until a constant weight was reached (Nair et al., 2025). The dried leaves were then cut into 1–2 cm pieces and pulverized into a uniform powder using a high-speed blender to increase the surface area for efficient solvent penetration (Kouakoua et al., 2024).

Preparation of Extracts

For the ethanolic maceration, 50 g of powdered *B. balsamifera* leaves were immersed in 500 mL of 99.5% ethanol (Montealegre et al., 2017) and a separate batch of 50 g of powdered *P. niruri* leaves was immersed in 500 mL of 96% ethanol (Olukunle et al., 2021). Both mixtures were contained in sealed 500 mL glass containers

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and are macerated for 72 hours at a controlled room temperature of 25°C with manual agitation performed twice daily to maximize solvent-solute interaction and refresh the extraction interface (Abubakar & Haque, 2020).

The resulting mixtures undergo primary filtration using a clean, double-layer muslin cloth to remove large particulate matter (Altemimi et al., 2017). Then the solvents were removed using a rotary evaporator at 45°C. To ensure efficient evaporation and prevent thermal degradation, a rotation speed of 120 rpm was maintained under 17.5 kPa (Tan et al., 2020). After the evaporation process, the concentrated residues were labeled as "*B. balsamifera* ethanolic extract" and "*P. niruri* ethanolic extract," respectively. These extracts were stored in light-resistant amber bottles at 2 to 8°C inside a desiccator to prevent phytochemical degradation and moisture absorption (Nkhili et al., 2020).

Preparation of Concentrations

To evaluate the efficacy of different formulation ratios alongside single-extract baselines at a fixed therapeutic dose, separate stock solutions for each plant were first prepared to ensure stoichiometric accuracy. A Sambong stock solution was created by dissolving 120 mg of the dried *Blumea balsamifera* crude extract in 100 mL of distilled water, yielding a concentration of 1200 µg/mL. Similarly, a Sampa-sampalukan stock solution was prepared by dissolving 120 mg of the dried *Phyllanthus niruri* crude extract in 100 mL of distilled water, which also yields 1200 µg/mL (Bawari et al., 2019).

From these individual stocks, the three experimental treatments (75:25, 50:50, and 25:75) were formulated in 10 mL aliquots into labeled test tubes according to the Simplex Lattice-based design principles (Gandhi et al., 2022). The 75:25 Ratio (Sambong: Sampa-sampalukan) were prepared by mixing 7.5 mL of the Sambong stock with 2.5 mL of the Sampa-sampalukan stock; the 50:50 Ratio (Sambong: Sampa-sampalukan) were prepared by combining 5.0 mL of the Sambong stock with 5.0 mL of the Sampa-sampalukan stock; and the 25:75 Ratio (Sambong: Sampa-sampalukan) were prepared by mixing 2.5 mL of the Sambong stock with 7.5 mL of the Sampa-sampalukan stock. All of these solutions were passed through a 0.45 µm syringe filter prior to use to remove particulate matter that may interfere with optical readings (Azwanida, 2015).

When 10 mL of any of these prepared mixtures is added to the assay (Total Volume = 30 mL), the final concentration of the combined extract in the 50 mL beaker is fixed at 400 µg/mL (Bawari et al., 2019).

Preparation of Calcium chloride and Sodium oxalate Stock Solutions

To simulate the physiological conditions of stone formation *in vitro*, the equimolar 0.01 M supersaturated solutions were prepared to serve as the primary reactants. First, a 0.01 M Calcium chloride solution was prepared by accurately weighing 0.147 g of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) using an analytical balance as then this quantity was dissolved in distilled water and diluted to the mark in a 100 mL Erlenmeyer flask. Separately, a 0.01 M Sodium Oxalate solution was prepared by weighing 0.134 g of sodium oxalate ($Na_2C_2O_4$). This salt was dissolved in distilled water and similarly diluted to volume in a distinct 100 mL Erlenmeyer flask. These stock solutions function as the source of calcium and oxalate ions, and were utilized across all experimental and control setups to induce crystallization (Sikarwar et al., 2017).

Experimental Controls and Spectrophotometric Quantification

To validate the assay and provide a comparative baseline for inhibition, two distinct control groups were established. The baseline serves as the baseline for maximum crystal growth in the absence of any inhibitor. This setup involves the 1 mL volume reserved for the treatment agent is replaced with 1 mL of distilled water, which is mixed with 1 mL of the calcium chloride dihydrate solution and 1 mL of the sodium oxalate solution in the cuvette. Since distilled water exerts no inhibitory effect, the turbidity measured from this group represents 0% inhibition ($ODcontrol$). It serves as the mathematical denominator for calculating the percentage inhibition of the plant extracts (Sikarwar et al., 2017).

In the positive control group, utilizing Potassium citrate which is a clinically standard anti-urolithiatic agent, was included to validate the assay's sensitivity. A stock solution of 1,200 µg/mL was prepared by dissolving 120 mg

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of potassium citrate powder in 100 mL of distilled water. For the assay, 1 mL of this stock solution was introduced to the reaction mixture (containing 1 mL calcium chloride and 1 mL sodium oxalate). Absorbance readings were recorded at 2-minute intervals starting from the point of treatment induction at 2 minutes and concluding at the 10-minute mark. The inhibition data derived from this group serve as a benchmark for the efficacy, ensuring the experimental system is capable of detecting valid crystal inhibition (Mao et al., 2021).

Testing of Inhibitory Effects on Calcium oxalate Crystal Growth

The assessment of crystal growth inhibition was conducted using a static turbidimetric assay to monitor the increase in crystal turbidity over time within a stable environment. The reactions were performed directly within quartz cuvettes placed inside a UV-Vis spectrophotometer, where 1 mL of the Calcium chloride solution was combined with 1 mL of the Sodium oxalate solution to initiate primary nucleation. To evaluate the growth phase, the mixture was then allowed to stand for two minutes before 1 mL of the treatment agent (consisting of the *Blumea balsamifera* and *Phyllanthus niruri* extract ratios, distilled water, or potassium citrate) was introduced to bring the final assay volume to 3 mL (Nirumand et al., 2018).

Quantification of crystal growth was performed by measuring absorbance at a wavelength of 620 nm against a solvent blank to track changes in optical density. The cuvette remained undisturbed inside the cell holder throughout the duration of the test to ensure the reliability of the growth kinetics and eliminate variables associated with mechanical agitation. Absorbance readings were recorded at 2-minute intervals starting from the point of treatment induction at 2 minutes and concluding at the 10-minute mark. This specific timing protocol ensures the data captured reflects the progression of the crystal growth phase (Bawari et al., 2019). A lower absorbance value in the treatment groups relative to the baseline signifies the successful inhibition of calcium oxalate crystal growth by the plant extracts (Mao et al., 2021). Based on these optical density readings, the Percentage of Growth Inhibition were calculated using the following formula:

Figure 1. Percentage of Growth Inhibition Formula

$$\%\ Inhibition = \left(\frac{ODcontrol - ODtreatment}{ODcontrol}\right) \times 100$$

The percentage of crystal growth inhibition was calculated using the turbidimetric formula to quantify the efficacy of each treatment ratio. The percentage of inhibition is determined by subtracting the mean absorbance of the treatment group (ODtreatment) from the mean absorbance of the baseline ($ODcontrol$), dividing the result by the mean absorbance of the baseline, and multiplying by 100 (Khajuria et al., 2020). All experimental assays were performed in triplicate to ensure the precision and reproducibility of the gathered data.

Determination of Synergistic Interaction of the Combined Plant Ratios

To evaluate the nature of the phytochemical interaction at the standardized concentration of 400 µg/mL, the observed mean percentage inhibition data from the turbidimetric assays for each tested ratio (75:25, 50:50, and 25:75) were comparatively analyzed (Bawari et al., 2019). This analysis specifically assessed how the magnitude of inhibitory effect in each ratio changed relative to established proportional expectations.

The categorization of the interaction was based on established thresholds for interpreting combined herbal effects in turbidimetric models. A combination was classified as Synergistic if the observed inhibition demonstrated a large, non-proportional increase that significantly surpassed the combined activity of both standard ratio and proportional effects, indicating a reinforced biological action (Golong-Roa., 2018). Combinations exhibiting only a marginal or moderate increase that followed a simple additive pattern were categorized as Additive, suggesting the phytochemicals work independently without substantial amplification. Antagonistic classification would be assigned if the combined inhibitory effect was lower than proportionally expected due to potential interference. In addition, following the established turbidimetric evaluation models, combinations that failed to produce positive inhibition and instead increased absorbance relative to controls were designated in this study as Pro-crystallization / Enhanced Turbidity (Upadhyay et al., 2023).

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Statistical Treatment

To ensure the reliability and precision of the experimental results, all assays for the three formulation ratios (75:25, 50:50, 25:75), as well as the positive and baseline, were conducted in triplicate (n=3). The raw absorbance data obtained from the UV-Vis spectrophotometer at each 2-minute interval (2, 4, 6, 8, and 10 minutes) were expressed as the Mean ± Standard Deviation (SD) (Mishra et al., 2019). The percentage of crystal growth inhibition was calculated using the turbidimetric formula: % $Inhibition$ = [$ODcontrol$] - [$ODtreatment$] / [$ODcontrol$] times 100, where [$ODcontrol$] represents the mean absorbance of the baseline (Dhunna et al., 2023).

To evaluate the inhibitory effects on crystal growth across the three formulation ratios and the positive control over the specified time series, a Two-Way Analysis of Variance (ANOVA) was used. This statistical model was used to determine the main effects of the treatment groups and time intervals as well as the interaction between these two factors. Since a significant main effect was detected for the treatment ($p < 0.05$), a post hoc Tukey's Honest Significant Difference (HSD) test was conducted to identify specific pairwise differences between group means (Pallant., 2020). All statistical procedures were performed using SPSS version 26.0. A p-value of less than 0.05 was considered the threshold for statistical significance at a 95% confidence interval (Banerjee & Chitnis., 2020).

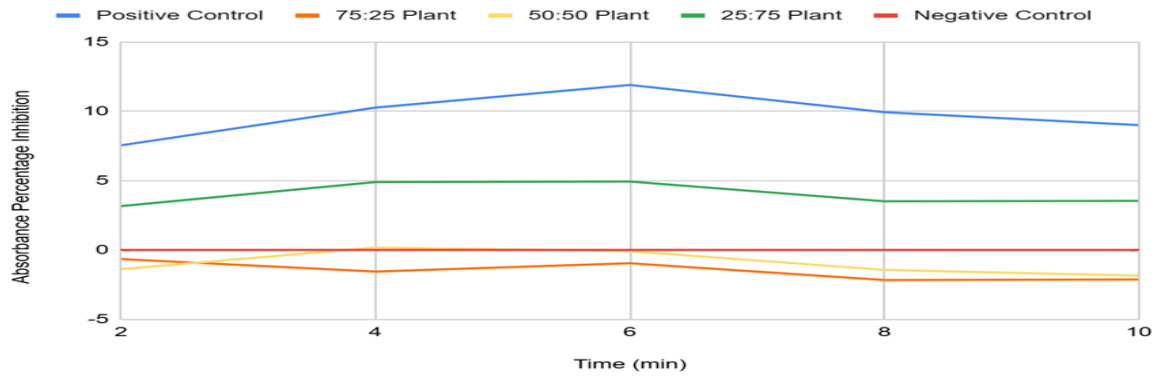
RESULTS AND DISCUSSION

Table 1. Percentage Inhibition of the Calcium oxalate Growth Phase Exhibited by the Various Treatment Groups and Controls

Treatment	Percentage Growth Inhibition (% ± SD)
75:25 (Sambong : Sampa-sampalukan)	-1.50 ± 1.61%
50:50 (Sambong : Sampa-sampalukan)	-0.92 ± 2.51%
25:75 (Sambong : Sampa-sampalukan)	4.01 ± 1.87%
Positive Control (Potassium citrate)	9.72 ± 3.59%
Negative Control (Distilled water)	0.00 ± 0.00%

Table 1 shows the percentage inhibition of the calcium oxalate growth phase at a concentration of 400 µg/mL. The data shows a clear hierarchy in anti-urolithiatic activity among the treatment groups, with the Positive Control (Potassium citrate) demonstrating the highest overall inhibitory activity, maintaining a final mean inhibition of 9.72%. Among the plant combinations, the 25:75 ratio (Sambong: Sampa-sampalukan) was the most effective, showing a final inhibition of 4.01%. In contrast, the 50:50 ratio and 75:25 ratio exhibited the lowest activity, with final results of -1.50% and -0.92%, respectively. The Negative Control (Distilled water) showed 0.00% inhibition.

Figure 2. Comparative Absorbance Percentage Inhibition of each Treatment Recorded at 2-Minute Intervals over a 10-Minute Period



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The positive control, potassium citrate, exhibited the highest efficacy (9.72%, blue line), showing a peak of activity at the 6-minute mark before stabilizing. This high performance is expected, as recent clinical reviews confirm that potassium citrate remains the gold standard for increasing urinary citrate levels as citrate acts by forming soluble complexes with calcium ions, effectively reducing the supersaturation of calcium oxalate and physically blocking crystal surfaces to prevent further aggregation and growth (Alelign and Petros., 2018).

Among the experimental formulations, the results demonstrate that the anti-urolithiatic effect of the herbal mixture is highly proportion-dependent. The 25:75 ratio, which contains the highest concentration of Sampa-sampalukan (*Phyllanthus niruri*), reached a positive final inhibition (4.01%, green line) that surpassed the other combinations which consistently remains in the positive percentage region. This outcome aligns that *P. niruri* is rich in polyphenols and flavonoids that actively interfere with the nucleation and aggregation phases of calcium oxalate crystals, even altering their morphology to make them less adhesive to renal cells (Nirumand et al., 2018).

The negative final inhibition observed in the 75:25 and 50:50 ratios (-1.50% and -0.92%, respectively) indicates significantly weaker inhibitory activity at these specific proportions. The 75:25 ratio (orange line) and the 50:50 ratio (yellow line) largely fluctuate below the zero-percent baseline, confirming their negative inhibition status during the growth phase. At these ratios, the concentration of *P. niruri* is likely insufficient to overcome the growth kinetics of the crystals within the observed timeframe. Furthermore, Sambong (*Blumea balsamifera*) may function more effectively through phase-shifting properties as Sambong extracts encourage the formation of calcium oxalate dihydrate (COD) crystals, which are less pathogenic and easier to void, rather than providing direct growth inhibition of the more harmful monohydrate (COM) form (Montealegre and De Leon., 2024).

The negative control (distilled water) yielded a final inhibition of 0.00%. This is where no inhibitory agents are present to interfere with the crystallization process, establishing the maximal rate of crystal growth. A stable 0% baseline is critical in urolithiasis research to confirm that the kinetic suppression observed in treated groups is due to the specific therapeutic properties of the plant extracts rather than experimental artifacts (Chandra et al., 2020).

Table 2A. Two-Way Analysis of Variance (ANOVA) for the Inhibitory Effects Among Combination Ratios and the Positive Control

Source of Variation	SS	df	MS	F	P value	Remarks
Treatment	1179.9	3	393.3	64.27	0.0001	Significant
Time	53.41	4	13.35	2.18	0.085	Not significant
Interaction (Treatment × Time)	41.27	12	3.44	0.56	0.86	Not significant
Error (Within)	244.68	40	6.12			
Total	1519.26	59				

The statistical significance of the inhibitory effects among the various treatments was evaluated using a Two-Way Analysis of Variance (ANOVA). The analysis reveals that the Treatment factor is the only statistically significant variable, yielding a P-value of 0.0001 ($p < 0.05$). This confirms that the anti-urolithiatic efficacy observed in the study is a direct consequence of the specific plant ratios and the positive control used, rather than random variation. Both Time ($p = 0.085$) and the Interaction between Treatment and Time ($p = 0.860$) were found to be not significant which indicates that the extracts act rapidly (within the first 2 minutes) and remain chemically stable throughout the 10-minute assay without losing efficacy over time.

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Table 2B. Post Hoc Multiple Mean Comparison Test of the Mean Difference in the Inhibitory Effects Among the Three Combination Ratios and the Positive Control

Comparison	Mean Difference (%)	Significance
Positive Control vs 25:75	5.71	Significant
Positive Control vs 75:25	11.22	Significant
Positive Control vs 50:50	10.64	Significant
25:75 vs 75:25	5.51	Significant
25:75 vs 50:50	4.93	Significant
75:25 vs 50:50	0.58	Not Significant

To identify specific points of divergence, a Post Hoc Multiple Mean Comparison test was conducted. The results indicate that the Positive control and the 25:75 ratio differ significantly from all other groups. The comparison between the 75:25 and 50:50 ratios yielded a non-significant result which suggests that beyond a certain threshold of Sambong concentration, the inhibitory capacity remains consistently low or negative within this experimental model.

In the crystal growth phase, the significance of the Treatment factor ($p = 0.0001$) underscores the importance of phytochemical concentration in disrupting the growth of the crystal. A successful growth inhibition is achieved when bioactive compounds adsorb onto the active growth sites of the crystal lattice to create a physical barrier (Krieger et al., 2015).

The non-significant remark for Time ($p = 0.860$), however, suggests that the inhibitory pressure exerted by the treatments was established almost immediately and did not fluctuate drastically as the 10-minute window progressed. This indicate that when time is not a significant factor in an inhibition assay, the concentration of the inhibitor acts as the primary dominant force, stabilizing the growth rate early in the process (Alelign and Petros., 2018).

The non-significant Interaction remark ($p = 0.860$) provides evidence to the stability of the herbal extracts. This demonstrates that the inhibitory behavior of each ratio was uniform and consistent throughout the duration of the experiment, meaning no specific treatment gained or lost its effectiveness as the minutes passed. A lack of significant interaction in botanical studies implies that the secondary metabolites responsible for preventing crystal growth remained chemically stable and provided a constant inhibitory influence against the deposition of calcium and oxalate ions (Chandra et al., 2020).

The significant variance established by the ANOVA confirms that the crystal inhibition is dependent on the concentration gradients. The 25:75 ratio over the 50:50 and 75:25 ratios suggests a "dose-response" relationship specifically to the amount of *P. niruri*. The efficacy of polyherbal formulations is often non-linear as certain ratios can lead to potentiation, while others result in phytochemical quenching where active compounds essentially cancel each other out (Ahmed et al., 2023).

The results of the Post Hoc Multiple Mean Comparison identify specific statistical differences between the treatment groups. The non-significant difference between the 75:25 and 50:50 ratios is highly notable, as both formulations resulted in negative inhibition values. This indicates that at these specific proportions is where the extracts failed to provide a protective barrier against crystal expansion. The negative percentages suggest that the mixture may have created an environment conducive to ion deposition. Certain plant extracts when used in specific ratios or high concentrations, can increase the turbidity or ionic strength of the medium, which may inadvertently support the growth kinetics of calcium oxalate crystals (Chandra et al., 2020).

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The lack of statistical divergence between these two groups suggests a plateau in their pharmacological behavior during the growth phase. This notes that the effectiveness of herbal combinations is often non-linear where in some instances, a high concentration of one plant component can counter the inhibitory metabolites of another (Zaid et al., 2021). In this study, the higher concentration of *B. balsamifera* in the 75:25 and 50:50 ratios likely reached a threshold where it no longer supported the disruption of the crystal lattice, leading to the similar negative inhibitory performance observed in both groups. This shows that the inhibitory capacity against crystal growth is highly sensitive to the balance of phytochemicals present in the mixture (Nirumand et al., 2018).

The significant difference between the 25:75 ratio and the positive control indicates that while the herbal mixture is effective, it has not yet reached the pharmacological potency of the synthetic standard, potassium citrate. This was expected given the refined, single-target nature of clinical chelating agents compared to the complex, multi-component nature of crude plant extracts. However, the statistical progress observed from the 50:50 ratio to the 25:75 ratio suggests that the anti-urolithiatic activity is highly sensitive to the concentration of *P. niruri*. The presence of specific lignans and polyphenols in *P. niruri* is what drives the disruption of the crystal lattice (Nirumand et al., 2018).

Table 3. Interaction Analysis of the Combined Plant Extracts

Combination	Mean Inhibition (%)	Comparison	Interpretation	Interaction Type
75:25:00	-1.5	vs 50:50 (-0.92)	Slight increase but still low/negative effect	Enhanced Turbidity/ Pro-crystallization
50:50:00	-0.92	vs 25:75 (4.01)	Moderate increase but not substantial	Enhanced Turbidity/ Pro-crystallization
25:75	4.01	Higher than both combinations	Large increase beyond proportional effect	Synergistic

Table 3 details the interaction analysis of the combined extracts of Sambong (*Blumea balsamifera*) and Sampa-sampalukan (*Phyllanthus niruri*) across the three ratios. The 75:25 ratio (Mean Inhibition: -1.50%) and the 50:50 ratio (Mean Inhibition: -0.92%) both exhibited negative inhibitory effects. These were classified as Enhanced Turbidity/ Pro-crystallization, respectively as they failed to produce positive inhibition and instead increased absorbance relative to controls. The 25:75 ratio demonstrated a mean inhibition of 4.01%, representing a substantial increase compared to the other formulations so this interaction is classified as Synergistic.

The classification of the 25:75 ratio as synergistic is the most critical finding of this analysis. Such synergy is often the result of "multitargeting," where diverse polyphenols work in tandem to simultaneously coat crystal surfaces and chelate free calcium ions (Ahmed et al., 2023). At this threshold, the active constituents, such as the lignans and flavonoids abundant in *P. niruri*, reach a concentration sufficient to actively interfere with the growth phase of calcium oxalate crystals. This is means that *P. niruri* modifies the surface charge of crystals, making them less likely to aggregate. The synergy result suggests that the minor proportion of *B. balsamifera* (25%) assisted this process by altering the microenvironment of the artificial urine, allowing the *P. niruri* metabolites to bond more effectively to the crystal lattice (Li et al., 2022).

This suggests that specific terpenes and saponins in *B. balsamifera* likely reduced the interfacial tension between the artificial urine and the crystal surface. This physicochemical shift facilitated a more rapid and uniform adsorption of the bulky polyphenol molecules from *P. niruri* onto the crystal lattice, creating a robust physical barrier against kinetic deposition that *P. niruri* could not achieve in isolation (Zaid et al., 2021).

While this specific combination has not been previously documented, this suggests that *B. balsamifera* has the capacity to subtly distort crystal geometry. By creating a more irregular surface, the Sambong component likely increased the density of active growth sites for the Sampa-sampalukan metabolites to "anchor" to (Montealegre and De Leon., 2024). This explains why the 25:75 ratio outperformed the other groups as it maximized the surface-coating potential of the primary inhibitor through the structural assistance of the secondary plant.

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The enhanced turbidity / pro-crystallization effect interaction observed in the 75:25 and 50:50 ratios indicates that the phytochemicals functioned cumulatively but were insufficient to disrupt the kinetic growth of calcium oxalate, potentially even facilitating ion deposition due to the higher concentration of organic solutes (Chandra et al., 2020). This suggests that when Sambong is the dominant or equal component, the bioactive compounds are not present in the correct proportions to overcome the natural growth kinetics of the crystals. While *B. Balsamifera* is a potent diuretic, its direct role in inhibiting the growth phase of existing crystals is limited compared to its ability to shift crystal morphology (Montealegre and De Leon., 2024).

While direct phytochemical binding kinetics were not measured in this assay, prior literature suggests that polyphenols and ellagic acid in *P. niruri* bind to active crystal growth sites to create a steric barrier. Furthermore, literature on *B. balsamifera* indicates that its terpene constituents may decrease surface tension, potentially facilitating more uniform adsorption of *P. niruri* metabolites onto the crystal lattice.

A main limitation of this study is its single-concentration (400 µg/mL) and short 10-minute time-frame design. While this static turbidimetric assay effectively models early crystal growth kinetics, these findings cannot be extrapolated to different concentration gradients or multi-hour crystal aggregation phases. Future multi-dose and kinetic studies are required to full map the dose-response profile.

CONCLUSION

Based on the statistical evidence, the study concludes that the 25:75 ratio of *Blumea balsamifera* and *Phyllanthus niruri* is the most effective formulation for inhibiting the growth phase of calcium oxalate crystals. This rejects the null hypothesis ($p < 0.0001$), confirming that varying the proportions of these extracts leads to significant differences in anti-urolithiatic potency.

The study further concluded that a higher concentration of *P. niruri* is a requirement for achieving synergy in this polyherbal mixture. Formulations with equal or dominant proportions of *B. balsamifera* fail to produce positive inhibitory results in this specific crystal growth model. Overall, the 25:75 ratio serves as a viable candidate for further pharmacological development as a standardized polyherbal treatment for urolithiasis, providing a localized, ratio-dependent approach to preventing stone expansion.

RECOMMENDATIONS

To further validate and expand the findings of this study, the researchers offer several recommendations for future research.

A recommendation of quantitative phytochemical analysis of the aqueous extracts should be conducted to determine the exact concentrations of bioactive metabolites. It is also recommended to extend the turbidimetric assay duration beyond the ten-minute interval to monitor long-term crystal stability and aggregation. The researchers additionally suggest transitioning to *in vivo* studies using animal models to assess the bioavailability and physiological performance of the extracts.

Future investigations should explore the use of different extraction solvents and methods to compare the yield of anti-urolithiatic compounds. The researchers recommend that extracts should also be tested against other varieties of kidney stones, such as calcium phosphate and uric acid crystals, to evaluate broad-spectrum efficacy. Additionally, it is highly recommended to conduct toxicity testing to establish the LD50 and ensure the safety profile of the formulation for renal and hepatic tissues.

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