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The Effectiveness of Pineapple Juice (Ananas comosus (L.) Merr) in Preventing the Formation of Calcium Oxalate Crystals In Vitro

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ABSTRACT
Background: Kidney stones (urolithiasis), particularly those composed of calcium oxalate (CaOx), remain a global health issue with a high recurrence rate. Non-pharmacological approaches using natural compounds are considered valuable alternatives to invasive treatments. Pineapple (Ananas comosus) contains citric acid and bromelain, which have potential inhibitory effects on CaOx crystallization, although scientific evidence is still limited.
Methods: A true experimental study with a post-test only control group design was conducted using four crystallization parameters (Tmax, SN, SA, and percentage of inhibition) across five groups: negative control, positive control (0.5% potassium citrate), and pineapple juice at concentrations of 25%, 50%, and 100%. Absorbance measurements were performed using a UV-Vis spectrophotometer at two-minute intervals for 60 minutes. Data were analyzed using Shapiro–Wilk, Levene's Test, Kruskal–Wallis, and Dunn's Post Hoc tests with a 95% confidence level.
Results: Pineapple juice exhibited strong inhibitory activity against CaOx crystal formation. All treatment concentrations reduced SN by more than 90% and achieved complete aggregation inhibition, along with increased Tmax compared to the negative control. The 25% concentration produced the most optimal inhibition effect, showing substantial SN reduction and a significant rise in Tmax. The Kruskal–Wallis test showed significant differences between groups for SN and Tmax, though pairwise comparison with Dunn's Post Hoc indicated no significant differences after correction.
Conclusion: Pineapple juice effectively inhibited CaOx crystallization in vitro by delaying nucleation and preventing crystal aggregation, suggesting its potential role as a natural non-pharmacological preventive agent for kidney stone formation.

KEYWORDS: Ananas comosus, Calcium Oxalate, Crystallization Inhibition, In Vitro, Kidney Stones.

INTRODUCTION

Kidney stones (urolithiasis) are solid masses formed due to crystal precipitation from urinary solutes and are recognized as one of the most common urological conditions worldwide. Approximately 80% of stones contain calcium, with calcium oxalate (CaOx) identified as the most dominant composition. Preventive efforts are important to reduce incidence and recurrence, particularly because most therapeutic approaches remain invasive and involve high healthcare costs.[1]

The development of kidney stones, especially CaOx stones, is closely related to elevated urinary concentrations of calcium and oxalate. When prevention is not initiated early, crystals can grow and aggregate into calculi, leading to complications such as urinary tract obstruction, infection, and renal impairment. This condition highlights the need for preventive strategies at the initial crystallization stage to achieve long-term benefits with minimal side effects.[2]

Global data demonstrate a rising trend in kidney stone cases. The 2013–2014 National Health and Nutrition Examination Survey (NHANES) reported increasing prevalence in several European countries, including 10% in Spain and 1.72% in Italy. In Germany, prevalence increased from 4.0% to 4.7% over two decades, while in France, 9.8% of individuals over 45 years were reported to have a history of kidney stones.[3] In East Asia, the prevalence ranges from 5–19.1% in developed countries such as Japan and South Korea.[4] In Indonesia, Basic Health Research (Riskesdas) 2013 reported a prevalence of 0.6% among individuals aged ≥15 years, and in Kupang, East Nusa Tenggara, kidney stones accounted for 7.5% of urological surgical cases. [5,6]

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Despite the high recurrence rate, preventive strategies have not received optimal clinical attention[7]. Management remains dominated by lithotripsy and surgical intervention, which do not prevent recurrence. This situation supports the increasing demand for natural-based and non-pharmacological preventive approaches. One commonly applied option is potassium citrate, known to increase urinary pH and reduce CaOx aggregation[8,9].

Natural compounds in fruits such as lemon and lime have shown the ability to inhibit CaOx crystal formation due to their citrate content.[10] Similar effects were also observed following consumption of lemon and tomato juice[8]. Pineapple (Ananas comosus) contains citric acid and bromelain, which are suggested to play a role in inhibiting crystallization, similar to the mechanism found in citrus fruits[10,11]. Research exploring pineapple as a preventive agent for kidney stone formation is still limited, indicating the need for further investigation.[10]

Based on this rationale, the present study aims to evaluate the effectiveness of pineapple juice in inhibiting calcium oxalate crystal formation using an in vitro model. By exploring pineapple's potential as a natural anti-crystallization agent, this research is expected to contribute to the development of non-pharmacological prevention strategies for kidney stones.

METHODS

This study employed a pre-experimental design using a one-group pretest–posttest approach. The research was conducted at This study employed a true experimental approach using a post-test only control group design. Five groups were assessed, consisting of a negative control without treatment, a positive control using 0.5% potassium citrate, and three treatment groups administered pineapple juice at concentrations of 25%, 50%, and 100% (v/v). Each group was tested in three independent replicates to ensure reproducibility and reliability of the results.

The independent variable was the concentration of pineapple juice filtrate introduced into the crystallization system, while the dependent variables included four parameters of calcium oxalate crystallization: time to reach maximum absorbance (Tmax), nucleation slope (SN), aggregation slope (SA), and percentage of inhibition. Factors such as pH, temperature, and the chemical composition of synthetic urine were controlled throughout the experiment to minimize confounding influences.

Synthetic urine was prepared by dissolving calcium chloride ($CaCl_2$), sodium chloride (NaCl), and sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$) in deionized water. Sodium oxalate ($Na_2C_2O_4$) was used as a 20 mM stock solution to initiate crystallization. Fresh pineapple fruits were peeled, blended, and filtered to obtain clear juice, which was then diluted to concentrations of 25%, 50%, and 100%. A 0.5% potassium citrate solution (5 g/L) served as the positive control due to its known ability to inhibit stone formation.

All components of the synthetic urine were dissolved in 100 mL of distilled water and stirred until homogeneous. The solution pH was adjusted to 5.7 ± 0.1 using 1N HCl or 1N NaOH, and the temperature was maintained at 37°C in a water bath. The respective treatments were added to each group, while the negative control group received no additives. Crystallization was induced through simultaneous addition of $CaCl_2$ and $Na_2C_2O_4$ at a final concentration of 4 mmol/L.

Absorbance measurements were recorded using a UV-Vis spectrophotometer at a wavelength of 620 nm at two-minute intervals over a period of 60 minutes. Absorbance-time curves were analyzed to determine Tmax, SN, SA, and inhibition percentage using linear regression. Data analysis was conducted using SPSS software. Normality and homogeneity were assessed using the Shapiro–Wilk and Levene's tests. As the data did not fulfill parametric requirements, the Kruskal–Wallis test was applied to determine differences among treatment groups, followed by Dunn's post hoc test for multiple comparison adjustments. Statistical significance was set at $p < 0.05$.

RESULT

This study was conducted from September 18 to October 8, 2025, at the Laboratory of the Medical Education Study Program, Nusa Cendana University, and the Pharmacy Laboratory of Poltekkes Kemenkes Kupang. The primary objective was to observe calcium oxalate (CaOx) crystallization parameters by measuring absorbance using a UV-Vis spectrophotometer at a wavelength of 620 nm. The analysis was carried out to determine the effect of pineapple juice at different concentrations on nucleation and aggregation rates under in vitro conditions.

Descriptive data for CaOx crystallization parameters across treatment groups are presented in Table 1.

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Table 1. Calcium Oxalate Crystallization Parameters at Different Pineapple Juice Concentrations

Inhibitor Concentration (%)	Nucleation (SN)	Slope Aggregation (SA)	Slope Growth Inhibition (%)	Aggregation Inhibition (%)	Tmax (minutes)
Negative Control	0.00813	-0.00084	0.00	0.00	14
Pineapple Juice 25%	0.00041	0.00000	95.00	100.00	44
Pineapple Juice 50%	0.00045	0.00011	94.46	100.00	26
Pineapple Juice 100%	0.00071	0.00120	91.21	100.00	58
Potassium Citrate 0.5%	0.00024	0.00007	97.10	100.00	60

Based on Table 1, the negative control group reached the fastest crystal formation time (Tmax) at 14 minutes, indicating uninterrupted nucleation. In contrast, pineapple juice treatment successfully extended the induction phase of crystallization. The highest Tmax was observed in the 100% concentration group (58 minutes), closely approaching the positive control of potassium citrate (60 minutes). All pineapple juice concentrations demonstrated strong inhibitory activity, with nucleation reduction above 90% and aggregation inhibition reaching 100%.

Statistical testing was performed to determine differences among treatment groups. The test results are summarized in Table 2.

Table 2. Normality, Homogeneity, and Kruskal-Wallis Test Results

Variable	Normality p-value	Homogeneity p-value	Kruskal-Wallis p-value
Tmax (minutes)	0.145	0.003	0.031
Nucleation Slope	<0.001	0.006	0.042
Aggregation Slope	0.012	0.015	0.197

The results indicate that SN and SA variables were not normally distributed, and all variables showed non-homogeneous variances ($p < 0.05$). Therefore, the Kruskal-Wallis test was applied. Significant differences were found between groups in the Tmax ($p = 0.031$) and SN parameters ($p = 0.042$), while no significant difference was observed for SA ($p = 0.197$). However, further post hoc analysis using Dunn's test with Bonferroni and Holm correction revealed no statistically significant pairwise differences between treatment groups. This outcome is likely influenced by the limited number of replicates ($n = 3$) in each treatment group.

DISCUSSION

The results of this study demonstrate that pineapple juice (Ananas comosus (L.) Merr.) possesses significant inhibitory activity against the crystallization of calcium oxalate (CaOx) in vitro.[13] Spectrophotometric analysis showed that pineapple juice effectively suppressed the crystallization process, indicated by the reduction in absorbance values across all treatment groups compared to the negative control. In the negative control group, absorbance continued to rise until reaching the maximum peak (Tmax) at 14 minutes, with a nucleation slope (SN) of 0.00813, indicating rapid nucleation and aggregation in the absence of inhibitors. This is consistent with the mechanism of lithiasis formation under calcium–oxalate supersaturation.[13]

Administration of pineapple juice resulted in a significant extension of nucleation time, reaching 44 minutes at 25% concentration and up to 58 minutes at 100%. The nucleation rate decreased by more than 90%, and crystal aggregation was completely inhibited (100%) in all treatment groups. These findings suggest that pineapple juice disrupts two important stages of CaOx formation: nucleation and aggregation.[11] This is in line with previous studies reporting that citrate-containing fruits reduce CaOx nucleation and aggregation.[11] Similar effects have also been described in plant extracts rich in organic acids and phenolic compounds.[14]

The inhibitory activity of pineapple is strongly associated with its bioactive components, primarily citric acid and bromelain. Citric acid forms soluble calcium–citrate complexes, lowering free calcium ion availability and reducing supersaturation.[15] It may also increase the pH of the medium, creating an environment less favorable for CaOx deposition.

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Bromelain, a proteolytic enzyme present in pineapple, may interfere with the organic matrix and crystal surfaces, reducing aggregation.[16] Additional compounds, including flavonoids and polyphenols, may contribute through antioxidant mechanisms that suppress early nucleation.[11]

All treatment concentrations (25%, 50%, and 100%) exhibited strong inhibitory responses. The 100% concentration reached a Tmax of 58 minutes, approaching the 60-minute result obtained with potassium citrate, while even 25% concentration produced >90% growth inhibition. This indicates that crystallization inhibition is not strictly linear with concentration, and may also be influenced by viscosity, molecular interaction, and particle dynamics.[9]

Kruskal-Wallis analysis showed significant differences in Tmax and SN among treatment groups ($p < 0.05$), but Dunn's post-hoc test did not show significant pairwise differences after adjustment. This is likely due to the small sample size ($n = 3$), which reduces statistical power. Despite this limitation, descriptive results consistently showed >90% inhibition in all pineapple juice groups compared to the control, confirming the strong biological effect of pineapple juice in inhibiting CaOx crystallization. These results support the potential of pineapple juice as a natural preventive agent for kidney stones by interfering with early crystallization processes.[11]

CONCLUSION

Based on the findings of this study, pineapple juice (Ananas comosus (L.) Merr.) demonstrated strong inhibitory activity against calcium oxalate (CaOx) crystallization in vitro. Pineapple juice at concentrations of 25%, 50%, and 100% successfully delayed the crystallization process, as indicated by the prolonged Tmax and significant reduction in nucleation rate (SN). All treatment concentrations achieved more than 90% inhibition of crystal growth and demonstrated complete inhibition (100%) of crystal aggregation. These results indicate that the bioactive components of pineapple, particularly citric acid and bromelain, play a key role in preventing early lithogenesis by forming soluble complexes with calcium and interfering with crystal assembly.

Despite the promising outcomes, this study has limitations. The experiment was conducted using an in vitro model with a limited sample size, which may affect the strength of the statistical interpretation. Therefore, further research is recommended through in vivo studies or clinical trials to validate the efficacy and safety of pineapple juice under physiological conditions. Future investigations should also include quantitative analysis of citric acid, bromelain, and other phytochemicals to better understand the dose–response mechanism and inhibition pathway. Overall, pineapple juice represents a potential natural, affordable, and non-pharmacological alternative for the prevention of calcium oxalate kidney stones.

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