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Polyfruit peel blend for inhibiting calcium oxalate crystallization in kidney stone formation: An eco-pharmaceutical perspective

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Abstract

Urolithiasis, or kidney stone disease, poses a persistent global health concern due to its high recurrence rate and the financial burden of conventional treatments. This study explored the synergistic in vitro anti-lithiatic potential of solid-phase extraction (SPE) concentrates derived from banana (*Musa spp.*), mango (*Mangifera indica*), and camansi (*Artocarpus camansi*) fruit peels. The extracts were assessed for their ability to inhibit calcium oxalate nucleation and aggregation using UV-Vis spectrophotometry at 620 nm. Following collection, the fruit peels underwent ethanol maceration, rotary evaporation, and SPE purification. Treatments, prepared individually and in combinations at 100 µg/mL, were tested against calcium oxalate crystal formation. Results showed that the polyfruit blend demonstrated significantly greater inhibition of nucleation (58.63%) and aggregation (63.07%) compared to individual extracts, second only to potassium citrate as the positive control. Quantitative phytochemical analysis confirmed the presence of saponins, flavonoids, and tannins, contributing to the observed bioactivity. ANOVA and Scheffé post hoc tests validated significant differences ($p < 0.01$) among treatment groups. This research bridges traditional pharmacognosy and modern biotechnology, offering an opportunity to develop health tourism products from agricultural waste. It catalyzes interdisciplinary collaborations among pharmacists, medical practitioners, tourism professionals, and farmers, showcasing how locally sourced bioactive compounds can shape a sustainable and health-oriented tourism economy while uplifting rural agricultural sectors. The study highlights the therapeutic and economic potential of fruit peel waste in preventive nephrology and recommends further in vivo and formulation studies for clinical translation and commercialization.

Keywords: *anti-lithiatic activity, calcium oxalate inhibition, polyfruit peel extracts, solid- phase extraction (SPE), eco-pharmaceutical*

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1. Introduction

Kidney stone disease or urolithiasis is a global burden affecting millions of people with rising incidence owing to dietary and lifestyle changes (Awedew, 2024; Zhang et al., 2022; Ma & Chen, 2025; Dirie et al., 2024; Akram & Somani, 2025; Lazer et al., 2025). Traditional treatment methods like surgery and extracorporeal shock wave lithotripsy though efficient come with a cost and the risk of side effects or recurrence (Assmy et al., 2016). With growing demand for safe, inexpensive, and plant-based solutions, researchers began focusing on natural compounds with anti-lithiatic activities (Anand et al., 2021; Zalavadiya et al., 2013; Zeppa et al., 2025; Sulaiman et al., 2022; Prabhu et al., 2025; El Menyiy et al., 2021; Savickiene & Raudone, 2024). This study analyzes the feasibility of fruit waste, specifically polyfruit peel extracts, as a sustainable and therapeutic option, considering both clinical as well as ecological issues.

Fruits like banana peel, mango peel, and camansi peel contain high levels of bioactive compounds such as polyphenols, flavonoids, and antioxidants with diverse therapeutic activities like anti-inflammatory and anti-crystalization activities (El Menyiy et al., 2021). Phytochemicals have been revealed to inhibit the nucleation of calcium oxalate and its subsequent development and accretion, major steps in the formation of a kidney stone (Kachkoul et al., 2018; Saha, & Verma, 2013). Further, solid-phase extraction (SPE) has been widely employed in purifying and concentrating bioactive compounds from complex plant samples to improve their bioefficacies (Abdelmohsen et al., 2022; Rosendo et al., 2023; Badawy et al., 2022; Naviglio et al., 2023; Njewa et al., 2025; Hlatshwayo et al., 2025; Patra et al., 2022). While the single fruit peel extracts show a promising bioactivity, several studies examined their synergistic activity when blended (Salazar Llorente et al., 2025; Mia et al., 2025; Bello et al., 2023; Durmus et al., 2024; Sha et al., 2023; Blicharz-Kania et al., 2025; Balogun & Kang, 2024). Also, there has not been an examination of the in vitro anti-lithiatic activity of SPE-concentrated polyfruit peel preparations, specifically against the nucleation and aggregation of calcium oxalate. Such a gap hampers the production of optimized, potent, and low-cost plant- derived interventions for the prevention of kidney stones.

Investigation of the synergistic interaction among bioactive compounds present in blended fruit peel extracts can result in a potent therapeutic drug for the control of kidney stones (Nazir et al., 2025; Habiba et al., 2025). The use of SPE methods also increases the potency and standardization of extracts, essential for reproducibility and clinical implications.

This study not only advances nephrology and pharmacognosy but also contributes to valorization of agricultural residues in accordance with sustainability objectives. Subsequently, this study endeavors to assess the synergistic anti-lithiatic effect of polyfruit peel solid-phase extraction concentrates from banana, mango, and camansi in in vitro tests focusing on the nucleation and aggregation of calcium oxalate.

This study aims to evaluate the synergistic in vitro anti-lithiatic potential of solid-phase extraction (SPE) concentrates derived from polyfruit peels (banana, mango, and camansi) by assessing their inhibitory effects on calcium oxalate nucleation and aggregation. This study specifically aimed to prepare solid-phase extraction (SPE) concentrates from banana, mango, and camansi fruit peels, both individually and in combination. It sought to quantify the bioactive compounds in each fruit peel, including saponin in banana peels, flavonoid in mango peels, and tannin in camansi peels. The study also aimed to determine the in vitro inhibitory effects of both individual and combined SPE concentrates on calcium oxalate nucleation and aggregation. Furthermore, it sought to assess the synergistic anti-lithiatic potential of the combined polyfruit peel SPE concentrates compared to the individual extracts. Finally, the study aimed to compare the anti-lithiatic activity of the treatment groups with a standard positive control to evaluate their relative efficacy.

2. Methods and Procedures

2.1. Plant Collection

The process commenced with the selection of fresh and undamaged fruits of banana (Musa spp.), mango (Mangifera indica), and camansi (Artocarpus camansi) from a local marketplace. Each fruit was carefully inspected to ensure the absence of molds, bruises, or any signs of microbial spoilage. Following selection, the fruits were first rinsed under running tap water to eliminate dirt and surface impurities, then washed with distilled water to remove residues that could potentially interfere with later analyses.

Peeling was performed manually using a sterile stainless steel knife. For bananas, only the outer skin was removed, avoiding the fibrous layer. Mangoes were peeled with care to exclude any pulp, while camansi fruits underwent brief blanching in boiling water for 2–3 minutes to facilitate shell removal. Once peeled, the fruit peels were chopped into uniform pieces measuring approximately 1–2 square centimeters. The chopped peels were evenly distributed on stainless steel trays and dehydrated at 40°C using an automated dehydrator.

After drying, the peels were stored in sterile, airtight Ziplock bags. Each bag was labeled with the fruit type, date of collection, and collection site. Samples were stored at 4°C until further processing, which included rotary evaporation, solid-phase extraction, quantitative phytochemical analysis, and in-vitro assays (Abubakar & Haque, 2020).

2.2. Plant Extraction

The samples were then pulverized with a grinder into a coarse powder to aid in efficient penetration by the solvents in extraction (Harborne, 1998). 100 grams of the crushed fruit peels were weighed and packed into a clean covered conical flask. 1 liter of 70% ethanol (700 mL ethanol and 300 mL distilled water) was added to this and used as the solvent used in extraction. The mixture was shaken and kept at room temperature for 48 hours to ease compound diffusion. The mixture was then centrifuged by filtering the mixture with Whatman No. 1 filter paper in order to part with the solid residues from the crude ethanolic extract (Do et al., 2014).

The filtrate was then processed by rotary evaporation. The filter extract was poured into a round-bottom flask and put in a rotary evaporator. The water bath was at 40°C under vacuum conditions to evaporate the ethanol and leave a semiconcentrate syrup-like extract (Azwanida, 2015). After concentration, the solid-phase extraction was carried out. A manual SPE column was prepared by a 10 mL syringe lined with a cotton plug at the bottom. A layer of silica gel was deposited in this column, followed by the addition of the C18 reversed-phase (octadecylsilane) packing. The column was first preconditioned with 5 mL of 70% ethanol. The concentrated extract was then packed into the column by gravity in approximately 5–10 mL. An extra 5 mL ethanol–water mixture was used in eluting the other compounds. The eluates were collected in labeled microcentrifuge (Alfonsi et al., 2008). These collected eluates were centrifuged at 5000 rpm for a 15-minute time frame at room temperature in order to eliminate insoluble particles. The produced supernatants were gently poured into drying containers. Automated drying was then achieved by utilizing a laboratory oven at 30°C until a stable semi-solid extract was reached (Sasidharan et al., 2011).

2.3. Treatment Preparation

Table 1 shows the composition of nine (9) treatments with the corresponding concentration. The diluent used to dissolve each treatment is distilled water, and consequently used as the negative control.

Table 1

Treatment composition

Treatment Code	Extract Composition	Total Concentration
A	Banana peel	100 µg/mL
B	Mango peel	100 µg/mL
C	Camansi peel	100 µg/mL
D	Banana + Mango peel	100 µg/mL
E	Camansi + Banana peel	100 µg/mL
F	Mango + Camansi peel	100 µg/mL
G	Banana + Mango + Camansi peel (polyfruit blend)	100 µg/mL
H	Potassium citrate	100 µg/mL
I	Negative control (Distilled Water	

2.4. Quantification of Saponins, Tannins, and Flavonoids Using UV-Vis

The quantification of saponins, tannins, and flavonoids was conducted using UV-Vis spectrophotometry. For saponins, a standard solution of diosgenin ranging from 0 to 100 µg/mL was prepared. To 1 mL of the standard or sample, 0.5 mL of 8% vanillin and 5 mL of 72% sulfuric acid were added. The mixture was then incubated in a water bath at 60°C for 10 minutes. After cooling to room temperature, the absorbance was measured at 544 nm using a UV-Vis spectrophotometer.

The saponin content was calculated based on the standard calibration curve, with diosgenin as the reference standard. For the quantification of tannins, tannic acid standards ranging from 0 to 100 µg/mL were first prepared. Then, 1 mL of the extract or standard was mixed with 5 mL of distilled water and 0.5 mL of 1% ferric chloride in 0.1 N HCl. Subsequently, 0.008 M potassium ferrocyanide was added, and the mixture was incubated at room temperature for 10 minutes. The absorbance was measured at 700 nm, using tannic acid as the reference standard (Makkar et al., 1995).

For flavonoid quantification, quercetin standards ranging from 0 to 100 µg/mL were prepared. A volume of 1 mL of the extract or standard was mixed with 4 mL of distilled water. Then, 0.3 mL of 5% sodium nitrite was added, and after 5 minutes, 0.3 mL of 10% aluminum chloride was added. After another 6 minutes, 2 mL of 1 M sodium hydroxide was introduced, and the total volume was adjusted to 10 mL with distilled water. Following 15 minutes of incubation, the absorbance was measured at 415 nm, using quercetin as the standard (Chang et al., 2002).

2.5. Calcium Oxalate Nucleation via Ultraviolet-Visible Spectrophotometry

To prepare the stock solutions, dissolve 6.5 mM of $CaCl_2 \cdot 2H_2O$ in 0.15 M NaCl containing 0.05 M Tris buffer (pH 6.5) to obtain the calcium solution. For the oxalate solution, dissolve 1 mM of sodium oxalate in the same buffer. Both solutions should be filtered through a 0.45 µm filter and stored at room temperature. For the test samples, dissolve or suspend the fruit peel extracts in distilled water to achieve the desired final concentration, for example, 100 µg/mL. Prepare control samples without extract and blank samples containing only the buffer.

In the nucleation assay, add 1 mL of the calcium chloride solution into a test tube or cuvette, followed by 1 mL of the plant extract solution or distilled water for the control. To initiate the reaction, quickly add 1 mL of the sodium oxalate solution while mixing gently, resulting in a total reaction volume of 3 mL. Incubate the mixture at 37°C for 30 minutes, and then measure the absorbance at 620 nm using a UV-Vis spectrophotometer. This wavelength corresponds to the turbidity caused by the formation of calcium oxalate crystals (Sabarinath et al., 2024; Chattaraj et al., 2023; Li et al., 2023; Hewagama & Hewawasam, 2022; Kaushik et al., 2017).

2.6. Calcium Oxalate Aggregation via Ultraviolet-Visible Spectrophotometry

To prepare calcium oxalate crystals in the pre-aggregation phase, equal volumes of 5 mM $CaCl_2$ in Tris-NaCl buffer (pH 6.5) and 0.5 mM $Na_2C_2O_4$ in the same buffer are mixed. The mixture is incubated at room temperature for 1 hour to allow the formation of small calcium oxalate monohydrate crystals. After incubation, the mixture is centrifuged at 3000 rpm for 10 minutes, and the supernatant is discarded. The resulting crystals are washed three times with distilled water and finally resuspended in the buffer to obtain a uniform suspension.

For the preparation of extract solutions, the polyfruit peel extract is dissolved in water at a known concentration, such as 100 µg/mL. If necessary, the solution is filtered to remove any debris. For the aggregation assay setup, in separate tubes or cuvettes, a reaction mixture is prepared with a final volume of 3 mL. The components include 1 mL of the crystal suspension, 1 mL of Tris-NaCl buffer, and 1 mL of the extract solution or control (distilled water). The mixture is then gently mixed and incubated at 37°C for 30 minutes. After incubation, absorbance is measured at 620 nm using UV-Vis spectrophotometry to assess the extent of aggregation (Sabarinath et al., 2024; Chattaraj et al., 2023; Li et al., 2023; Hewagama & Hewawasam, 2022; Kaushik et al., 2017).

3. Results and Discussion

Table 2 presents the quantification of bioactive compounds, saponins, tannins, and flavonoids, extracted from banana, camansi, and mango peels, respectively, using UV- Vis spectrophotometry.

Table 2

Mean percent nucleation of calcium oxalate

Treatment	mean_x (%)
A	11.40
B	23.94
C	27.22
D	36.44
E	44.93
F	49.26
G	58.63
H	75.25
I	0.87

The concentrations were determined through standard calibration curves and trend analysis based on their measured absorbance at specific wavelengths. The banana peel extract, analyzed at 544 nm, exhibited an absorbance of 0.419, corresponding to a saponin concentration of 55.98 µg/mL. The camansi peel extract, evaluated at 700 nm, showed an absorbance of 0.472, which translated to a tannin concentration of 73.63 µg/mL, the highest among the three samples. Meanwhile, the mango peel extract, measured at 415 nm, had the highest absorbance value of 0.554, resulting in a flavonoid concentration of 65.99 µg/mL. These findings suggest that camansi peel is the richest in its respective bioactive compound, followed by mango and banana peels. The results validate the presence and relative abundance of phytochemicals in the tested fruit peels, highlighting their potential for further pharmacological or nutraceutical applications.

The absorbance values were evaluated across nine treatment groups (A-I) to determine the extent of inhibition of calcium oxalate nucleation. These treatments showed varying mean values, indicating differences in their impacts. As summarized in Table 2, Treatment H recorded the highest mean value of 75.25, indicating the most prominent effect among all treatments. This suggests that Treatment H resulted in the highest measured response, possibly

linked to the strongest experimental influence or active component presence. In contrast, Treatment I displayed the lowest mean value of 0.87, pointing to the least effect, and possibly indicating minimal or negligible activity under the test condition. Meanwhile, Treatment A, the lowest among the active groups, had a mean value of 11.40, while Treatment B showed a moderate effect with a mean of 23.94. Treatment C and D followed with increasing responses of 27.22 and 36.44, respectively. Treatments E through G exhibited higher mean values in succession, 44.93 (E), 49.26 (F), and 58.63 (G), indicating a gradation in their effects leading up to the peak response observed in Treatment H. The standard errors were also calculated, with Treatment E having the highest standard error (0.183), reflecting more variability in its data, while Treatment A had the lowest among active treatments (0.043), indicating more consistent results.

Table 3

Analysis of variance of percent inhibition of calcium oxalate nucleation

Source	Sum of Squares	Degrees of Freedom	Mean Square	F statistic	p-value
treatment	39,319.0429	8	4,914.8804	52,304.6059	1.1102e-16
error	6.7656	72	0.0940		
total	39,325.8085	80		**α = 0.01**	

A one-way analysis of variance (ANOVA) was conducted to evaluate the effect of different treatments on the inhibition of calcium oxalate nucleation. The results demonstrated a highly significant effect of treatment, $F(8, 72) = 52{,}304.61$, $p < .001$. Given that the p-value (1.11×10^{-16}) is far below the alpha level of 0.01, the null hypothesis that there is no difference among the treatment means is strongly rejected. This finding indicates that there is a statistically significant difference among at least two of the nine treatment groups. The exceptionally large F statistic suggests that the variability among the treatment means is overwhelmingly greater than the variability within the groups. This provides robust evidence that the treatment factor plays a significant role in influencing the outcome. Therefore, it is necessary to conduct post hoc comparisons to determine which specific treatments differ significantly from one another.

To further explore the significant treatment effects identified in the ANOVA, a Scheffé post hoc test was performed to pinpoint which specific treatment pairs differed significantly

on the inhibition of calcium oxalate nucleation. The results, as shown in Table 4, reveal that all pairwise comparisons among the nine treatment groups (A to I) yielded p-values less than 0.01, indicating statistically significant differences in their responses. This means that each treatment was distinctly different from the others in terms of its measured outcome.

Table 4

Post Hoc Scheffe Test of % Nucleation

Treatments pair	Scheffé TT-statistic	Scheffé p-value	Scheffé inferfence	Treatments pair	Scheffé TT-statistic	Scheffé p-value	Scheffé inferfence
A vs B	86.7673	1.11E-16	** p<0.01	C vs G	217.359	1.11E-16	** p<0.01
A vs C	109.429	1.11E-16	** p<0.01	C vs H	332.4315	1.11E-16	** p<0.01
A vs D	173.27	1.11E-16	** p<0.01	C vs I	182.3523	1.11E-16	** p<0.01
A vs E	231.9966	1.11E-16	** p<0.01	D vs E	58.7266	1.11E-16	** p<0.01
A vs F	261.9771	1.11E-16	** p<0.01	D vs F	88.7072	1.11E-16	** p<0.01
A vs G	326.7881	1.11E-16	** p<0.01	D vs G	153.5181	1.11E-16	** p<0.01
A vs H	441.8605	1.11E-16	** p<0.01	D vs H	268.5905	1.11E-16	** p<0.01
A vs I	72.9233	1.11E-16	** p<0.01	D vs I	246.1933	1.11E-16	** p<0.01
B vs C	22.6618	1.11E-16	** p<0.01	E vs F	29.9806	1.11E-16	** p<0.01
B vs D	86.5027	1.11E-16	** p<0.01	E vs G	94.7915	1.11E-16	** p<0.01
B vs E	145.2293	1.11E-16	** p<0.01	E vs H	209.8639	1.11E-16	** p<0.01
B vs F	175.2099	1.11E-16	** p<0.01	E vs I	304.9199	1.11E-16	** p<0.01
B vs G	240.0208	1.11E-16	** p<0.01	F vs G	64.8109	1.11E-16	** p<0.01
B vs H	355.0932	1.11E-16	** p<0.01	F vs H	179.8833	1.11E-16	** p<0.01
B vs I	159.6905	1.11E-16	** p<0.01	F vs I	334.9004	1.11E-16	** p<0.01
C vs D	63.8409	1.11E-16	** p<0.01	G vs H	115.0724	1.11E-16	** p<0.01
C vs E	122.5676	1.11E-16	** p<0.01	G vs I	399.7113	1.11E-16	** p<0.01
C vs F	152.5481	1.11E-16	** p<0.01	H vs I	514.7838	1.11E-16	** p<0.01

For instance, Treatment H vs I exhibited the largest difference with a TT-statistic of 514.78, followed closely by H vs G (399.71) and H vs F (334.90), suggesting that Treatment H had the strongest divergence compared to other treatments. Even the smallest TT-statistic, D vs E (58.73), still indicated a statistically significant difference, underscoring the consistent uniqueness of each treatment group. These findings confirm the robustness of the initial ANOVA result, validating that the differences across all treatments are not due to random variation but represent true distinctions in effect.

The percentage values across nine different treatments were measured to assess their relative effects on the inhibition of calcium oxalate aggregation. The results, as shown in Table 5, revealed a progressive increase in the mean values from Treatment A to Treatment H, with Treatment H showing the highest effect at 75.90%.

Table 5

Mean percent aggregation of calcium oxalate

Treatment	mean_x (%)
A	10.92
B	19.85
C	22.56
D	31.66
E	45.27
F	48.63
G	63.07
H	75.9
I	1.85

This suggests that Treatment H exerted the strongest influence on the parameter being studied. Following this, Treatments G (63.07%) and F (48.63%) also demonstrated considerable impact. On the other hand, Treatment I recorded the lowest mean value of 1.85%, suggesting minimal effect and potentially indicating limited activity or response under the experimental condition. Treatment A, with a mean of 10.92%, showed only slight activity, while B (19.85%) and C (22.56%) indicated modest responses. Overall, these values show a clear separation in the effectiveness of treatments, with the response intensifying from Treatment A through H, and sharply declining with Treatment I. This pattern suggests that specific treatments, particularly H, G, and F, were more effective in eliciting the desired outcome, while Treatment I was the least effective among all groups.

As shown in Table 6, a one-way ANOVA was conducted to evaluate the effect of nine different treatments on the inhibition of calcium oxalate aggregation. The results indicated a highly significant effect of treatment, $F(8, 72) = 6{,}456.28$, $p < .001$. Given that the p-value (1.11×10^{-16}) is significantly lower than the alpha level of 0.01, the null hypothesis that there are no differences among treatment means is rejected.

Table 6

Analysis of variance of percent inhibition aggregation

Source	Sum of Squares	Degrees of Freedom	Mean Square	F statistic	p-value
treatment	43,419.3599	8	5,427.4200	6,456.2846	1.1102e-16
error	60.5262	72	0.8406		
total	43,479.8860	80		**α = 0.01**	

This indicates that there is a statistically significant difference among at least two of the treatment groups. The very large F statistic further supports the conclusion that the variability between treatment groups is substantially greater than the variability within groups. Therefore, post hoc comparisons are necessary to determine which specific treatment pairs differ significantly from each other.

Table 7

Post Hoc Scheffe test of % aggregation

Treatments pair	Scheffé TT-statistic	Scheffé p-value	Scheffé inferfence	Treatments pair	Scheffé TT-statistic	Scheffé p-value	Scheffé inferfence
A vs B	20.6601	1.11E-16	** p<0.01	C vs G	93.7446	1.11E-16	** p<0.01
A vs C	26.9188	1.11E-16	** p<0.01	C vs H	123.4225	1.11E-16	** p<0.01
A vs D	47.9827	1.11E-16	** p<0.01	C vs I	47.9154	1.11E-16	** p<0.01
A vs E	79.4776	1.11E-16	** p<0.01	D vs E	31.4949	1.11E-16	** p<0.01
A vs F	87.2504	1.11E-16	** p<0.01	D vs F	39.2677	1.11E-16	** p<0.01
A vs G	120.6633	1.11E-16	** p<0.01	D vs G	72.6806	1.11E-16	** p<0.01
A vs H	150.3413	1.11E-16	** p<0.01	D vs H	102.3586	1.11E-16	** p<0.01
A vs I	20.9966	1.11E-16	** p<0.01	D vs I	68.9793	1.11E-16	** p<0.01
B vs C	6.2586	7.52E-05	** p<0.01	E vs F	7.7728	2.85E-07	** p<0.01
B vs D	27.3225	1.11E-16	** p<0.01	E vs G	41.1857	1.11E-16	** p<0.01
B vs E	58.8175	1.11E-16	** p<0.01	E vs H	70.8636	1.11E-16	** p<0.01
B vs F	66.5903	1.11E-16	** p<0.01	E vs I	100.4743	1.11E-16	** p<0.01
B vs G	100.0032	1.11E-16	** p<0.01	F vs G	33.4129	1.11E-16	** p<0.01
B vs H	129.6811	1.11E-16	** p<0.01	F vs H	63.0908	1.11E-16	** p<0.01
B vs I	41.6568	1.11E-16	** p<0.01	F vs I	108.247	1.11E-16	** p<0.01
C vs D	21.0639	1.11E-16	** p<0.01	G vs H	29.6779	1.11E-16	** p<0.01
C vs E	52.5589	1.11E-16	** p<0.01	G vs I	141.66	1.11E-16	** p<0.01
C vs F	60.3317	1.11E-16	** p<0.01	H vs I	171.3379	1.11E-16	** p<0.01

To further investigate the significant differences found in the ANOVA results, a Scheffé post hoc test was conducted to determine which specific treatment pairs differed significantly. The results, as summarized in Table 7, reveal that almost all treatment comparisons yielded p-values less than 0.01, indicating statistically significant differences among most pairs. The largest observed difference was between Treatments H and I (TT = 171.34), followed closely by G vs I (141.66) and C vs H (123.42), highlighting strong contrasts in treatment effectiveness. Even the smallest statistically significant difference, such as E vs F (TT = 7.77, $p < 0.01$), confirms that the two treatments differ in their outcomes. Only one p-value (from E vs F) is slightly above the others but still statistically significant at $p < 0.01$, affirming the reliability of these distinctions. These results reinforce the conclusion that all treatments elicited significantly distinct effects.

The treatment involving distilled water served as the negative control in this study. It contains no phytochemicals or bioactive compounds and lacks any functional or structural groups capable of interacting with calcium or oxalate ions. Consequently, it does not inhibit either the nucleation (initial crystal formation) or aggregation (clumping) of calcium oxalate crystals. Its role is to provide a baseline for evaluating the efficacy of other treatments (Hewagama & Hewawasam, 2022).

Banana peel extract contains saponins, which are glycosidic compounds composed of a hydrophobic aglycone (sapogenin) and hydrophilic sugar moieties. Structurally, saponins feature hydroxyl (-OH), ether (-O-), and glycosidic linkages. Functionally, they act as mild surfactants, disrupting crystal aggregation by adsorbing onto the crystal surface and altering surface charge (zeta potential), which limits growth. However, their antioxidant activity is moderate and their calcium-chelating capacity is weaker than that of flavonoids or tannins, resulting in limited inhibition of nucleation (Rajeshwari et al., 2023).

Mango peel extract is rich in flavonoids such as quercetin and mangiferin. These compounds contain phenolic hydroxyl groups (-OH), ketone (=O), and aromatic ring structures. Flavonoids are potent antioxidants that scavenge free radicals produced during crystal formation, thereby reducing aggregation. Additionally, their hydroxyl groups chelate calcium ions, decreasing supersaturation and limiting crystallization. Despite these properties, flavonoids alone provide only moderate inhibition of nucleation (Sabarinath et al., 2024).

Camansi peel contains both hydrolyzable and condensed tannins, which are polyphenolic in nature and contain multiple hydroxyl (-OH), carboxylic acid (-COOH), and

aromatic ring groups. These functional groups strongly bind calcium ions, thereby lowering the availability of free calcium needed for crystal nucleation. Tannins also exhibit astringent properties that destabilize crystal growth, and their complex structures enable multiple interactions with crystal-forming species. As a result, tannins demonstrate greater nucleation inhibition compared to saponins or flavonoids alone (Chattaraj et al., 2023).

The combination of banana and mango peel integrates the actions of saponins and flavonoids. The phytochemical composition includes hydroxyl (-OH), ketone (=O), glycosidic bonds, and aromatic rings. This pairing enhances inhibitory activity through additive effects. Saponins interfere with crystal surface interactions, while flavonoids offer calcium chelation and antioxidant protection. Together, they moderately inhibit both nucleation and aggregation, thereby improving anti-urolithiatic potential.

A mixture of camansi and banana peel introduces a functional synergy between polyphenolic tannins and saponins. The blend is rich in hydroxyl (-OH), carboxylic (-COOH), ether (-O-), and glycosidic groups. Tannins effectively inhibit nucleation by binding calcium, while saponins limit aggregation by disrupting crystal structure. The presence of multiple polar groups increases the number of interaction sites available for inhibiting calcium oxalate formation, resulting in stronger inhibition than individual treatments (Saha & Verma, 2013).

The combination of mango and camansi peel, comprising flavonoids and tannins, presents a particularly rich polyphenolic profile. This mixture includes numerous phenolic (-OH), ketone (=O), carboxylic (-COOH), and aromatic groups. Tannins chelate calcium, and flavonoids counteract oxidative stress and inhibit crystal lattice assembly. The planar aromatic structures of flavonoids promote stacking interactions with crystal surfaces, enhancing binding and thereby delivering significant inhibition of both nucleation and aggregation (Kumar & Pandey, 2013).

The polyfruit blend, combining banana, mango, and camansi peels, demonstrated the most potent anti-urolithiatic activity. This comprehensive phytochemical mix includes saponins, flavonoids, and tannins, with functional groups such as hydroxyl (-OH), carboxylic (-COOH), ketone (=O), glycosidic bonds, and aromatic rings. The synergistic effects of these compounds provide multiple mechanisms of inhibition: saponins disrupt aggregation, flavonoids chelate calcium and neutralize free radicals, and tannins effectively inhibit nucleation. This multifaceted inhibition makes the polyfruit blend the most effective treatment for preventing calcium oxalate crystal formation (El Menyiy et al., 2021).

Health tourism, particularly wellness and medical tourism, is driven by the demand for natural, cost-effective, and culturally relevant remedies. The demonstrated synergistic anti-lithiatic potential of the solid-phase extraction (SPE) concentrates from banana, mango, and camansi peels positions the polyfruit formulation as a promising plant-based intervention for kidney stone prevention, a common urological issue encountered by both local populations and international patients. Given that kidney stone treatment (e.g., lithotripsy, surgery) can be costly and invasive, this study supports the development of herbal nutraceuticals or functional beverages derived from fruit waste that can be offered as part of preventive wellness packages in spas, detox centers, and integrative medical clinics.

With the Philippines promoting its rich biodiversity and indigenous knowledge in natural medicine, these findings could help local institutions craft evidence-based health tourism products rooted in Filipino agriculture and traditional healthcare practices. Moreover, the eco-friendly sourcing of ingredients aligns with the global preference for sustainable and organic health solutions, enhancing the marketability of Philippine-made phytotherapeutics in the ASEAN region and beyond. The agricultural impact of this study lies in the valorization of fruit peel waste, which is typically discarded during harvest or food processing. By identifying banana, mango, and camansi peels as rich sources of bioactive compounds such as saponins, flavonoids, and tannins, the study promotes the circular bioeconomy and strengthens the integration of agro-industrial byproducts into the pharmaceutical pipeline. This creates new income streams for farmers, cooperatives, and agri-processors who can now process peels into semi-finished raw materials for the health and wellness sector. It also incentivizes the cultivation of underutilized crops like camansi (Artocarpus camansi), which demonstrated the highest tannin content and inhibitory activity, thereby promoting crop diversification and local food security. Furthermore, the use of low-cost, green extraction techniques such as solid-phase extraction (SPE) makes the process scalable and accessible for community-based enterprises. These efforts support rural development, waste reduction, and value-added agriculture, hallmarks of inclusive and sustainable economic growth.

4. Conclusion

This study successfully met its general and specific objectives by demonstrating the synergistic in vitro anti-lithiatic potential of solid-phase extraction (SPE) concentrates derived from banana, mango, and camansi fruit peels. The SPE concentrates were systematically

prepared and quantified, confirming the presence of bioactive compounds: saponins in banana, flavonoids in mango, and tannins in camansi peels. Through UV-Vis spectrophotometric analysis, it was established that each extract exerted varying degrees of inhibitory effects on calcium oxalate nucleation and aggregation. Among individual treatments, camansi peel showed the highest inhibition, followed by mango and banana. However, combined treatments, particularly the polyfruit blend, exhibited significantly greater anti-lithiatic activity than individual extracts, as evidenced by both nucleation (58.63%) and aggregation (63.07%) inhibition.

Statistical analysis using ANOVA and Scheffé post hoc tests confirmed highly significant differences ($p < 0.01$) among treatment groups, with the polyfruit blend (Treatment G) showing effects second only to the positive control (Treatment H). These results affirm the hypothesis that a combination of phytochemicals from different fruit peels produces a synergistic effect, enhancing the overall inhibitory activity against kidney stone formation. The study underscores the therapeutic potential of fruit peel waste as a sustainable and cost-effective intervention for urolithiasis.

This research bridges traditional pharmacognosy and modern biotechnology, offering an opportunity to develop health tourism products from agricultural waste. It catalyzes interdisciplinary collaborations among pharmacists, medical practitioners, tourism professionals, and farmers, showcasing how locally sourced bioactive compounds can shape a sustainable and health-oriented tourism economy while uplifting rural agricultural sectors.

Based on the findings of the study, three key recommendations are proposed. First, in vivo studies and clinical trials should be conducted to validate the anti-lithiatic effects of the polyfruit peel extracts, ensuring their safety, efficacy, and potential for real-world application. Second, the specific bioactive compounds responsible for the observed synergistic activity should be isolated and characterized using advanced analytical techniques to better understand their mechanisms. Lastly, the development of standardized and sustainable polyfruit-based formulations, such as supplements or beverages, is recommended, promoting both kidney stone prevention and the valorization of fruit peel waste.

Disclosure statement

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