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In-vitro analysis of the anticoagulation properties of Kamias (*Averrhoa Bilimbi*)

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Abstract

This study investigated the potential of *Averrhoa bilimbi* (kamias) extract as a novel anticoagulant and compared its efficacy with the commercially available anticoagulant potassium oxalate. *Averrhoa bilimbi* is naturally rich in oxalic acid, which is known for its anticoagulant properties. Blood samples were obtained from two healthy donors, one male and one female. Given its feasibility, wide availability, and cost-effectiveness, an experimental design was employed to evaluate the in vitro anticoagulant properties of kamias through a series of controlled experiments. The study was conducted in three progressive experimental phases. The initial phase examined the anticoagulant activity of freshly prepared kamias filtrate derived from ground fruit. Although preliminary results indicated some anticoagulant potential, clot formation was observed, suggesting interference from an unidentified clotting factor. To address this limitation, a second phase, referred to as the "refinement phase," was implemented. In this phase, the kamias filtrate was treated with potassium hydroxide (KOH) to produce kamias extract oxalic acid (KEOx), which enhanced its ability to inhibit clot formation. This phase also enabled the identification of the clotting factor responsible for the unexpected coagulation observed earlier. In the final phase, procoagulant-free KEOx was tested and documented using standardized observation sheets to assess macroscopic and microscopic clot formation. Statistical analysis of the results indicated that KEOx exhibited anticoagulant activity comparable to that of standard anticoagulant blood collection tubes, although further refinement is needed to eliminate remaining interferences. The findings suggest that KEOx has potential applications in other laboratory settings, such as serving as an alternative dilution reagent for manual cell counting. Conversely, the natural procoagulant properties observed in untreated kamias also highlight its potential value in procoagulant research.

Keywords: *oxalic acid, oxalate, KEOx anticoagulant, calcium chelation*

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1. Introduction

Blood samples are obtained from patients prior to diagnostic testing through a procedure known as laboratory blood tube collection. Over the years, blood collection tubes have undergone continuous advancement, evolving from manually prepared glass tubes containing clot activators or anticoagulant additives to standardized evacuated systems that ensure more accurate blood-to-additive ratios (Moonla et al., 2025). Each clot activator and anticoagulant additive serves a specific purpose, and both tube manufacturers and laboratory professionals strive to provide appropriate, interference-free sample matrices for reliable laboratory testing (Bowen & Dasgupta, 2025). A clear understanding and correct use of clot activators and anticoagulant tube additives are therefore essential in clinical laboratories to preserve sample integrity and maintain optimal conditions before analysis (Lima-Oliveira et al., 2020).

Anticoagulants are substances added to blood or plasma to prevent clot formation (Heestermans et al., 2022; Günther & Ruppert, 2006), thereby minimizing changes in analyte concentration prior to analysis. Anticoagulation is primarily achieved through two mechanisms: inhibition of thrombin activity (as with heparin) and chelation of calcium ions, as seen with EDTA, citrate, and oxalate. Different laboratory tests require specific types of collection tubes, and proper blood collection follows the established “order of draw.” Tubes are color-coded to allow easy identification and appropriate use. In clinical practice, gray-top tubes contain potassium oxalate, which prevents coagulation by binding calcium, along with sodium fluoride, an antiglycolytic agent. For this reason, these tubes are routinely used for plasma glucose and lactic acid testing (Bayot, 2023).

Interest in herbal medicines has long contributed to advances in medicinal development. Oxalic acid occurs naturally and is produced by many organisms, including plants, animals, bacteria, and fungi. It is widely present in fruits, vegetables, nuts, and whole grains. One fruit particularly rich in oxalic acid is *Averrhoa bilimbi*, commonly known as bilimbi or kamias. Owing to its high oxalate content and wide availability, *Averrhoa bilimbi* has attracted research interest for potential applications in medical laboratory science (Alhassan & Ahmed, 2016; Anindita et al., 2024; Sagadevan et al., 2019; Solfaine et al., 2021).

Averrhoa bilimbi, a member of the Oxalidaceae family, is recognized worldwide by various names, including bilimbi in English, “blimblim” in French, and “kamias” or “kalamias” in Filipino. Often referred to as the cucumber tree or sorrel tree, it bears small,

green, highly acidic fruits commonly used in culinary practices to impart sourness to dishes. Beyond its culinary value, this distinctive fruit presents considerable scientific promise due to its biochemical properties (Alhassan & Ahmed, 2016).

Anticoagulant tube additives play a pivotal role in preserving blood samples prior to laboratory analysis. Oxalate is particularly significant because it prevents clot formation by chelating calcium, an essential cofactor in the coagulation cascade. Sá et al. (2019) reported that the oxalic acid content of Averrhoa bilimbi ranges from 8.45 to 11.20 mg/g, underscoring its potential medicinal relevance. These findings enhance the understanding of plant-derived anticoagulants and support further exploration of Averrhoa bilimbi as a viable laboratory reagent. Extensive experimental research has also highlighted its antibacterial and blood-thinning properties. Given its abundance, accessibility, and notable oxalic acid content, this study seeks to introduce KEOx as a practical, economical anticoagulant for laboratory applications.

The primary objective of this study is to extract the oxalate content of Averrhoa bilimbi and evaluate its potential as an alternative anticoagulant in comparison with commercially available potassium oxalate. The proposed Kamias-Extracted Oxalic Acid (KEOx) is expected to offer a cost-effective substitute without compromising anticoagulant efficacy. This research aims to develop a cost-effective and efficient source of oxalic acid that demonstrates minimal variation from synthetic and commercially available anticoagulants.

2. Literature Review

2.1. Anticoagulant Tube Additives and Their Role in Clinical Laboratory Practice

Anticoagulant tube additives are essential components of clinical laboratory workflows because they directly influence the quality and reliability of blood samples prior to analysis (Bowen & Remaley, 2014). By preventing clot formation, these additives preserve the physical and chemical characteristics of blood (Bowen & Adcock, 2016), allowing laboratory tests to reflect the patient's true physiological state. The selection of an appropriate anticoagulant is closely linked to the type of test being performed, as different analytical procedures require either whole blood or plasma in a stable, unclotted form. Failure to use the correct additive can lead to sample rejection, inaccurate results, or the need for repeat collection, all of which can delay diagnosis and patient management.

In addition to preventing coagulation, anticoagulant additives contribute to consistency and standardization in laboratory practice (Sarkar, 2025). Their use supports controlled sample handling, reduces pre-analytical variability, and ensures compatibility with automated analyzers. Proper handling of anticoagulant tubes, including correct filling, mixing, and storage, is equally important to maintain their effectiveness. As laboratory testing continues to expand in scope and complexity, the role of anticoagulant tube additives remains central to ensuring accuracy, efficiency, and overall quality in clinical diagnostics.

2.2. Oxalic Acid and Averrhoa bilimbi as a Potential Natural Anticoagulant

Oxalic acid is a well-documented calcium-chelating agent, and its anticoagulant action is based on its ability to bind free calcium ions (Grąz, 2024; İlhan et al., 2013; Liu et al., 2023), which are essential for multiple steps in the coagulation cascade. This mechanism has been widely applied in clinical laboratory practice through the use of oxalate-based anticoagulants, particularly in combination with antiglycolytic agents for biochemical testing. Several studies have demonstrated that calcium chelation effectively prevents fibrin formation and preserves blood samples for analytical purposes, making oxalates reliable anticoagulants in vitro (Al-Obaidy et al., 2023; Aizawa et al., 2020; Aaseth et al., 2023). The effectiveness of oxalic acid in inhibiting coagulation provides a strong biochemical rationale for exploring natural sources of oxalate as alternative anticoagulant agents, especially in settings where access to commercial reagents may be limited.

Averrhoa bilimbi has gained increasing research attention due to its exceptionally high oxalic acid content and associated biological activities. Quantitative analyses have confirmed that the fruit contains substantial levels of oxalic acid, supporting its potential use as a natural calcium-binding agent (Sá et al., 2019). In addition to its chemical composition, experimental studies have reported blood-thinning and antimicrobial properties of *Averrhoa bilimbi*, further reinforcing its relevance to medical and laboratory applications (Jayawardane et al., 2022; Labu et al., 2025; Luan et al., Lahlou et al., 2024; Alfiraza et al., 2025; Luan et al., 2021). Its widespread availability in tropical regions, low cost, and renewable nature strengthen the argument for its investigation as a plant-derived anticoagulant. Collectively, existing evidence supports the feasibility of *Averrhoa bilimbi* as a natural source of oxalic acid and justifies continued research into its extraction, standardization, and potential integration into laboratory anticoagulant systems.

3. Methodology

3.1. Study Design

The study was designed as an experimental investigation aimed at determining the in vitro anticoagulant properties of Kamias-Extracted Oxalic Acid (KEOx). Within this design, the researchers identified the optimum volume of KEOx required to achieve effective anticoagulation. An appropriate and validated observation sheet was used as the research instrument to collect essential data and other relevant information throughout the study.

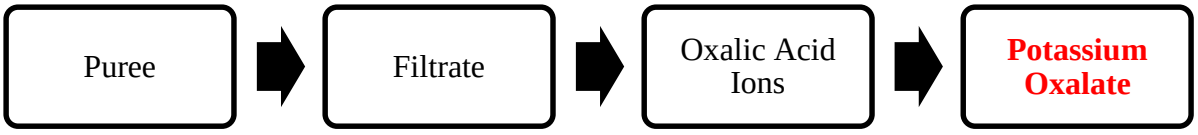
Venous blood samples were obtained from one male and one female student. The inclusion of both sexes was intentional, as biological differences may influence coagulation processes. For instance, males generally have higher muscle mass and lower body fat compared to females, which can affect blood volume and distribution and may influence the activity and metabolism of anticoagulant agents (Lundsgaard & Kiens, 2014; Oi Yan Chan et al., 2022; Wagner et al., 2022; Wróblewska et al., 2025; Hong et al., 2016; Faioni & Scimeca, 2025). Furthermore, sex hormones such as estrogen and progesterone in females are known to affect coagulation by influencing platelet activation and the synthesis of specific clotting factors (Gee et al., 2008; Coleman et al., 2024; Leng & Bray, 2005; Dupuis et al., 2019; Abou-Ismail et al., 2020).

The observation sheet served as the primary data collection tool during all experimental phases. It included detailed records of the date and time of experimentation, the optimum volume of KEOx solution, the volume of blood specimen used, the presence or absence of blood clots, and findings from both macroscopic and microscopic examinations. The use of this instrument facilitated systematic documentation, effective organization of records, and continuous monitoring of the study's progress.

3.2. Data Gathering Process

Figure 1

General overview of data collection procedure



Collection and preparation procedure. The researchers collected 400 grams of fresh green kamias fruits and ensured that only *Averrhoa bilimbi* specimens were used in the experiment. The fruits were harvested and sorted during the summer season in the Philippines, which corresponds to their peak growing period. Prior to processing, the fruits were thoroughly washed with water to remove dirt and other impurities. They were then cut into small pieces, and the stems and seeds were included in the homogenization process using a blender to produce a purée. The resulting purée was sieved and filtered using a cheesecloth to obtain clear kamias juice.

From the 400 grams of fresh green kamias fruits, the sieving and filtration process yielded approximately 250 mL of filtrate, as illustrated in Figure 3. The researchers assumed that 250 mL of kamias filtrate had an equivalent mass of 250 grams. According to Datiles (2022), the oxalic acid content of kamias fruit ranges from 8.57 to 10.32 mg per gram of filtrate, which contributes to its characteristic sour taste. Based on this estimated oxalic acid content in Averrhoa bilimbi, the researchers proceeded to calculate the corresponding oxalic acid ion concentration for the study.

$$250\ g\ x\ 8.5\frac{mg}{g} = 2125\ mg$$

$$50\ g\ x\ 10.32\frac{mg}{g} = 2580\ mg$$

$$\frac{2125\ mg\ x\ 2580\ mg}{2} = 2352.50\ mg$$

$$2352.50\ mg\ x\ \frac{1\ g}{1000\ mg} = 2.352\ grams$$

Based on the literature, it was concluded that a 250 mL volume of kamias filtrate contains approximately 2,352.50 mg, or 2.353 g, of oxalic acid ions (Daud et al., 2013). To obtain this filtrate, the kamias purée was poured into a clean cheesecloth and squeezed thoroughly until the maximum amount of liquid was extracted. This process yielded approximately 250 mL of filtrate containing about 2.35 g of oxalic acid ions. The filtrate was then treated to remove calcium ions by passing it twice through a cheesecloth-lined funnel filled with cation exchange resins. This step was performed to eliminate calcium interference and prevent unnecessary consumption of potassium hydroxide during subsequent reactions.

Stoichiometric principles were then applied in the following steps to determine the appropriate amount of potassium hydroxide required to react with the oxalic acid ions and produce potassium oxalate.

Find the moles of 2.35 g of oxalic acid. Oxalic acid (H2C2O4) is 90.04 g/mol

$$2.35\ g\ x\ \frac{90.04\ g/mol}{1\ moles\ of\ oxalic\ acid\ ions} = 0.26\ moles\ of\ Oxalic\ acid\ ions$$

Moles of Potassium Hydroxide (KOH) needed. The balanced reaction of producing oxalate is 2 Moles of KOH with 56.11 g/mol and 1 moles of Oxalic acid ions:

$$H_2C_2O_4 + 2KOH \rightarrow K_2C_2O_4 + 2H_2O$$

$$0.26\ moles\ of\ Oxalic\ acid\ x\ 2\ moles\ of\ KOH =$$

$$0.052\ moles\ of\ KOH 0.052\ moles\ KOH\ x \frac{56.11\ grams\ of\ KOH}{1\ moles\ of\ KOH} = 2.92\ grams\ of\ KOH$$

$$0.052\ moles\ KOH\ x \frac{56.11\ grams\ of\ KOH}{1\ moles\ of\ KOH} = 2.92\ grams\ of\ KOH$$

Preparation of 1 M KOH solution

$$\frac{0.052\ moles\ of\ KOH}{1000\ mL} = 0.052 \rightarrow 52\ mL\ solution$$

The researchers carefully weighed 56.11 grams of potassium hydroxide (KOH) flakes, which were slowly added to 800 mL of distilled water in a one-liter Erlenmeyer flask, leaving space for final adjustments. The solution was homogenized carefully until it became warm and then allowed to cool to room temperature. An additional 200 mL of distilled water was added to produce a 1 M KOH solution. Based on stoichiometric calculations, 52 mL of this KOH solution, containing 2.92 g of KOH, was sufficient to neutralize the 2.35 g of oxalic acid ions in 250 mL of Kamias filtrate, yielding approximately 4.79 g of potassium oxalate.

Phases of data gathering procedure with qualitative findings

Phase one: Filtrate as anticoagulant (Pilot Testing. In the initial phase, the researchers evaluated the anticoagulant potential of freshly extracted Kamias filtrate. Three different volumes of filtrate (0.5 mL, 1 mL, and 3 mL) were separately added to 3 mL blood samples, as shown in Table 1. The 0.5 mL treatment resulted in clotting within five minutes at room temperature. The 1 mL treatment demonstrated anticoagulant activity for approximately one hour before clot formation occurred. Interestingly, the highest volume (3 mL) did not prevent

clotting, suggesting that excessive filtrate may introduce factors that promote coagulation. Phase one indicated that Kamias filtrate has anticoagulant activity at an optimal volume but may induce clotting at higher concentrations.

Phase two: Kamias-Extracted Oxalic Acid (KEOx) as anticoagulant. According to Bakul et al. (2013) and Warren and Sargent (2011), *Averrhoa bilimbi* contains approximately 13 mg/g of calcium, which is higher than its oxalic acid content. This may explain the clotting observed with higher filtrate volumes in phase one. To address this, the researchers processed the filtrate to produce potassium oxalate using KOH. During this process, they observed the formation of precipitates. As noted by the Food Standards Australia New Zealand. (2015) and Krieger et al. (2015), potassium oxalate readily reacts with free calcium ions, forming insoluble calcium oxalate that settles at the bottom of the solution. Phase two thus revealed that the naturally high calcium content in *Averrhoa bilimbi* contributes to clot formation, emphasizing the need for calcium removal to enhance the anticoagulant activity of the extract.

Table 1

Cause of coagulation during Phase One

Volume (mL)	Result	Initial Interpretation	Reason
0.5	Clotted after 3 mins	Less oxalic acid	Less oxalic acid; Presence of Calcium
1	Shows anticoagulation activity for 1 hour	Right amount oxalic acid, unknown clotting	Right amount of Oxalic acid; Hindrance of Calcium ions
3	Clotted after 1 min and 30 secs	Unknown reason	High Unbalance number of Oxalic acid and Calcium ions

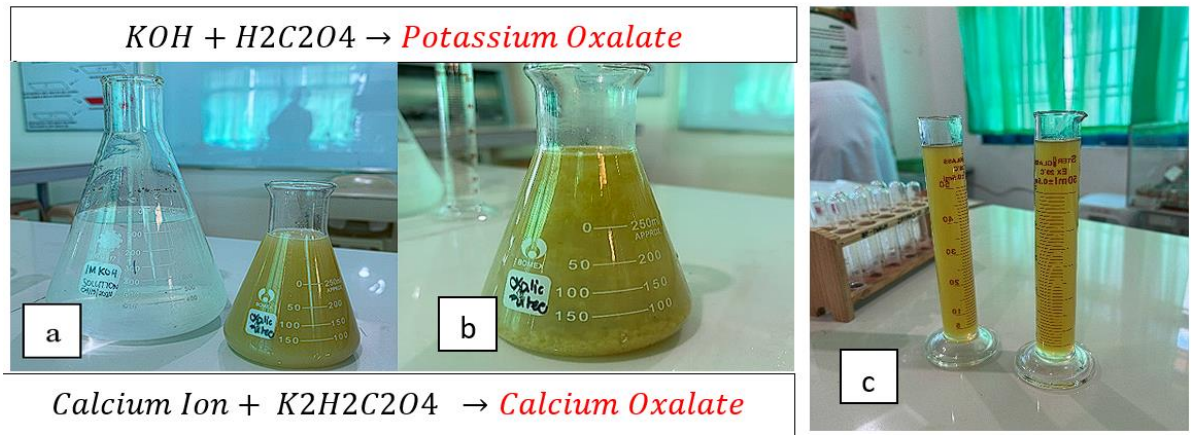
Table 1 shows that calcium plays a major role in clotting during the initial testing (Phase One). To address this, the researchers developed a new anticoagulant and used cation exchange resin beads (a water softener) to remove calcium ions present in the Kamias filtrate. The filtrate was passed through a cheesecloth funnel containing the resin beads. After calcium removal, both untreated and treated KEOx anticoagulants were collected. The filtrate was decanted into another Erlenmeyer flask to minimize the formation of precipitate ($CaC_2O_4 \cdot H_2O$). The filtered solution was then transferred into 10 mL test tubes and centrifuged to remove any remaining calcium oxalate precipitate. This step helped ensure the effective elimination of residual calcium, improving the purity of the KEOx anticoagulant.

The viability of KEOx Anticoagulant reagents of two variability was observed by the researchers for three weeks. First week after extraction, both KEOx anticoagulants exhibits anticoagulant activity on its first and second week. However, on its third week, KEOx anticoagulant without cation exchange treatment shows coagulation at any volume. This is due to the usage of potassium oxalate by the Kamias' calcium ions. KEOx treated with Ion exchange beads exhibits viability because it maintains ability to prevent coagulation on the third week post-extraction of kamias. KEOx anticoagulant remains its ability to prevent clot just by removing the calcium ions in the filtrate. Therefore, no wasting of potassium oxalate is done.

In Kamias fruits, the calcium content level is almost the same as the oxalic acid. That is why the researchers decided to let the extracted filtrate of kamias pass through an ion exchange resin beads for the removal of calcium ion present in the filtrate. Therefore, calcium is, without a doubt, the culprit of potassium oxalate wasting in the KEOx anticoagulant that is not treated with the resin beads.

Figure 2

Comparison of untreated and treated KEOx



Legend: (a) Initial KEOx anticoagulant. Filtrate that forms potassium oxalate that is gradually consumed by calcium ions to form calcium oxalate. (b.) Removal of calcium ions using water softener to prevent the wasting of potassium oxalate. (c.) Filtered Filtrate from the resin beads (no calcium oxalate formation).

The study aimed to isolate oxalic acid from Kamias extract to produce KEOx through the reaction of oxalic acid ions with potassium hydroxide. This process selectively precipitated free oxalic acid ions as potassium oxalate, separating them from other components and producing a potassium oxalate-rich extract that functions as a natural anticoagulant. Although

a secondary reaction between calcium ions and potassium oxalate can occur, it is considered irrelevant because potassium oxalate effectively chelates calcium, resulting in a purified anticoagulant solution.

Following extraction, the researchers tested the anticoagulant activity of KEOx under conditions designed to minimize interference that could induce clotting. Control groups included two negative controls, Ethylenediamine Tetraacetic Acid (EDTA, Purple Top) and Sodium Fluoride/Potassium Oxalate (Gray Top), and one positive control consisting of 50 µL of normal saline solution (NSS). In the test procedure, 50 µL of potassium oxalate-rich KEOx was added to a 10 mL test tube containing 1 mL of freshly extracted blood. Macroscopic clot formation was assessed every 20 minutes for two hours using an applicator stick to detect fibrin clot formation.

Table 2

Different KEOx anticoagulant concentration

Tubes	KEOx Concentration	Amount of KEOx Anticoagulant	Amount of NSS
KEOx #1	100	1 mL	0
KEOx #2	75	0.75 mL	0.25 mL
KEOx #3	50	0.5 mL	0.5 mL
KEOx #4	25	0.25 mL	0.75 mL

Phase three: Determination of optimum KEOx volume. To identify the lowest effective anticoagulant-to-blood ratio, the researchers prepared four different concentrations of KEOx, 100%, 75%, 50%, and 25%, as outlined in Table 2. This approach also accounted for the presence of glucose in Kamias fruits, which is approximately 2.8 g per 100 g (Malaysian Food Composition Database) and could interfere with plasma glucose measurements. The concentrations were titrated by gradually adding small amounts of KEOx to 1 mL blood samples until complete anticoagulation was achieved, as verified through both macroscopic and microscopic examination (Table 3). This procedure enabled the determination of the minimal KEOx volume required to prevent coagulation while minimizing interference from other components in the Kamias extract.

Table 3

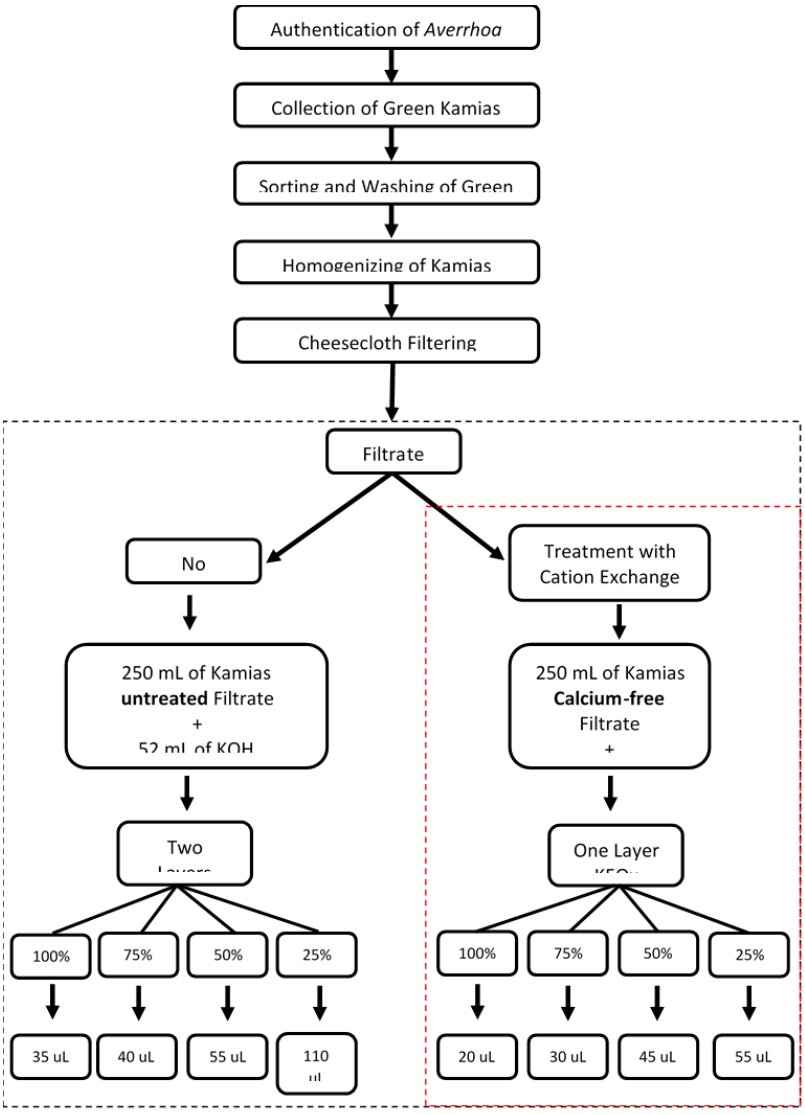
Titration of the optimum volume of KEOx

ANTICOAGULANT: BLOOD			PRESENCE OF BLOOD CLOT							
			Macroscopic Exam				Microscopic Exam			
Ratio	Anticoagulant (uL)	Blood (uL)	100	75	50	25	100	75	50	25
1:100	10	1000	+	+	+	+				
1:50	20	1000	-	+	+	+	-			
	25	1000	-	-	+	+	-	+		
1:33	30	1000	-	-	+	+	-	-		
1:29	35	1000	-	-	-	+	-	-	-	
1:25	40	1000	-	-	-	-	-	-	-	+
1:20	50	1000	-	-	-	-	-	-	-	-
1:18	55	1000	-	-	-	-	-	-	-	-

Note: Blue Negative – No fibrin clot latches on applicator stick; Red Negative – No Trapped RBCs is present

Figure 3

Flow chart of processing KEOx anticoagulant from Kamias



Note: Black box indicates the experimental phase while the gathered data on the red box were subjected to statistical treatment

3.3. Microscopic Examination

For microscopic evaluation, 100 µL of normal saline solution (NSS) was combined with 50 µL of whole blood on a clean glass slide. The mixture was gently homogenized and covered with a cover slip. Observations were conducted under high-power magnification to assess the presence or absence of microclots and to evaluate the effectiveness of KEOx as an anticoagulant at the cellular level. This procedure provided detailed qualitative insights into the anticoagulant activity of KEOx beyond macroscopic assessments.

3.4. Statistical Treatment

The study employed a two-sample t-test to statistically compare the anticoagulant effect of Kamias-Extracted Oxalic Acid with that of commercially available anticoagulant blood collection tubes. The analysis also examined the relationship between the anticoagulant activity of KEOx and the initial blood glucose level, assessing whether the presence of glucose in the extract influenced anticoagulation performance. This statistical approach allowed the researchers to determine the significance of differences in anticoagulant efficacy between natural and standard laboratory reagents.

4. Results and Discussions

Table 4

50 uL of KEOx anticoagulant to prevent Blood clots in terms of Macroscopic (MAC) clots

Tubes	1 Hour	2 Hours	3 Hours	24 Hours
NSS	0	0	0	0
Grey Top	0	0	0	0
Lavender Top	0	0	0	0
100% KEOx	0	0	0	0
75% KEOx	0	0	0	0
50% KEOx	0	0	0	0
25% KEOx	0	0	0	1

Note: MAC (0 = No clot, 1 = Small clot, 2 = Medium clot, 3 = Large clot)

These findings indicate that KEOx exhibits significant anticoagulant activity. At higher concentrations, clot formation was completely suppressed, demonstrating strong interference with the coagulation cascade. The 25% KEOx concentration, however, displayed a different

pattern: although it markedly delayed clot formation, small clots were observed after 24 hours. This suggests a potential dose-dependent effect, where lower concentrations may be less effective at maintaining complete anticoagulation. Alternatively, the delayed clotting could be due to the presence of minor procoagulant compounds in the Kamias extract that become active when the concentration of KEOx is reduced.

Table 5

50 uL of KEOx anticoagulant to prevent Blood clots in terms of Microscopic (MIC) clots

Tubes	1 Hour	2 Hours	3 Hours	24 Hours
NSS	3	3	3	3
Grey Top	0	0	0	0
Lavender Top	0	0	0	0
100% KEOx	0	0	0	0
75% KEOx	0	0	0	1
50% KEOx	0	0	0	1
25% KEOx	0	1	1	3

Note: MIC (0 = No trapped RBCs, 1 = minimal trapped RBCs, 2 = Moderately trapped RBCs, 3 = Extensive trapped RBCs)

Microscopic analysis of clot formation revealed a complex, dose-dependent effect of KEOx on red blood cell (RBC) entrapment. Higher concentrations of Kamias extract (100%, 75%, and 50%) effectively suppressed RBC entrapment within fibrin clots for up to three hours. In contrast, the 25% KEOx concentration initially inhibited RBC trapping but exhibited the highest level of trapped RBCs within 24 hours. This observation suggests that the anticoagulant effect of KEOx may be both dose- and time-dependent, although further investigation is needed to fully understand the underlying mechanisms.

The results indicate the presence of two functional components within KEOx: a dominant anticoagulant factor active at higher concentrations, and a potentially weaker procoagulant component of Kamias that becomes evident at lower KEOx concentrations. As shown in Table 5, the filtrate was not initially treated before reacting with potassium hydroxide, allowing some calcium ions to interact with oxalate, reducing the effective formation of potassium oxalate. Even after treatment with cation exchange resin beads, residual calcium may persist due to a regeneration effect (Abusultan et al., 2024), potentially contributing to delayed clotting. Additionally, the lower dose of potassium oxalate in the 25%

KEOx preparation may be insufficient to fully chelate the available calcium ions, further explaining the observed increase in RBC entrapment over time. These findings highlight the importance of both concentration and calcium management in optimizing KEOx as an anticoagulant.

Table 6

Acceptability testing of the Kamias-Extract Oxalic Acid in terms of macroscopic and microscopic examination

Mean KEOx Anticoagulant	t	p	Descriptive Equivalent
Macroscopic Examination (MAC)			
25%	1.000	.356	Not Significant
Microscopic Examination (MIC)			
75%	1.000	.356	Not Significant
50%	1.000	.356	Not Significant
25%	1.987	.094	Not Significant

KEOx at 100% concentration demonstrated potent anticoagulant activity, closely resembling the effects observed with standard Grey Top (sodium fluoride/potassium oxalate) and Lavender Top (EDTA) tubes. The collected data indicated no significant differences between KEOx and the commercial anticoagulants. Macroscopic analysis of 25% KEOx yielded a p-value of 0.356, while microscopic comparisons showed p-values of 0.356 for 75% and 50% KEOx, and 0.094 for 25% KEOx. Since all p-values exceed the alpha threshold of 0.05, these results suggest that KEOx possesses inherent anticoagulant properties, both macroscopically and microscopically.

The strong anticoagulant effect of KEOx can be attributed to the high oxalic acid content in *Averrhoa bilimbi*. Singh (2023) reports that 100 grams of Kamias contains 8–11 mg of oxalic acid, which also contributes to the fruit's characteristic sourness. During the study, a standardized volume of 100 µL of KEOx was used for all concentrations, as this volume was sufficient to achieve optimal anticoagulation. Additionally, variations in calcium levels between individuals necessitate the presence of extra chelating agents to bind excess calcium ions, further enhancing the inhibition of clot formation. These findings highlight the potential of KEOx as a natural, effective alternative to commercially available anticoagulants in laboratory settings.

Table 7

Blood to anticoagulant ratio of different concentration of KEOx anticoagulant in terms of its optimum volume

Specimen	Anticoagulant (mL)	Blood (mL)	Anticoagulant: Blood Ratio
100% KEOx	0.020	1	1:50
75% KEOx	0.030	1	1:33
50% KEOx	0.035	1	1:29
25% KEOx	0.050	1	1:20

Following the procedures outlined in Tables 5 and 6, the researchers determined the optimum volume of each KEOx concentration required to achieve effective anticoagulation. For 100% KEOx, 0.020 mL (20 µL) was sufficient to neutralize calcium ions in the blood. The 75% KEOx concentration required 0.030 mL, while 50% KEOx needed 0.035 mL (30 µL) to prevent clot formation. The 25% KEOx specimen required 0.050 mL (50 µL) to achieve anticoagulation.

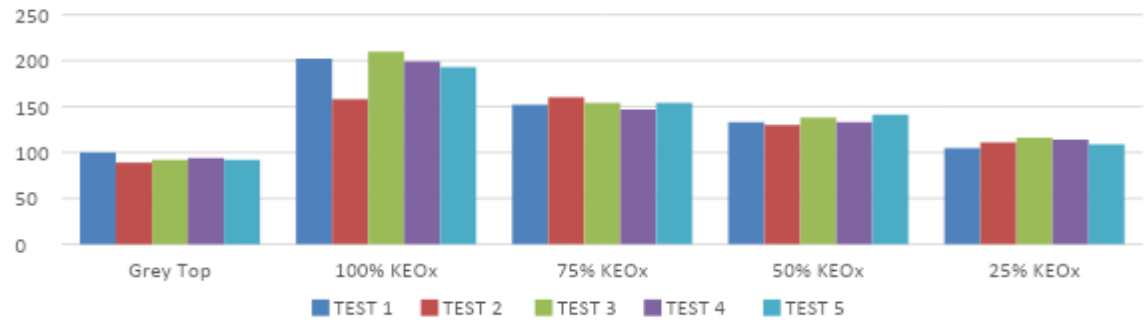
Based on these findings, 75% and 50% KEOx are considered the most suitable concentrations because they balance anticoagulant effectiveness with minimal interference. Higher concentrations, such as 100% KEOx, could potentially affect blood glucose measurements, while the lower 25% KEOx may cause a dilution effect in biochemical tests. Specifically, 25% KEOx contains approximately 0.378 mg of potassium oxalate per 50 µL of anticoagulant, which is theoretically sufficient to chelate calcium in 1 mL of blood. However, variations in calcium levels among individuals may require slightly higher volumes, an additional 5–10 µL, to ensure complete calcium chelation. This variability likely explains the macroscopic and microscopic clot formation observed with 25% KEOx. Commercial grey-top tubes typically include excess potassium oxalate to account for individual differences in calcium concentration, ensuring reliable anticoagulation (Lima-Oliveira et al., 2021).

In the study, the positive control (grey-top tube) was collected before initial glucose testing, showing an average glucose level of 93 mg/dL as measured with a glucose meter. When ripe Kamias was used as the source of KEOx, the researchers anticipated a potential glucose increase due to the fruit's natural sugar content. As data were collected, the 100% KEOx sample showed a marked rise in glucose, averaging 192 mg/dL. The 75% KEOx sample had an initial average glucose of 153 mg/dL, while 50% KEOx averaged 135 mg/dL. KEOx

#4 (25% concentration) yielded an average glucose level of 109 mg/dL, which was comparatively lower but still elevated despite its reduced KEOx content.

Figure 4

Comparison of initial glucose testing of KEOx anticoagulant and grey top tube



These initial glucose measurements were conducted to assess whether the natural sugars present in Kamias extract could significantly affect plasma glucose levels during anticoagulation testing. The findings highlight the need to monitor glucose interference in KEOx anticoagulant preparations and suggest that further separation or purification techniques may be required to minimize the impact of Kamias sugars on laboratory assays.

Table 8

Acceptability testing between the initial glucose levels of different KEOx and commercially available potassium oxalate

Mean Grey Top Glucose Concentration	KEOx blood Glucose Concentration	t	p	Descriptive Equivalent
93 mg/dL	192 mg/dL	-2.644	.038	Significant
93 mg/dL	153 mg/dL	-6.881	.000	Significant
93 mg/dL	135 mg/dL	-9.739	.000	Significant
93 mg/dL	109 mg/dL	-12.323	.000	Significant

The comparison of initial glucose levels between KEOx and commercially available potassium oxalate revealed significant differences across all concentrations. The 100% KEOx sample had a p-value of 0.038, while 75%, 50%, and 25% KEOx concentrations all showed p-values of 0.000. Since these values fall below the alpha level of 0.05, the results indicate that KEOx contains high sugar content despite demonstrating anticoagulant activity. This is

consistent with the natural composition of Averrhoa bilimbi, which, according to Singh (2024), contains approximately 10 g of carbohydrates per 100 g of fruit, including 3.56 mg of glucose. However, the presence of glucose is less relevant in assays targeting lactic acid because sodium fluoride stabilizes lactic acid levels during testing.

5. Conclusion

The experimentation confirmed that the raw Kamias filtrate without potassium hydroxide initially demonstrated contradictory anticoagulant activity due to the interaction between calcium ions and oxalic acid ions. In phase two, the addition of potassium hydroxide produced potassium oxalate, although some was wasted through interaction with Kamias calcium, a process the researchers termed “processu chelation,” highlighting the plant’s potential procoagulant properties. This was addressed using cation exchange resin beads, isolating potassium oxalate for effective calcium chelation, referred to as “in vitro chelation.”

Statistical analysis supported acceptance of the null hypothesis, showing no significant difference between the anticoagulant activity of Kamias-Extracted Oxalic Acid (KEOx) and commercial anticoagulants, both macroscopically and microscopically. Overall, Kamias extract exhibits intrinsic anticoagulant properties but also contains procoagulant elements. The isolation of potassium oxalate allowed the extract to function effectively as an anticoagulant, producing results comparable to standard commercial blood collection tubes. These findings demonstrate the potential of KEOx as a natural and cost-effective alternative anticoagulant.

The study recommends further refinement of KEOx to remove interfering compounds such as glucose and phenolic substances, isolating only the active anticoagulant component, potassium oxalate. Achieving a powdered form of the extract is suggested to improve stability, handling, and standardization for laboratory applications. Additionally, the study suggests incorporating sodium fluoride-based lactic acid testing and kinetic glucose assays to evaluate the combined effectiveness of KEOx and sodium fluoride as a potential substitute for commercial Grey Top blood collection tubes. KEOx also shows promise in broader laboratory applications, particularly as an alternative dilution reagent for white blood cell and platelet counts, potentially replacing reagents like acetic acid or ammonium oxalate. For example, oxalic acid can react with ammonium carbonate to form ammonium oxalate, providing a safer and natural option for laboratory procedures.

Furthermore, Averrhoa bilimbi may serve as a potential procoagulant source. Qualitative findings revealed that calcium oxalate forms as a secondary product when potassium oxalate chelates calcium present in Kamias. By removing calcium ions during the "in processu chelation," an insoluble precipitate is formed, highlighting the plant's procoagulant potential. Future studies could explore methods to extract this calcium and investigate its application as a clot activator, offering opportunities for new research into natural procoagulants derived from Kamias.

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Institutional Review Board Statement

This study was conducted in accordance with the ethical guidelines of Calayan Educational Foundation, Inc. The conduct of this study has been approved and given relative clearance(s) by Calayan Educational Foundation, Inc.

AI Declaration

The author declares the use of artificial intelligence (AI) in writing this paper. In particular, the authors utilised ChatGPT for language editing, restructuring sentences, and improving clarity. The authors take full responsibility for ensuring proper review and editing of the AI-generated content.

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