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In vitro bacteriostatic properties of Kamias (*Averrhoa bilimbi*) fruit ethanolic extract against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*

Ryan Jay G. Mostoles*[1], Priscilla S. Abellera[2]

[1]*Lorma Colleges, Lingsat, San Fernando City, La Union, Philippines*

[2]*Don Mariano Marcos Memorial State University, Bacnotan, Philippines*

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Abstract

A study was conducted to (a) determine the phytochemical constituents found in Kamias ethanolic extract that give its bacteriostatic property, and to (b) determine the degree of bacteriostatic property of the Kamias ethanolic extract in different concentrations (25%, 50%, 75%, and 100%) based on the zone of inhibition. The results of the study showed that the constituents which gave the bacteriostatic property of Kamias are flavonoids, saponins, and tannins. Among the different concentrations that were tested, the concentration that contained 100% Kamias ethanolic extract exhibited the highest zone of inhibition. The 100% concentration of the Kamias ethanolic extract with a mean zone of inhibition of 11.4mm was revealed to be the most effective inhibitor of *Staphylococcus aureus* as compared to the other concentrations. No zone of inhibition was exhibited on the different concentrations against *Escherichia coli* and *Pseudomonas aeruginosa* with a mean zone of inhibition of 6mm. Kamias ethanolic extract, as an organic component for hand sanitizer, has phytochemical constituents responsible for its bacteriostatic activity, and the highest concentration of Kamias ethanolic extract possessed a bacteriostatic property with the ideal concentration and potential in formulating the hand sanitizer as an organic ingredient.

* **Corresponding Author:** Angelina T. Gonzales ✉ agonzales@dmmmsu.edu.ph

Introduction

A bacteriostatic agent is a biological or chemical agent that stops bacteria from reproducing and killing them. When bacteriostatic antimicrobials are used, the duration of therapy must be sufficient to allow host defense mechanisms to eradicate the bacteria. According to the study by Silver (2016), it was documented the use of natural products as new anti-bacterial drugs.

To date, about 3.4 billion people, which represent about 88% of the world's inhabitants, in the developing world depend on plant-based traditional medicines. In China, traditional herbal preparations account for 30-50% of the total medicinal consumption, while in Europe, North America, and other industrialized regions, over 50% of their population uses complementary or alternative medicine (World Health Organization, 2022).

In the Philippines, traditional healers have used herbal preparations as antiseptics and cures for pneumonia, diarrheas, and other bacterial infections. In the study of Lazarte *et al.* (2020), the commonly used medicinal plants by traditional healers in the Philippines include guyabano, tsaang gubat, sambong, and ulasimang bato. These herbal plants were cultivated throughout the Philippines and used by Filipino traditional healers for various ailments. The study also revealed the anti-bacterial activity of guyabano, ulasimang bato, sambong, and tsaang gubat leaf extracts against common drug-resistant bacteria. Locally, the province of La Union demonstrated many plant species, which are also important in local healing practices. According to the study of Ducusin (2017), gatas-gatas/tawa-tawa (*Euphorbia hirta* L.) and malmalukong/takip-kuhol (*Centella asiatica* L.), guava (*Psidium guajava* L.) and lagundi (*Vitex negundo* L.) were identified types of medicinal plants used by local people. In the study, Kamias is locally found in the province of La Union. It is abundant in most places of the province. It is considered an herbal plant with many medicinal benefits.

One common plant in urban areas is the Kamias (*Averrhoa bilimbi*). *A. bilimbi* of the Oxalidaceae family is medicinally used as a folk remedy for many symptoms. It is used as an anti-bacterial, antiscorbutic, astringent, post–partum protective medicine. It is also used for the treatment of fever, mumps, pimples, inflammation of the rectum and diabetes, itches, boils, rheumatism, syphilis, bilious colic, whooping cough, hypertension, stomach ache, aphthous ulcer, and as a cooling drink. The chemical constituents of *A. bilimbi* include amino acids, citric acid, cyanidin–3–O–h–D–glucoside, phenolics, potassium ions, sugars, and vitamin A. In the Philippines, *A. bilimbi* treats scabies and is applied to poisonous creature bites (Garg *et al.,* 2022).

Pathogenic microorganisms such as bacteria and viruses have always been a big societal problem. Various bacteria cause various diseases which interactions between host and microorganisms such as *S. aureus*, *E. coli* and *Pseudomonas* species can transmit. In the study, the microorganism which was used is *S. aureus* with American Type Culture Collection (ATCC) number 25923, *E. coli* (ATCC 9637), and *P. aeruginosa* (ATCC 27853).

Staphylococci are gram-positive aerobic organisms. *Staphylococcus aureus* (*S. aureus*) is the most pathogenic, which typically causes skin infections and sometimes pneumonia, endocarditis, and osteomyelitis. It commonly leads to abscess formation. Some strains elaborate toxins that cause gastroenteritis, scalded skin syndrome, and toxic shock syndrome.

Escherichia coli (*E. coli*) is a bacterium commonly found in the gut of humans and warm-blooded animals. Some strains, however, such as Shiga toxin-producing *E. coli* (STEC), can cause severe foodborne disease. It is transmitted to humans primarily by consuming contaminated foods, such as raw or undercooked ground meat products, raw milk, and contaminated raw vegetables and sprouts.

Pseudomonas species are Gram-negative rod-shaped bacteria widespread in water and soil. Infections of the skin or soft tissues by *Pseudomonas aeruginosa* and other Pseudomonas spp. Range from superficial discolorations to serious and life-threatening because these bacteria are invasive and toxigenic. *P. aeruginosa* is commonly found transiently on the skin and readily colonizes moist skin.

The study was conducted to determine the phytochemical constituents found in Kamias (*A. bilimbi*) fruit ethanolic extract that gives its bacteriostatic property, and (2) determine the degree of bacteriostatic property of the Kamias ethanolic extract in different concentrations.

Materials and Methods

Research design

This study utilized the experimental type of research to determine the in vitro bacteriostatic property of Kamias (*A. bilimbi* L.) fruit extract.

An experiment was conducted to test the in vitro bacteriostatic properties of Kamias fruit ethanolic extract against *S. aureus*, *E. coli*, and *P. aeruginosa*. The antibacterial susceptibility of *S. aureus*, *E. coli*, and *P. aeruginosa* was tested on the strength of the in vitro bacteriostatic property of the different concentrations (25%, 50%, 75%, and 100%) of Kamias fruit ethanolic extract.

Materials and procedures

The materials and equipment used in performing the procedures are Kamias (*A. bilimbi*) fruits, 95% ethanol, blood agar plate, Mueller Hinton agar, mannitol salt agar, incubator, autoclave, vernier caliper, ruler, test tubes (15×125mm), test tube rack, disposable Pasteur pipettes, disposable petri dish, forceps, digital weighing scale, inoculating loop, blender, filter paper, Erlenmeyer flask, beaker, mixing vessel, stirring rods, refractometer, digital pH meter, viscometer, and pill tile spreadable device.

Collection and extraction process of Kamias fruits

A fresh and healthy fruits of the Kamias were harvested from the vicinity of the researcher's barangay in Ipet, Sudipen, La Union. The harvested fruits were placed in a clean plastic bag and washed with distilled water to remove the dust. Fruits of Kamias were air-dried and weighed. Using the extraction process employed in the study conducted by Jacela *et al.* (2021) and Gonzales (2018), *A. bilimbi* fruits were subjected to alcoholic extraction. Ethanolic extraction was done by soaking 300 grams of Kamias fruits in 1L of 95% ethanol for 48 hours. After exhaustive extraction, the fruits were filtered and concentrated. The solution was heated in a water bath until all the ethanol was evaporated and the pure extract was left. Ethanol was used as an extracting medium in this study due to its ability to easily penetrate the cellular membrane and extract intercellular ingredients from the plant material.

Phytochemical analysis of Kamias ethanolic extract

Kamias fruit ethanolic extract was subjected to phytochemical screening to evaluate the presence of phytochemical metabolites, specifically alkaloids, carbohydrates, flavonoids, tannins, phenols, saponins, organic acids, fixed and essential oils. The extract was subjected to Mayer's test, Hager's test, Wagner's test, and Dragendorff's test to detect the presence of alkaloids; Molisch test, Benedict's test, and Fehling's test for the detection of carbohydrates; Lead Acetate test and Alkaline Reagent test for the detection of flavonoids and tannins; Ferric Chloride test for the detection of phenols; Froth test and Gelatin test for the detection of saponins and organic acids; Filter paper test for the detection of fixed and essential oils. The Kamias ethanolic extract was submitted to the College of Pharmacy of LORMA Colleges for the standardized procedure of phytochemical analysis. The official phytochemical test result was obtained and released.

Results and discussion

Phytochemical constituents of Kamias (A. bilimbi L.*) fruit ethanolic extract*

Phytochemical constituents are the natural bioactive compounds found in plants. In vitro bacteriostatic properties against *S. aureus*, *E. coli*, and *P. aeruginosa* were done through phytochemical testing on the Kamias fruit ethanolic extract. The phytochemical analysis evaluates plant chemicals that have protective properties against microorganisms.

The summary of the phytochemical test results Kamias (*A. blimbi* L.) fruit ethanolic extract is presented in the Table 1 below.

Table 1. Phytochemical analysis results of Kamias *(A. blimbi* L.*)* fruit ethanolic extract

Type of test	Result
A. Detection of Alkaloids	
1. Mayer's Test	-
2. Hager's Test	-
3. Wagner's Test	-
4. Dragendorff's Test	-
B. Detection of Carbohydrates	
1. Molisch Test	+
2. Benedict's Test	+
3. Fehling's Test	+
C. Detection of Flavonoids and Tannins	
1. Lead Acetate Test	-
2. Alkaline Reagent Test	+
D. Detection of Phenols	
1. Ferric Chloride Test	-
E. Detection of Saponins and Organic Acids	
1. Froth Test	+
2. Gelatin Test	+
F. Detection of fixed and essential oils	
1. Filter Paper Test	-

Legend: (+) present; (-) not present

Table 1 shows the complete panel of test results of the qualitative phytochemical analysis of Kamias *(A. blimbi* L.) ethanolic extract. It can be gleaned from the table the different phytochemical tests performed for the detection of alkaloids, carbohydrates, flavonoids, tannins, phenols, saponins, organic acids, fixed oils, and essential oils. The analysis provided a notable positive result of the Molisch test, Benedict's test, Fehling's test, Alkaline reagent test, Froth test, and Gelatin Test.

The Kamias ethanolic extract turned negative for Mayer's, Hager's, Wagner's, and Dragendorff's tests, thus indicating the absence of any alkaloids. The extract reacted positively to all the tests for carbohydrates, implying the presence of reducing sugars or carbohydrates with aldehyde functional groups. Lead acetate and alkaline reagent test turned negative and positive, respectively. The interpretation of the official results declared the presence of flavonoids and tannins. The ferric chloride test turned negative, signifying the absence of phenolic substances. On the other hand, the Gelatin test turned positive, and foam formation in the froth test appeared, thus both indicating the presence of saponins and organic acids in the extract. The extract also turned negative results in filter paper tests, thus indicating the absence of fixed and essential oils.

The test result implies that the plant sample contains carbohydrates and reducing sugars, flavonoids, saponins, and tannins. However, the plant sample contains neither alkaloids, proteins, polyphonic compounds, fixed or essential oils. This complements the findings in the study of Cheong *et al.* (2022), wherein they detected the presence of flavonoids, tannins, triterpenoids, and steroids but no alkaloids in the Kamias fruit extract. The study mentioned that flavonoids are anti-inflammatory, anti-allergic, analgesic, and antioxidant. Also, the saponin compound in the plant has supported the usefulness of Kamias in managing inflammation caused by bacteria.

Moreover, similar observations are reported in the study of Setyawan *et al.* (2021) too. The study demonstrated the phytochemical diversity of *A. bilimbi* fruit extract, enriched with different secondary metabolites, especially flavonoids, phlorotannins, tannins, and saponins. The study's methanol extract of *A. bilimbi* has detected a diverse component of flavonoids, coumarins, and phenols, followed by chloroform extract, which exhibited the same component. The study concluded that *A. bilimbi* is a source of phytopharmaceutical production and showed significant pharmacological activities and contains immense phytochemicals that can be utilized to produce novel drugs and agents to combat microorganism that causes various diseases.

Phytochemistry is a distinct discipline that deals with various organic substances accumulated in plants. The plants may be considered a biosynthetic laboratory. Not only are their chemical compounds, such as carbohydrates, protein, and lipids, used as food by men but also many compounds like glycosides, alkaloids, flavonoids, etc., are used as medicines by man in various ways and means.

According to their functions in plant metabolism, phytochemicals are basically divided into two groups. The primary constituents comprise common sugars, amino acids, proteins, and chlorophyll, whereas the secondary constituents comprise alkaloids, terpenoids, saponins, phenolic compounds, flavonoids, tannins, etc.

Bacteriostatic components of Kamias (A. bilimbi L.*) fruit ethanolic extract*

Table 2 shows the bacteriostatic component tested in Kamias *(A. bilimbi* L.*)* fruit ethanolic extract: flavonoids, phenols, tannins, saponins, alkaloids, and organic acid. The table registered a positive presence of the compound's flavonoids, tannins, saponins, and organic acids. Phytochemical constituents such as tannins, flavonoids, phenols, and other aromatic compounds or plants' secondary metabolites serve as defense mechanisms against many microorganisms. The antimicrobial properties of the plant are present due to these metabolites.

Table 2. Bacteriostatic components of Kamias (*A. bilimbi* L.*)* extract

Bacteriostatic component	Result
Flavonoids	+
Phenols	-
Tannins	+
Saponins	+
Alkaloids	-
Organic Acid	+

Flavonoids have various properties that are of great health benefits. Flavonoids are a group of plant metabolites that provide health benefits and antioxidant effects. These properties are found in many fruits and vegetables. Flavonoids are well-known as antibacterial agents against a wide range of pathogenic microorganisms. Flavonoids can bind to bacterial extracellular proteins, inactivate proteins to inhibit bacterial cell metabolism and be involved with bacterial cell walls. With the increasing prevalence of untreatable infections induced by antibiotic resistance bacteria, flavonoids have attracted much interest because of the potential to be substituted for antibiotics.

According to the study of Setyawan *et al.* (2021), flavonoids have been recognized as potential natural sources of antimicrobial drugs. It can exert antibacterial activities through multiple mechanisms, such as disruption of the cytoplasmic membrane, inhibition of nucleic acid synthesis, inhibition of energy metabolism, inhibition of cell wall synthesis, and inhibition of cell membrane synthesis. Their activity is due to their ability to react with extracellular and soluble proteins and complex bacterial cell walls leading to the death of the bacteria.

Saponins are widely isolated from the medicinal plant, containing bioorganic molecules with considerable molecular weight. Saponin is derived from *Saponaria vaccaria,* a plant that abounds in saponins and was once used as soap. Saponins have many health benefits. Saponins have pharmacological activities as immunomodulators, anticarcinogens, anti-inflammatory, antiviral, antimicrobial, antiprotozoal, hypoglycemic, hypocholesterolemic, and antioxidant. Saponin applications were observed due to various biological, medicinal, and pharmaceutical actions (Aziz *et al*., 2019).

Tannins are naturally occurring complex organic compounds possessing nitrogen-free polyphenols of high molecular weight. Tannins are abundant in leafy vegetables and possess antimicrobial activity. Tannins are water-soluble polyphenols that differ from most other natural phenolic compounds in their ability to precipitate proteins such as gelatin. Tannins are widely distributed in plant flora. These substances may inhibit microorganisms, interfere with some metabolic processes, or modulate gene expression and signal transduction pathways. The different mechanisms proposed so far to explain tannin antimicrobial activity include inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth, or direct action on microbial metabolism through inhibition of oxidative phosphorylation (Kaczmarek, 2020).

Based on the data gathered, the presence of significant bacteriostatic components such as flavonoids, saponins, and tannins in Kamias *(A. bilimbi* L.*)* fruit ethanolic extract contributes to its bacteriostatic activity against selected microorganisms. The findings of the current study are consistent with those of Safitri and Leliqia (2021), wherein their study revealed the ability of the Kamias extract that exhibit antibacterial activity, suggesting the presence of polar and non-polar antibacterial compounds such as flavonoids, saponins, and tannins.

Further, it is also apparent in the study of Galvez (2016) on antibacterial activity and phytochemical screening of selected folkloric plants that the presence of the secondary metabolites of their chosen plants in the study with antibacterial potentials is responsible for their antimicrobial properties. According to the study, if at least one of the extract's constituents is present in a particular plant, it is more likely to have antimicrobial or bacteriostatic properties against bacterial isolate. Therefore, the detected presence of bioactive compounds of flavonoids, saponins, and tannins in the extract can be associated with the antibacterial activity or bacteriostatic property of Kamias.

This section presents the bacteriostatic components of Kamias fruit extract. The mechanism of action of the bacteriostatic phytochemicals present can explain the bacteriostatic component of the fruit extract.

Kamias (A. bilimbi L.*) fruit ethanolic extract against S. aureus, E. coli, and P. aeruginosa*

Table 3 shows the antimicrobial property of different concentrations (25%, 50%, 75%, and 100%) of Kamias *(A. bilimbi* L.*)* Fruit ethanolic extract against *S. aureus, E. coli, and P. aeruginosa* through Kirby-Bauer disc diffusion assay.

The degree of bacteriostatic sensitivity of Kamias fruit ethanolic extract against *P. aeruginosa* in various concentrations based on the zone of inhibition revealed that all concentrations (25%, 50%, 75%, and 100%) exhibited no zone of inhibition with a mean zone of inhibition of 6mm, thus it is interpreted to be resistant or inactive as indicated in standard zones of inhibition.

Table 3. Sensitivity Testing Results in Different Concentrations of *Kamias* Ethanolic Extract against *S. aureus, E. coli, and P. aeruginosa*

Concentrations	Zone of Inhibition (mm)						Interpretation
	Replication						
	1	2	3	4	5	M	
P. aeruginosa							
25%	6	6	6	6	6	6	R
50%	6	6	6	6	6	6	R
75%	6	6	6	6	6	6	R
100%	6	6	6	6	6	6	R
E. coli							
25%	6	6	6	6	6	6	R
50%	6	6	6	6	6	6	R
75%	6	6	6	6	6	6	R
100%	6	6	6	6	6	6	R
S.aureus							
25%	6	6	6	6	6	6	R
50%	6	6	6	6	6	6	R
75%	10	11	9	9	8	9.4	R
100%	12	13	11	11	10	11.4	I

Legend: Susceptible/Very active (>19mm); Active (14-19mm); Intermediate (10-13mm); Resistant/inactive (<10mm); R- resistant; I- Immediate

The study's findings are congruent with the result of Elmido *et al.* (2018) study on the inhibition of *P. aeruginosa* by the extracts of indigenous plants from the Cordillera Region, Philippines. The findings of their study showed that 4 out of the 13 plant extracts screened showed significant inhibition zones. The study concluded that the plants tested against *P. aeruginosa* have low antibacterial activity. The study explained that *P. aeruginosa* is an opportunistic pathogen that has attained severe therapeutic challenges in the fields of medicine and pharmacy.

Moreover, it has been reported that *P. aeruginosa* develops resistance to multiple classes of antibiotics. The composition of its outer membrane is structural lipopolysaccharides, making the cell impermeable to lipophilic solutes. Furthermore, it has characteristic inherent multiple efflux pumps that prevent the intracellular accumulation of antibacterial agents. These factors contribute to its rapid resistance against antibacterial drugs.

In this study, a resistant result of the sensitivity test shows that the inhibition of *P. aeruginosa* was not achieved by the Kamias ethanolic extract, hence incapable of becoming a bacteriostatic agent.

The degree of sensitivity of Kamias fruit ethanolic extract against *E. coli* showed that all concentrations (25%, 50%, 75%, and 100%) exhibited no zone of inhibition with a mean of 6mm; thus, it is interpreted to be resistant or inactive, as indicated in standard inhibition zones. According to Pakbin *et al.* (2021), *E. coli* is a remarkable and diverse organism that acquires a combination of genetic elements to become a pathogen capable of causing a range of diseases, from gastroenteritis to extra intestinal infections of the urinary tract, bloodstream, and central nervous system. The pathogenicity of *E. coli* isolates shares many virulence strategies.

Moreover, it has been reported that phenolic acids, a major component in plants, have antioxidant and antimicrobial activity against Gram-negative bacteria such as *E. coli*. In the study of Zhang *et al.* (2021) on the analysis of the antibacterial effect and mechanism of compound phenolic acid on *E. coli*, their study showed that the inhibitory effect of each phenolic acid compound was high against all examined strains of *E. coli*.

Therefore, the pathogenicity and virulence factor of *E. coli* and the absence of phenolic compound of Kamias extract, as indicated in the phytochemical test result, can be attributed to an antibacterial mechanism that significantly increases its resistance to *E. coli*. Hence, this shows further that the concentrations of Kamias ethanolic extracts are ineffective.

As shown in Fig. 1 and presented in Table 3, 25% and 50% show no zone of inhibition against *S. aureus* for the five trials with a mean of 6mm; thus, it is interpreted to be resistant or inactive as indicated in standard zones of inhibition. On the other hand, 75% shows a zone of inhibition with a mean of 9.4 mm but is interpreted as resistant. 100% of Kamias ethanolic extract concentration is identified as intermediate. The first trial resulted in a value of 12 mm, the second trial at 13 mm, the third at 11 mm, the fourth at 11mm, and the last at 10mm. The mean zone of inhibition is 11.4mm which is interpreted as intermediate. An intermediate interpretation means that some organisms are sensitive to it. This intermediate action of 100% concentration of Kamias ethanolic extract shows that it has the potential for developing sensitivity to the bacteria and demonstrating a positive antibacterial activity.

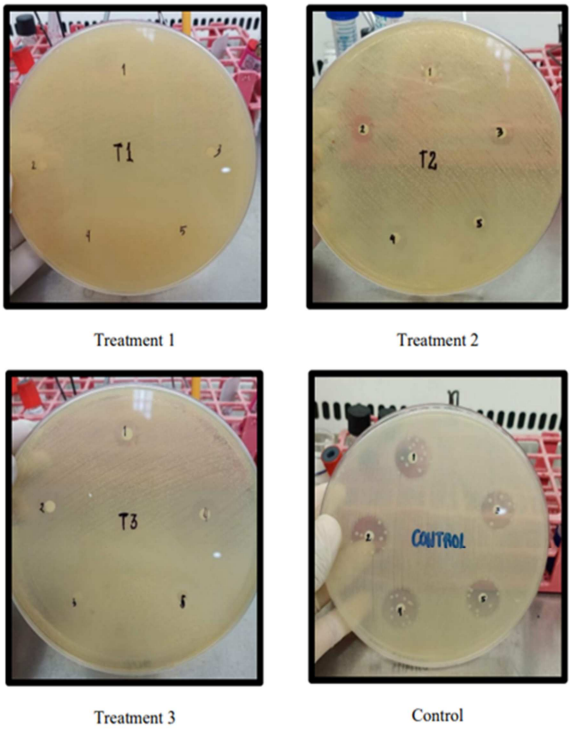

Fig. 1. Zone of inhibition of Kamias *(A. bilimbi* L.*)* fruit ethanolic extract against *S. aureus, E. coli, and P. aeruginosa*

The study's findings are similar to the study of Cheong *et al.* (2022) on the antibacterial effect of extracts from *A. bilimbi* fruits ethyl alcohol extract against selected bacteria, specifically on *S. aureus*. The results of their study revealed that the fruit extract showed good inhibitory activity against the tested pathogen. *S. aureus* was observed to be the most susceptible organism and an indication of the potential of the extract as a drug that can be used against this organism. The same result corroborates with the study of Safitri and Leliqia *et al.* (2021) on antibacterial activity and toxicity study of *A. bilimbi*

L. leaves and fruit extracts. The results demonstrated the potential of *A. bilimbi* extracts against some Gram-positive and negative bacteria. The extracts of *A. bilimbi*'s leaves and fruits showed positive antibacterial activity against the Gram-positive *S. aureus.*

Based on the result, only 100% of Kamias ethanolic extract exhibited the highest inhibition against *S. aureus* and no zone of inhibition against *E. coli* and *P. aeruginosa*. Thus, the researcher found the Kamias extract impotent as a bacteriostatic agent against *E. coli* and *P. aeruginosa*. Therefore, the result of the sensitivity test against *S. aureus* means that the 100% concentration of Kamias ethanolic extract possesses a potential bacteriostatic activity which can be used as a bacteriostatic component for the formulation of hand sanitizer and subjected for susceptibility testing against *S. aureus* to determine its bacteriostatic efficacy. More so, it would be futile to subject the formulations of Kamias hand sanitizer for susceptibility testing against *E. coli* and *P. aeruginosa* as the potency is not enough to inhibit the growth of bacteria, as indicated in the ethanolic extract sensitivity test result.

Conclusion

The study aimed to: (a) determine the phytochemical constituents found in Kamias fruit ethanolic extract that give its bacteriostatic property; (b) determine the degree of bacteriostatic property of the Kamias ethanolic extract in different concentrations (25%, 50%, 75%, and 100%) based on the minimum zone of inhibition. The analysis of the gathered data yielded the following pertinent findings: (1) the Kamias fruit ethanolic extract contains carbohydrates, reducing sugars, flavonoids, saponins, and tannins. Among these, flavonoids, saponins, and tannins are the bacteriostatic properties; and (2) a mean ZOI of 11.4mm was exhibited on the 100% concentration of Kamias ethanolic extract against *S. aureus* and interpreted as intermediate. No zone of inhibition was exhibited on the different concentrations against *E. coli* and *P. aeruginosa* with a mean ZOI of 6mm and interpreted as resistant or inactive.

Based on the findings, the following conclusions were drawn: (1) the Kamias ethanolic extract, as an organic component for hand sanitizer, has phytochemical constituents responsible for its bacteriostatic activity, and (2) the highest concentration of Kamias ethanolic extract possessed a bacteriostatic property with the ideal concentration and potential in formulating the hand sanitizer as an organic ingredient.

As shown in results and discussion, 25% and 50% show no zone of inhibition against *S. aureus* for the five trials with a mean of 6mm; thus, it is interpreted to be resistant or inactive as indicated in standard zones of inhibition. On the other hand, 75% shows a zone of inhibition with a mean of 9.4 mm but is interpreted as resistant. 100% of Kamias ethanolic extract concentration is identified as intermediate. The first trial resulted in a value of 12 mm, the second trial at 13 mm, the third at 11 mm, the fourth at 11mm, and the last at 10mm. The mean zone of inhibition is 11.4mm which is interpreted as intermediate. An intermediate interpretation means that some organisms are sensitive to it. This intermediate action of 100% concentration of Kamias ethanolic extract shows that it has the potential for developing sensitivity to the bacteria and demonstrating a positive antibacterial activity.

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