Antibacterial activity of formulated *Psidium guajava* (guava) hand sanitizer gel on *Staphylococcus aureus*

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Date Received: 10th of April, 2017 **Date Accepted:** 28th of November, 2017

ABSTRACT

The primary purpose of the study was to detect the bactericidal potential of a formulated guava hand sanitizer gel on *Staphylococcus aureus*. The efficacy of this formulation was tested against a bacterial strain through the well-diffusion method. Inhibition of *Staphylococcus aureus* growth was compared across two independent variables: (a) the type of sanitizer (experimental guava hand sanitizer gel vs. three other commercial brands); and (b) the type of alcohol utilized (experimental guava hand sanitizer gel vs. isopropyl vs. ethyl alcohol). The dependent variable which is the reduction of *S. aureus* growth rate, was measured by the zone of inhibition in each of the bacterial strains. Experimental design was used to measure the bactericidal potential of guava hand sanitizer gel. Through a two-way ANOVA, no significant difference was found between groups of commercial hand sanitizers and the formulated guava hand sanitizer gel (Brand A with a zone of inhibition of of 9.7 mm, Brand B with 9.3 mm, Brand C with 9 mm, and the new formulation with a zone of inhibition of 9 mm). These findings reveal that the reference standard commercial product (Brand A) with the highest zone of inhibition manifests a similar antibacterial property with the formulated guava hand sanitizer gel as revealed by the bacterial reduction rate. In conclusion, the formulated hand sanitizer possesses antibacterial potential to inhibit the multiplication of *S. aureus*. Further studies should be conducted on other therapeutic activity parameters and the elucidation of structure for the active constituent with potential as a bactericidal.

Keywords: formulated guava hand sanitizer gel, hand sanitizer commercial product, *Staphylococcus aureus*

I. INTRODUCTION

Sanitation has been a challenge to most health care providers since most infectious diseases are caused by pathogenic bacteria. The effectiveness of hygiene depends primarily on compliance. Staying healthy is the key to success, and the last thing people want to engage in is being in direct contact with microorganisms. Hand sanitizers are popular products used in fighting against most bacteria that people may come into contact with. However, very few sanitizers in the market are based on botanical ingredients.

The study was therefore conducted to determine the reduction growth rate of *Staphylococcus aureus* using guava leaf extract formulated into guava hand sanitizer gel. There have been previous studies conducted where the focus is on the essential parts of plants. The manifestation of most plants with high potentials on antimicrobial activity is the presence of phytochemicals that inhibit the growth of microorganisms, especially bacteria.

The leaf of the guava or *Psidium guajava*, locally known as "bayabas" is a plant of the family *Myrtacea*. Research studies have shown that almost all of its parts

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have medicinal values. The phytochemicals of the plant show a potential for antibacterial activity (Guinto, & Chua, 2013). Guava leaves (*Psidium guajava L*) have active chemical compounds such as saponins, flavonoids, tannins, eugenol, and triterpenoids. Tannin and flavonoids are polyphenolic compounds (1.4%) that dominate guava leaves (Mailoa, Maherdradatta, Laga, & Pjide, 2013). The growth of bacteria is inhibited by some types of phenolic compounds that possess antibactericidal property. The formulation of guava sanitizer gel containing the extract showed the same phytochemicals of antimicrobial potential with some other plants. The focus of the study is to determine how effective the bactericidal potential of the newly formulated product. Hand sanitizer is a topical preparation containing an agent that can reduce the production of microorganisms, especially bacteria. The present study compares the bactericidal activity of the newly formulated product against most commercial hand sanitizers on *Staphylococcus aureus*.

The spread of *Staphyloccocus aureus* in various part of the body such as skin, hair and nose causes various infections from minor to fatal diseases. Several studies conducted have also revealed that hand sanitizers containing ethanol or ethyl alcohol produce an impact in reducing microbial growth and are more effective than soap and water (Turner, Fuls, & Rodgers, 2010). A study on the effectiveness of using hand sanitizers showed that employees using alcohol-based hand sanitizer at least five times each workday were about two-thirds less likely to acquire sickness than those who continued to just wash their hands. Constant use of hand sanitizers also led to a decline of the flu virus (Swartzberg, 2014). Another study revealed that hand sanitizers could reduce common colds (Turner, Fuls, & Rodgers, 2010). Hand washing is effective against diseases but if the question is on the availability of clean and running water, hand sanitizers comes into the picture as an alternative for the first line of health defense. In some findings, exposure of patients to *Staphylococcus aureus* in the hospital setting is high compared with the 30% found in the nose and skin (Tortora, Funke, & Case, 2010).

The emergent infection caused by bacteria leaves a dilemma in health issues especially with regards to skin infection. Among the contagious bacteria in the environment, *Staphylococcus aureus* is widely spread. The spread of bacteria to one's blood stream may also cause infection to other organs especially on the skin. A penetration of these bacteria can be prevented through hand washing. Mammals are hosts to a series of microorganisms recognized as residents' microorganisms. The body also selects other microorganisms, identified as short-term microorganisms, through contact with other objects. Other microbes known as fecal microorganisms are similarly present within the intestines of human beings and mammals. These microbes may contaminate a person's hands, arms, or fingers and in turn be able to transmit to some individuals and something that they get in touch with.

The researchers identify the need to address this gap by providing an organic formulation of guava hand sanitizer gel that can be used as an effective way other than hand washing. Using hand wash is essential in decreasing destructive microbes within the hands and for lowering the threat of transporting destructive microbes to some individuals and other parts of the body. The formulated guava hand sanitizer gel is therefore recommended as an intervention to address the gap in reducing the production of *Staphylococcus aureus*.

The study determined the antibacterial activity of formulated guava hand sanitizer gel on *Staphylococcus aureus*. This study specifically sought to: (A) Find out the zones of inhibition produced on *Staphylococcus aureus* using: (a) commercial hand sanitizers; (b) formulated guava hand sanitizer gel; and (c) type of alcohol with high batericidal potential (ethyl alcohol and isopropyl alcohol); and (B) Determine the significant difference in the zones of inhibition produced on *S. aureus*.

II. METHODOLOGY

An experimental research design was used in this study. This method describes and analyzes variables under controlled conditions and it is specifically made up of manipulating experimental variables under highly measured conditions to determine how and why a certain event occurs (Shadish, Cook, & Campbell, 2002). The research method used in this study focused on the reduction on the growth of *Staphylococcus aureus* using guava extract formulated into gel as compared with different brands of commercial hand sanitizers containing ethyl and isopropyl alcohol. The treatments were administered only with positive controls and the experimental group since administering negative control presumably will not show any significant antibacterial activity.

Collection of Plant Material. The fresh leaves of *Psidium guajava* (guava) plant was collected at Gullas Drive, Banilad Mandaue City. The fresh leaves of the guava plant was collected and washed thoroughly to remove unwanted contaminants and other particles

which may affect in determining the microbial activity of the plant sample. It was finely chopped or cut into smaller pieces using a knife and washed again to maintain contaminant free.

Preparation of samples and extract. Two hundred grams of finely cut fresh *F. septica* leaves were macerated with 95% ethyl alcohol for 72 hours. The mixture was then filtered and the marc was discarded. The filtrate was evaporated until it reached a syrupy consistency. Excess solvent was removed by placing the remaining volume in a hot air oven (at 40˚C). The residue was re-macerated twice using the same strength and volume of ethyl alcohol. All filtrate was collected and evaporated. The compacted extract was liquified by adding 20% between surfactant and distilled water. Flammability and iodoform tests were performed to determine any traces of the solvent. All extracts were placed in a tightly closed container and kept refrigerated at 2-8 degree Celsius until further use. One percent w/v solution of extract of *F. septica* was subjected to phytochemical screening for the detection of various classes of constituents. The extract was used in the formulation of guava hand sanitizer gel.

Preparation of commercial hand sanitizers. Ethanol and isopropanol are the common ingredients found in most hand sanitizers. They eliminate microbes without causing the skin tissues any harm, thus making them an effective hand sanitizer ingredient and disinfectant. Polyacrylic acid, a gelling agent, helps provide easier application of hand sanitizers on the skin. The use of scent oils as a means to reduce the odor of alcohol is a fresher improvement in hand sanitizer products. Hand sanitizers containing 60 percent or lesser are considered effective in bacterial and fungal eliminations. The hand sanitizers used in this study were of 3 varying brand names manufactured by different manufacturer. The brand names are Naturals Hand Sanitizer, Green Cross Hand Sanitizing Gel and Alcogel Hand Sanitizer.

Formulation of guava hand sanitizer gel. Gelatin powder was placed in a beaker and dissolved with a portion of glycerin with the aid of heat. This was previously pre-dissolved in alcohol. The remaining alcohol and glycerin were added. Using the spatula it was vigorously stirred until the ingredients were fully mixed together. The guava leaf extract was added to the mixture which was then transferred to a container.

Procurement of pure culture. The microbial cultures required for testing were procured at Vicente Gullas Memorial Hospital, an accredited facility. Upon acquisition, these pure cultures were placed in a sealed container to prevent contamination while being transported and labeled accordingly for security purposes. These were preserved using refrigeration and paraffin method before being brought out for biological testing. Staphylococcus aureus was obtained through isolation of pure culture by the use of quadrant streak isolation procedure, T streak method, or the continuous streaking method.

Preparation of culture media and Inoculation of bacteria. Before obtaining a culture of *Staphylococcus aureus*, preparation of the culture medium was done. Twenty grams of nutrient agar powder as dissolved in 120 ml distilled water and brought to boiling for complete dissolution. The agar solution was autoclaved at 121 degrees centigrade for 15 minutes. The sterilized solution was poured with 20 ml in each petri dish and covered with foil. The agar was cooled at room temperature and set aside for refrigeration. After the preparation of the culture medium, the pure culture was obtained by inoculation. All materials such as the inoculation loop and plates were sterilized and cooled. Streaking the plate followed, after which the loop was re-sterilized again. The surface of the agar was repeatedly streaked for triplicates using the sterilized inoculation loop and plates. There was an overlap of streaking on the surface with the first and second section a few times. The loop was disinfected by flaming and allowed to cool. The sterilized and cooled loop was then drawn on the third section of the agar surface. Overlap with the second section was observed. Prior to sensitivity testing, a colony of *Staphylococcus aureus* was obtained through cultured media in nutrient agar placed in plates for 18 to 24 hours incubation at thirty seven degrees centigrade. After the overnight incubation, the colony was streaked and compared with the standard 0.5 Mc Farland standard solution for its bacterial characteristics (Biswas & Rogers, 2013).

Antibacterial susceptibility testing. The National Committee for Clinical Laboratory Standards was the reference in determining antimicrobial susceptibility through the Well Diffusion Method. The new formulated guava hand sanitizer gel and the commercial brand hand sanitizer gels were tested using standard Mueller Hinton II plates to detect the antibacterial activity of these testing solutions. The plates with inoculated bacteria that had been adjusted to the 0.5 Marcland standard were provided with fifty uL aliquots in 6mm diameter punched wells with the testing solutions. All plates were inoculated with the test bacteria. The surface of the agar plate was streaked over the entire sterile agar surface rotating the plate to ensure an even distribution of inoculum

with a final swab around the rim. Three plates of inoculated bacteria for each experimental condition were also applied to the surface to determine the bactericidal property of these solutions. In the same manner, inoculated plates for propyl and isopropyl alcohol were compared for higher sensitivity. All of the plates were sealed with parafilm, labeled, and placed in the incubator set at a temperature of 37°C. Zones of inhibition were examined after twenty four hours. The bactericidal activity of the formulated gel and commercial gels were measured using a ruler by determining the zone of inhibition. This was carried out in at least an average of three parallel independent trials.

Measurement of the zone of inhibition. A larger zone of bacteria-free area that surrounds a hand sanitizer disk means that the bacteria are more sensitive to the sanitizer that the disk contains. To measure the diameter of the disk, a ruler was used. The disk diameter and the diameter of surrounding area was measured in millimeters (mm) or the zone that is inhibited while keeping the plate's lid in place. The zone of inhibition produced on *S. aureus* by the formulated guava hand sanitizer gel, commercial brands A, B and C and the types of alcohols (i.e., ethyl alcohol and isopropanol) were measured.

III. Results and Discussion

The formulated guava hand sanitizer gel is an alternative topical antibacterial agent. It has organic properties derived from its own natural phytochemicals which has an effect comparable with the commercial hand sanitizer brands. Table 1 shows the zone of inhibition for commercial product Brand A. This was used as a reference to determine the antibacterial activity of the formulated guava hand sanitizer gel and other brands B and C. This brand was used as a reference standard since it is one of the leading commercial hand sanitizers in the market with proven efficacy.

Table 1
Baseline value on the zone of inhibition for standard commercial product (Brand A)

Standard Commercial Product	Zone of Inhibition in Millimeter	
Hand sanitizer Brand A (Ethyl Alcohol)	Before Treatment	After Treatment
Trial 1	No inhibition	10 mm
Trial 2	No inhibition	9 mm
Trial 3	No inhibition	10 mm
	$\bar{x}$	9.7 mm

Table 1 presents the baseline value on the zone of inhibition. The table reflects the three trials obtaining a mean of 9.7 mm. The standard commercial product that is used in the study is brand A which is ethyl-alcohol based.

Table 2
Zone of inhibition for other commercial products and guava sanitizer gel with the reference standard (Brand A)

	Brand A Ref standard (mm)	Brand B (mm)	Brand C (mm)	Formulated guava hand sanitizer gel (mm)
Trial 1	10	9	8	9
Trial 2	9	9	9	10
Trial 3	10	10	10	8
$\bar{x}$	9.7	9.3	9	9

Table 2 shows the measurements on the zones of inhibition by the three varying commercial brands on *S. aureus*. In the table, all commercial products including the formulated hand sanitizer showed the same bactericidal effect with reference standard brand A.

Table 3
Zone of inhibition using different types of solvent

Types of Solvent	Ethyl Alcohol (mm)	Isopropyl Alcohol (mm)
Trial 1	9	9
Trial 2	9	11
Trial 39mm	11	13
$\bar{x}$	9.7	11

Table 3 shows the measurement on the zones of inhibition using different types of solvents namely ethyl alcohol -based and isopropyl alcohol with a percentage strength of seventy percent (70%). Ethyl and isopropyl alcohol are the two most common types of alcohol found in most antiseptic agents. The measurements presented in table 3 obtained from the three different trials showed that isopropyl alcohol has a higher measurement in the zones of inhibition than ethyl alcohol does. The chemical structure of the compound varies with its branching property. As branching increases so does antibacterial property, therefore isopropanol or isopropyl alcohol, having the longer branching than ethanol or ethyl alcohol, has a higher antibacterial property than ethyl alcohol.

Table 4
Two-way Analysis on the effect of hand sanitizer using different types of alcohols

Types of Variance	Zone of Inhibition	df	t-value		Alcohol	Interpretation
Ethyl Alcohol	$\sum$ = 29 mm	9.67 mm	1.33	1.33	4	=2.13 Insignificant
Isopropyl Alcohol	$\sum$ = 35 mm	11.67 mm	1.33	1.33	4	=2.13 Insignificant

Seventy percent (70%) of ethyl Alcohol was found to have no significant difference with seventy percent (70%) isopropyl alcohol (t= 2.13, p>0.01). This means that ethyl alcohol and isopropyl alcohol-based hand sanitizers are both equally effective in inhibiting the growth of *S. aureus*.

Antibactericidal Activity. The findings of the study indicates that the ethanolic extracts obtained from the

leaves of *Psidium guajava* which was formulated into a hand sanitizer gel, show inhibitory activity against *Staphylococcus aureus* bacteria. The bactericidal activity of the formulated gel is caused by the impermeability of the bacteria to permeate to any lipopolysaccharide membrane in the presence of active component of *Psidium guajava* which is the tannins. Tannin has an effect of inhibition on the substrate of bacteria. It has also a direct action inhibiting phosphorylation activity of bacteria forming its cell wall which can cause multiplication of bacteria. Disruption of the cell wall prevents the process of multiplication of the bacteria. The formulated gel of *psidium guajava* has bactericidal activity that limits the multiplication of *Staphylococcus aureus*.

IV. Conclusion

The study was conducted to determine the reduction rate on the growth of *Staphylococcus aureus* using the formulated guava hand sanitizer gel. The findings revealed the inhibitory activity of the plant extract on *S. aureus*. The formulated hand sanitizer gel obtained from the extract of guava leaves showed the same activity with the reference standard on the measurement of the zone of inhibition. It also showed no significant difference compared to hand sanitizer commercial brands B and C used in the study. A comparison of the solvents ethyl alcohol and Isopropyl alcohol showed no significant difference in the mean zones of inhibition. This is an indication that hand sanitizer is an effective antibactetial agent against *Staphylococcus aureus*.

The reduction of bacteria as indicated by the appearance of zone of inhibition produced using formulated guava hand sanitizer gel on *Staphylococcus aureus* is a manifestation of the antibacterial activity of the plant's active constituent such as tannins and other phytochemicals that have potential for antibacterial activity. The mechanism includes the rupture of bacterial cell wall and membranes of bacteria with irregular disruption of intracellular matrix when treated with the plant extracts.

Originality Index: 97%
Similarity Index: 3%
Paper ID: 967043510
Grammar: Checked

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