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# Application of guava leaf extract on hard candy to inhibit upper respiratory tract infection caused by bacteria

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## Abstract

Guava (*Psidium guajava* L.) leaf has long been the subject of diverse research initiatives as herbal medicine due to its antimicrobial activity against various bacteria. An Upper Respiratory Tract Infection (URTI) is a contagious infection of the upper respiratory tract, which can be caused by bacteria, i.e., *Streptococcus pyogenes* and *Pseudomonas aeruginosa*. In this research, guava leaves were extracted sequentially using solvents with different polarities. Furthermore, this research aimed to determine the antibacterial activity of guava leaf extract against URTI caused by bacteria (*S. pyogenes* and *P. aeruginosa*) and to formulate it into hard candy at various Minimum Inhibitory Concentrations (MIC), which were 2 MIC, 3 MIC, 4 MIC, and 5 MIC, to obtain hard candy with the highest antibacterial activity and organoleptic acceptability. To assist in the organoleptic acceptability aspect of the hard candy, lemongrass oil was used in varying concentrations (0%, 0.15%, 0.3%) as a flavoring agent. Results showed that ethanol guava leaf extract had the strongest antibacterial activity against both *S. pyogenes* (MIC=721.21 ppm) and *P. aeruginosa* (MIC=3085.91 ppm). Moreover, hard candy added with guava leaf extract at a concentration of 4 MIC and 0.15% lemongrass oil had the highest overall acceptability and antibacterial activity against *S. pyogenes* (inhibition diameter of 11.92 ± 0.56 mm) and *P. aeruginosa* (10.77 ± 0.69 mm).

**Keywords:** Antibacterial; Ethanol extract; Extraction; Minimum Inhibitory Concentration; *P. aeruginosa*; *S. pyogenes*.

## Highlights

- Ethanol guava leaf extract had the strongest inhibition against URTI-causing bacteria, *i.e.*, *S. pyogenes* and *P. aeruginosa.*
- Hard candy added with 4 MIC extract and 0.15% lemongrass oil had the best properties
- Hard candy added with guava leaf extract is potential to be developed commercially since it had higher acceptance score compared to control.

## 1 Introduction

An Upper Respiratory Tract Infection (URTI) is a contagious infection of the upper respiratory tract, which includes the nose, sinuses, pharynx, and larynx, caused by bacteria or viruses. The most common URTIs include acute bronchitis, the common cold, influenza, laryngitis, pharyngitis, and Respiratory Distress Syndromes (RDS). These are often transmitted through airborne respiratory droplets (from sneezing or coughing), skin-to-skin contact, or by touching a contaminated surface (Zhou & Li, 2015). *Streptococcus pyogenes* and *Pseudomonas aeruginosa* are the most found bacteria in patients suffering from URTI (Limsuwan & Voravuthikunchai, 2013; Zhou & Li, 2015; Ali et al., 2015).

These bacteria have been known to be broadly resistant to antibiotic treatment, therefore, various plant-based treatments options have been proposed in previous studies as an alternative to treat URTIs, such as the use of fingerroot (*Boesenbergia pandurata* (Roxb.) Schltr.*), tiwai* onion *(Eleutherine americana* (Aubl.) Merr. ex K. Heyne*)* and rose myrtle *(Rhodomyrtus tomentosa* (Aiton) Hassk.) extracts (Limsuwan & Voravuthikunchai, 2013), *Gnaphalium oxyphyllum* DC., *G. americanum* Mill., and *Crescentia alata* Kunth extracts (Rojas et al., 2001) against *S. pyogenes.* Extracts from horseradish (*Armoraciae rusticanae* G.Gaertn., B.Mey. & Scherb.) root, nasturtium plants (Conrad et al., 2006), jabuticaba skin (Oliveira et al., 2018) and *E. globulus* (Pereira et al., 2014) have also shown antibacterial efficacy against *P. aeruginosa*.

Guava (*Psidium guajava* L.) leaves have been used traditionally in many countries to manage various diseases and infections. Pharmacological research (both *in vivo* and *in vitro*) has been widely used to demonstrate the potential of guava leaf extract for the co-treatment of various ailments of high prevalence worldwide, including cardiovascular diseases, diabetes mellitus, cancer, and parasitic infections (Biswas et al., 2013; Mohammed & Taha, 2017; Ghosh et al., 2010). Previous studies have also shown the antibacterial efficacy of guava leaves against pathogenic bacteria such as *Staphylococcus aureus* and *Bacillus cereus* (Biswas et al., 2013); *S. mutans, Escherichia coli*, and *Salmonella enteritidis* (Dhiman et al., 2011); *V. parahaemolyticus* (Farjana et al., 2014), and *Aeromonas hydrophila* (Mahfuzul Hoque et al., 2007). According to Ugoh & Nneji (2013), the strong bactericidal activity exhibited by guava leaves may be due to its protein degradation activity, which depends on the method of extraction.

Despite established evidence of their pharmacological efficacy, plant extracts are often unpalatable and do not elicit good responses in terms of other sensorial properties such as texture and mouthfeel. Hard candies are an attractive option as the high sugar content greatly enhances flavor and palatability, while the hard, brittle glass structure allows the consumer to savor the product for a long period of time during consumption (Hartel et al., 2018). In this research, formulating guava leaf extract into hard candy may offer antibacterial benefits for those with URTI in a manner that is both palatable and easy to consume.

To improve its sensorial properties, additional flavoring is often incorporated into candy formulation in the form of extracts, essential oils, or organic acids to increase consumer appeal and product value. Lemongrass (*Cymbopogon citratus* (DC.) Stapf) is often described to impart a flavor reminiscent of lemon and lemon mint, with subtle ginger-like notes (Charles, 2012). Lemongrass can be used as a flavoring agent in sugar confectioneries such as hard candy in the form of essential oils or extracts (Natisri et al., 2014). This research aimed to determine the antibacterial activity of guava leaf extract against URTI caused by bacteria (*S. pyogenes* and *P. aeruginosa*) and to formulate guava leaf extract into hard candy, added with lemongrass oil, to improve its organoleptic acceptability.

## 2 Material and methods

The materials used in this research include fresh guava leaves obtained from *Balai Penelitian Tanaman Rempah dan Obat* (Balittro), Bogor, Indonesia, food-grade solvents (ethanol, hexane, and ethyl acetate), lemongrass oil ("Natura Aromatik"), granulated sugar, glucose syrup, distilled water, and citric acid. Chemical used for analysis include Nutrient Agar (NA), Brain Heart Infusion Agar (BHIA), Nutrient Broth

(NB), Trypticase Soy Broth (TSB), defibrillated sheep blood, 2% aluminium chloride ($AlCl_3$), quercetin, Folin-Ciocalteu reagent sodium carbonate ($Na_2CO_3$), gallic acid, and gaseous nitrogen. The pure cultures of *S. pyogenes* ATCC 19615 (Thermo Fisher Scientific Culti-Loops™) and *P. aeruginosa* FNCC 0063 (obtained from Institut Pertanian Bogor, Indonesia) were used.

### 2.1 Extraction of Guava Leaves

Extraction of the guava leaves was done according to the method described by Biswas et al. (2013) and Uthayarasa et al. (2010), with some modifications. Before to extraction, guava leaves were processed into powder. Fresh guava leaves were cut into ±1 cm pieces and dried using a cabinet dryer at 50°C for 24 h. Dried guava leaves were then size reduced using "Fomac" miller and sieved using mesh no.40 to obtain guava leaves powder.

The extraction process for guava leaves was performed using sequential extraction with three different solvents of varying polarities, starting from hexane, ethyl acetate, and ethanol, respectively. Fifty grams of guava leaf powder were mixed with hexane (1:5 w/v) in an Erlenmeyer flask, macerated using a platform shaker (250 rpm at 27°C) for 24 h, and filtered using Whatmann no.1 filter paper. Hexane was then removed using a "Buchi R-210" rotary evaporator and further removed using gaseous nitrogen. The crude hexane extract was obtained, while the residue was then mixed with the second solvent, which was ethyl acetate. The previous steps were repeated using ethyl acetate and then ethanol to obtain ethyl acetate extract and ethanol extract. Each extract obtained was analyzed for its antibacterial activity (Jeyaseelan et al., 2012; Uthayarasa et al., 2010) against *S. pyogenes* and *P. aeruginosa* to determine the best guava leaves extract.

### 2.2 Preparation of Hard Candy added with Guava Leaves Extract

Hard candy was made by adding several concentrations of best guava leaves extract and lemongrass oil. The aim was to determine the best concentration at which the two extracts should be used in the formulation of hard candy that exhibit an antibacterial effect against *S. pyogenes* and *P. aeruginosa* while still retaining its appeal in terms of its sensory qualities. The hard candy formulation and manufacturing process used in this study was according to Kailey et al. (2019) with some modifications. The selected guava leaf extract was used at concentrations of 2MIC, 3MIC, 4MIC, and 5MIC, whereas the lemongrass oil was used at concentrations of 0%, 0.15%, and 0.3% (v/v). The control was formulated with no guava leaf extract and 0% lemongrass oil. The formulation of hard candy made in this research could be observed in Table 1.

**Table 1.** Formulation of hard candy.

| Ingredients | Amount (%) |
|---|---|
| Granulated sugar | 60 |
| Water | 15 |
| Glucose syrup | 25 |
| Citric acid | 0.2 |
| Guava leaf extract | 2MIC, 3MIC, 4MIC, 5MIC |
| Lemongrass oil | 0, 0.15, 0.3 |

Source: Kailey et al. (2019), with modifications.

To make the hard candy, the granulated sugar, water, and glucose syrup were all placed in a saucepan and stirred continuously until a temperature of 150°C was reached. The pan was then removed from the stove and the citric acid was added along with the guava leaf extract and lemongrass oil. The mixture was then poured immediately into silicone molds and left to cool at room temperature until completely hardened. The hard candy obtained was then analyzed for its antibacterial activity against *S. pyogenes* and *P. aeruginosa,* texture

(Figiel & Tajner-Czopek, 2006), colour (Nollet & Toldra, 2009; Nielsen, 2010), and organoleptic properties (Heymann & Lawless, 2010; Granato et al., 2012).

### 2.3 Antibacterial activity of guava leaves extract and guava leaves hard candy

The determination of antibacterial activity for all the guava leaf extracts as well as the guava leaves hard candy was conducted using the well-diffusion method following Jeyaseelan et al. (2012) and Uthayarasa et al. (2010), with some modifications. Guava leaf extract was diluted with its extraction solvent to give concentrations of 50,000 ppm, 100,000 ppm, 150,000 ppm, 200,000 ppm, and 250,000 ppm. DMSO was added to the hexane solution at 2% concentration, and to the ethyl acetate solution at 1% concentration.

To perform the antibacterial analysis, the bacteria were first refreshed by inoculating the culture into test tubes containing liquid media. The liquid medium used for *S. pyogenes* was Trypticase Soy Broth (TSB) with 5% defibrillated sheep blood, whereas *P. aeruginosa* was refreshed in Nutrient Broth (NB). The bacteria were incubated at 37 ºC for 24 hours, followed by a secondary refreshment in new test tubes containing the appropriate media. The bacteria were incubated again at 37ºC until the log phase was reached. According to Masson et al. (2013), *S. pyogenes* could reach the log phase in TSB at approximately 5 hours, while *P. aeruginosa* reached the log phase in NB at approximately 6 hours.

For the analysis of the antibacterial activity of extracts, media were first autoclaved at 121ºC for 2 hours and cooled down to approximately 40 ºC prior to incorporation of 0.02% refreshed bacterial suspension. The bacteria-infused agar was then poured into sterile Petri dishes and allowed to set fully. Six wells were punched into each plate using a sterile borer 6 mm in diameter: five wells in a circular order for each extract concentration; and one in the center for the negative control (solvent). Each well was then filled with 60 µl of the extract using a micropipette. The plates were then incubated at 37 ºC for 24 hours, and the antibacterial activity of the extracts was determined by measuring the clear zones around each well using a Vernier calliper. The MIC values were determined using the linear regression equation (Bloomfield, 1991), in which the extract concentration was expressed on the ln $M_o$ curve (X-axis), whereas the squared value of the inhibition zone ($Z^2$) was expressed on the Y-axis. The intersection point of the X or Mt axis will appear on the curve. The MIC value was determined by multiplying the $e^{Mt}$ by 0.25. MBC (Minimum Bactericidal Concentration) is equal to 4 x MIC.

For the antibacterial analysis of the hard candy, 0.78 g of hard candy from each treatment was crushed, weighed, and dissolved in 1 mL of sterile water in test tubes. A "Thermolyne Maxi Mix II Type 37600" vortex mixer was used. Plating was performed after lemongrass oil was added. For each concentration of lemongrass oil (0%, 0.15%, and 0.3%), five holes were bored into the agar: one for each guava leaf extract concentration (from 2 MIC to 5 MIC) and one in the center for the negative control (hard candy without guava leaves extract and lemongrass oil addition).

### 2.4 Texture and colour analysis of guava leaves hard candy

The texture profile of the guava leaves hard candy was determined using a Texture Analyzer (TA-XT Plus Stable Micro System), according to a method by Figiel & Tajner-Czopek (2006). In the analysis of hard candy, a 35 mm cylindrical probe was used. The pre-test speed, test speed, and post-test speed were all set to 1.0 mm/s and the probe was set to compress the sample to 60% of its height. The sample was then subjected to a single compression cycle, which imitates the bite of human jaws, and the maximum peak was used to measure the hardness of the hard candy. Furthermore, colour analysis of the hard candy samples was conducted using a "Konica Minolta CR-400" chromameter to determine the lightness value (L), according to a method by Nollet & Toldra (2009) and Nielsen (2010).

### 2.5 Organoleptic analysis of guava leaves hard candy

Two types of sensory analyses were conducted to evaluate the organoleptic properties of the guava leaves hard candies, which were scoring and hedonic. The scoring test was adapted from Heymann & Lawless (2010) and was conducted using 12 panellists. Each panellist received all the candy samples simultaneously along with a neutral palate cleanser (mineral water) and a questionnaire. The sensory parameters and scoring equivalent for each parameter are shown in Table 2. The data obtained were analyzed statistically through Analysis of Variance (ANOVA) and Duncan test (at 5% significance level) using SPSS version 23.0.

**Table 2.** Sensory parameters for scoring test.

| Scores | Parameters | | | | |
|---|---|---|---|---|---|
| | Colour | Foreign Aroma | Foreign Taste | Texture | Aftertaste |
| 1 | Extremely not dark | Extremely undetectable | Extremely undetectable | Extremely not hard | Extremely not bitter |
| 2 | Not dark | Undetectable | Undetectable | Not hard | Not bitter |
| 3 | Slightly not dark | Slightly undetectable | Slightly undetectable | Slightly not hard | Slightly not bitter |
| 4 | Slightly dark | Slightly detectable | Slightly detectable | Slightly hard | Slightly bitter |
| 5 | Dark | Detectable | Detectable | Hard | Bitter |
| 6 | Extremely dark | Extremely detectable | Extremely detectable | Extremely hard | Extremely bitter |

The hedonic test used in this study was adapted from Granato et al. (2012), with some modifications. This test was conducted simultaneously with the scoring test. Evaluation was conducted using an untrained panel comprised of 12 assessors using a 7-point hedonic scale (1 for “dislike it extremely” and 7 for “like it extremely”). Parameters assessed include colour, aroma, taste, texture, aftertaste of the hard candies, and overall acceptance.

## 3 Results and discussion

### 3.1 Antibacterial activity of guava leaf extracts

Guava leaf extracts were obtained sequentially in three stages using hexane, ethyl acetate, and ethanol. This resulted in hexane, ethyl acetate, and ethanol guava leaf extracts. Based on Halim et al. (2021), the yield of hexane, ethyl acetate, and ethanol guava leaf extract were $2.52 \pm 0.03\%$, $2.82 \pm 0.31\%$, and $60.87 \pm 4.89\%$, respectively. This indicates that ethanol extract had the highest yield. These extracts were then analyzed for antibacterial activity on both *S. pyogenes* and *P. aeruginosa* using the well-diffusion method, where the MIC and MBC were determined for the selection of the best extract for candy formulation. The clear zone formed was used to calculate MIC and MBC values and the results can be observed in Table 3.

**Table 3.** MIC and MBC values of ethanol, ethyl acetate, and hexane extracts against S. pyogenes and P. aeruginosa.

| Extracts | *S. pyogenes* | | *P. aeruginosa* | |
|---|---|---|---|---|
| | MIC (ppm) | MBC (ppm) | MIC (ppm) | MBC (ppm) |
| Ethanol | 721.21 | 2884.84 | 3085.91 | 12343.64 |
| Ethyl Acetate | 742.91 | 2971.64 | 3168.44 | 12673.75 |
| Hexane | 766.24 | 3064.97 | 3349.2 | 13396.8 |

Based on the results, ethanol extract possessed the lowest MIC and MBC against both *S. pyogenes* and *P. aeruginosa*, followed by ethyl acetate extract, then hexane extract. This means ethanol extract exhibited the strongest antibacterial activity against both bacteria. The results of the antibacterial analysis imply that ethanol was the best solvent to extract antibacterial compounds in guava leaf.

Halim et al. (2021) reported that guava leaf extracts contain the phenolic and flavonoid compounds, in which ethanol extract had the highest amount compared to ethyl acetate and hexane extracts. The amount of phenolics and flavonoids in ethanol extract were 355.0347 ± 10.8186 mg GAE/g extract and 234.2960 ± 2.5074 mg QE/g extract, respectively. Biswas et al. (2013) have reported a significant increase in the total amount of phytochemicals in plant extracts with an increase in polarity. Some compounds that were reported to possess antibacterial activity in guava leaf extract included tannins, flavonoids, saponins, and various other components such as triterpenoids and acids (Ghosh et al., 2010; Dhiman et al., 2011; Bisht et al., 2016). These compounds can inhibit bacterial growth through penetration of the lipid bilayer of the cell membrane. Weakening of this membrane leads to increased permeability, which causes leakage of vital cell components (Biswas et al., 2013).

When compared, all extracts were shown to have smaller MICs and MBCs against *S. pyogenes* compared to *P. aeruginosa*, which indicated a higher susceptibility to bacterial inhibition from the extracts. Biswas et al. (2013) reported that stronger antibacterial activity was found in guava leaf extract against Gram-positive bacteria compared to Gram-negative bacteria. This is because Gram-negative bacteria such as *P. aeruginosa* have an outside lipopolysaccharide membrane that serves as a permeability barrier; this restricts the penetration of the extruding extract. Gram-positive bacteria such as *S. pyogenes*, on the other hand, possess a mesh-like peptidoglycan layer that is more easily permeated by the plant extract (Mailoa et al., 2014).

Based on the results, the extract that produced the lowest MIC, *i.e.*, ethanol extract was selected to be used for hard candy making. To ensure effective inhibition of both bacteria, the MIC for *P. aeruginosa* (3085.91 ppm), which is higher than that of *S. pyogenes*, was selected for use in hard candy formulation.

### 3.2 Antibacterial activity of guava leaf hard candy

The antibacterial activity of hard candies formulated with guava leaf ethanol extract and lemongrass oil at varying concentrations was analyzed by measuring the diameter of clear zones around each well for both *S. pyogenes* and *P. aeruginosa* using the well-diffusion method. Hard candy without guava leaf extract and lemongrass oil was used as a control. In this research, the control hard candy showed no inhibition towards *S. pyogenes* and *P. aeruginosa.*

Furthermore, statistical analysis using Univariate showed that guava leaf extract concentration significantly affects ($p \leq 0.05$) the antibacterial activity of the hard candy against both *S. pyogenes* and *P. aeruginosa*. However, lemongrass oil concentration and the interaction between lemongrass oil concentration and guava leaf extract concentration were both shown to have no significant effect ($p > 0.05$) on the antibacterial activity of the hard candy against both bacteria. This indicated that guava leaf extract concentration was the sole determinant of the antibacterial efficacy of hard candy. Results of Post Hoc analysis using Duncan can be observed in Figure 1.

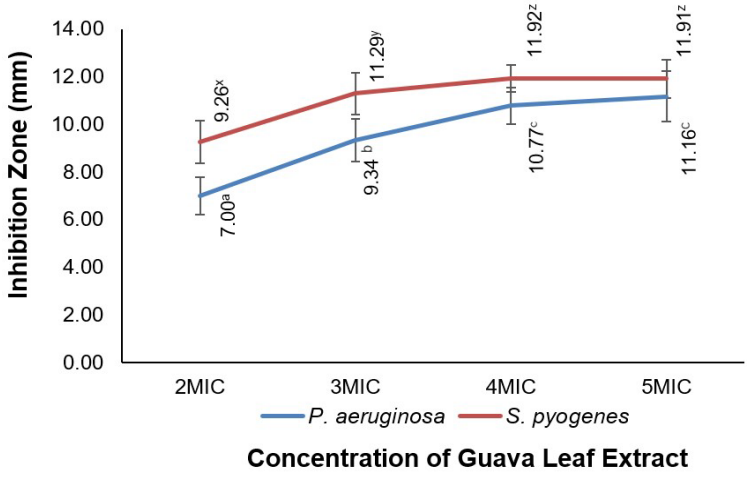


**Figure 1.** Effect of guava leaf extract concentration on the diameter of inhibition zone for *P. aeruginosa* and *S. pyogenes*. Note: Different letter notations on the same line colour indicate a significant difference ($p \leq 0.05$).

Figure 1 shows that the incorporation of guava leaf ethanol extract into hard candy formulation was effective to inhibit both *S. pyogenes* and *P. aeruginosa*. A steady increase in inhibition zone diameter, which indicates greater antibacterial activity, is shown from 2 MIC to 4 MIC. However, the use of 5 MIC guava leaf extract did not show a significant increase in antibacterial activity compared to 4 MIC.

Based on its inhibition diameter, it can be inferred that the incorporation of 4 MIC and 5 MIC of guava leaf extract into hard candy showed moderate antimicrobial activity against both bacteria (diameter zone 10-15 mm) (Paudel et al., 2014). This inhibition zone is also higher compared to the addition of 5 MIC guava leaf extract in jelly candy making to inhibit *S. mutans,* with an inhibition zone of 9.68 ± 0.54 mm (Halim et al., 2021). An interesting result in this research is that the addition of lemongrass oil at different concentrations insignificantly affected the inhibition zone of guava leaf hard candy. Lemongrass oil has been reported to have antimicrobial activity against *E. coli* and *S. aureus* (Leimann et al., 2009), *S. mutans* (Chaudhari et al., 2012), *B. cerveius* (Premathilake et al., 2018) and antifungal activity against *Colletotricum truncatum*, *Fusarium* spp., *Penicillium* spp., *Crysosporium* spp (Premathilake et al., 2018), *Ascosphaera apis* and *Pseudogymnoascus destructans* (Gabriel et al., 2018). However, Singh et al. (2011) and Shendurse et al. (2021) reported that *P. aeruginosa* was resistant to lemongrass oil, whereas Unachukwu et al. (2017) reported that lemongrass oil could weakly inhibit *S. pyogenes* but could not inhibit *P. aeruginosa.* This might explain why addition of lemongrass oil in this research did not affect the antimicrobial activity of guava leaf hard candy.

## 3.3 Effect of guava leaf extract and lemongrass oil concentration on hard candy characteristics

### 3.3.1 Lightness

Hard candies formulated with varying concentrations of guava leaf extract and lemongrass oil were analyzed in terms of colour using a chromameter and the data were expressed in terms of lightness (L). Figure 2 shows that the hard candies gradually decrease in lightness value with an increase in guava leaf extract concentration. This is due to the pigments found naturally in guava leaves that were solubilized in ethanol during the extraction process, such as tannins and chlorophyll. These pigments are naturally dark in colour, and so provide a darker colour to the hard candy in higher concentrations (Kamble et al., 2015).

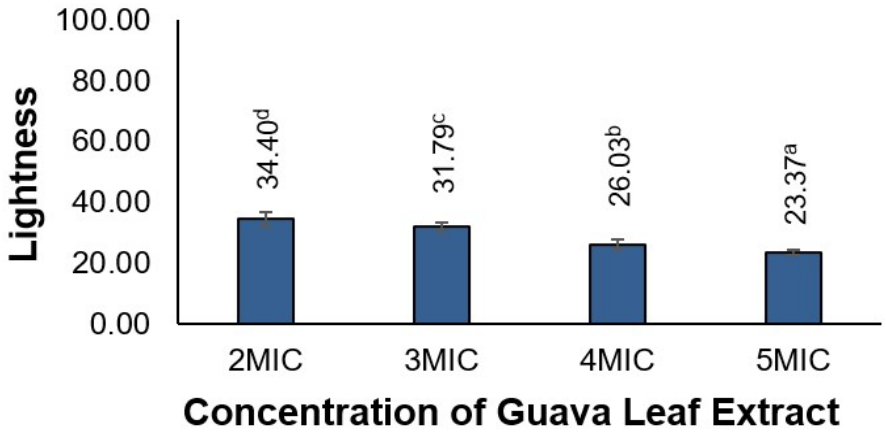


**Figure 2.** Effect of guava leaf extract concentration of hard candy lightness. Note: Different letter notations indicate a significant difference ($p \leq 0.05$).

### 3.3.2 Texture

The texture of the guava leaf hard candies was analyzed in terms of hardness. From texture analyzer measurement, the hardness value shows the force required for human jaws to cause the hard candy structure to shatter in between molars. Based on statistical analysis, the concentration of guava leaf extract and lemongrass oil did not have a

significant effect on the hardness of the hard candy ($p > 0.05$). Additionally, no significant interaction was found between the two in terms of the effect on hardness. The average values for hardness ranged from 10557.39 g ± 256.76 to 10786.69 g ± 248.04 (Table 4). According to Rathod et al. (2018), hard candies typically have hardness values ranging from 10000-15000 kg/cm, which is indicative of sufficient hardness in the candy to maintain its structural integrity and resist breakage within the oral cavity. Higher hardness values are generally unfavorable as breakage upon biting is a desirable quality in hard candies (Rathod et al., 2018)

**Table 4.** Effect of GLE concentration and LO concentration on hardness of guava leaf hard candy.

| GLE Conc. | LO Conc. (%) | Hardness (g) |
|---|---|---|
| 2MIC | 0 | 10717.51 ± 315.75 |
| | 0.15 | 10786.69 ± 248.04 |
| | 0.3 | 10557.39 ± 256.76 |
| 3MIC | 0 | 10602.86 ± 144.36 |
| | 0.15 | 10675.44 ± 240.62 |
| | 0.3 | 10590.21 ± 208.95 |
| 4MIC | 0 | 10686.48 ± 142.88 |
| | 0.15 | 10585.40 ± 255.36 |
| | 0.3 | 10700.12 ± 181.06 |
| 5MIC | 0 | 10642.97 ± 237.77 |
| | 0.15 | 10707.62 ± 238.72 |
| | 0.3 | 10659.88 ± 279.96 |

Notes: - GLE = guava leaves extract, LO = lemongrass oil.

### 3.3.3 Organoleptic characteristics

The sensory acceptance of the formulated hard candies was assessed by 12 panelists based on both hedonic and scoring tests. Results obtained from the hedonic test, which assesses the acceptability of the product based on personal preference, determine the preferred hard candy formulation. Statistical analysis results in Table 5 show that both guava leaf extract and lemongrass oil significantly affected ($p \leq 0.05$) the scoring value in terms of colour, aroma, and aftertaste, but did not significantly affect ($p > 0.05$) the scoring value in terms of texture and taste. However, Table 6 shows that both guava leaf extract and lemongrass oil significantly affected ($p \leq 0.05$) the hedonic value in terms of colour, taste, and overall acceptance, but did not significantly affect ($p > 0.05$) the hedonic value in terms of aroma, aftertaste, and texture.

**Table 5.** Effect of GLE concentration and LO concentration on scoring ratings of guava leaf hard candy.

| GLE Conc. | LO Conc. (%) | Color | Aroma | Taste | Texture | Aftertaste |
|---|---|---|---|---|---|---|
| 2MIC | 0 | $3.2 \pm 0.9^{ab}$ | $2.6 \pm 1.4^{ab}$ | $3.2 \pm 1.0^{a}$ | $4.9 \pm 0.3^{a}$ | $1.8 \pm 0.7^{a}$ |
| | 0.15 | $3.0 \pm 1.29^{a}$ | $2.4 \pm 0.8^{a}$ | $2.7 \pm 1.2^{a}$ | $5.2 \pm 0.6^{a}$ | $1.9 \pm 1.0^{a}$ |
| | 0.3 | $2.7 \pm 1.0^{a}$ | $2.6 \pm 0.9^{ab}$ | $3.2 \pm 1.1^{a}$ | $5.2 \pm 0.6^{a}$ | $2.4 \pm 1.2^{a}$ |
| 3MIC | 0 | $3.3 \pm 0.9^{ab}$ | $2.6 \pm 1.4^{ab}$ | $3.2 \pm 1.0^{a}$ | $4.9 \pm 0.3^{a}$ | $1.8 \pm 0.7^{a}$ |
| | 0.15 | $2.8 \pm 0.9^{a}$ | $2.3 \pm 0.9^{a}$ | $2.9 \pm 1.2^{a}$ | $4.8 \pm 1.0^{a}$ | $2.6 \pm 1.4^{ab}$ |
| | 0.3 | $3.3 \pm 0.9^{ab}$ | $2.2 \pm 0.8^{a}$ | $3.5 \pm 1.0^{a}$ | $5.0 \pm 0.6^{a}$ | $2.7 \pm 1.0^{ab}$ |
| 4MIC | 0 | $3.8 \pm 0.8^{b}$ | $2.4 \pm 1.2^{a}$ | $3.0 \pm 1.2^{a}$ | $5.2 \pm 0.4^{a}$ | $1.6 \pm 0.5^{a}$ |
| | 0.15 | $4.9 \pm 0.7^{cd}$ | $2.2 \pm 0.6^{a}$ | $3.8 \pm 1.0^{a}$ | $5.2 \pm 0.6^{a}$ | $2.9 \pm 1.3^{b}$ |
| | 0.3 | $4.6 \pm 0.5^{c}$ | $2.2 \pm 0.9^{a}$ | $3.8 \pm 1.0^{a}$ | $5.1 \pm 0.5^{a}$ | $2.7 \pm 1.1^{ab}$ |
| 5MIC | 0 | $4.9 \pm 0.8^{cd}$ | $3.6 \pm 1.3^{b}$ | $3.2 \pm 1.2^{a}$ | $5.2 \pm 0.4^{a}$ | $2.9 \pm 1.4^{b}$ |
| | 0.15 | $5.4 \pm 0.5^{d}$ | $2.8 \pm 1.2^{b}$ | $4.0 \pm 1.2^{a}$ | $5.2 \pm 0.6^{a}$ | $2.6 \pm 1.4^{ab}$ |
| | 0.3 | $5.6 \pm 0.5^{d}$ | $2.6 \pm 1.0^{ab}$ | $3.2 \pm 1.1^{a}$ | $5.1 \pm 0.7^{a}$ | $2.9 \pm 1.1^{b}$ |

Notes: - GLE = Guava Leaves Extract, LO = Lemongrass Oil. - description of each parameter can be observed in Table 2. Data are presented in value ± SD. - different letter notations on the same column/parameter indicate a significant difference ($p \leq 0.05$).

**Table 6.** Effect of GLE concentration and LO concentration on hedonic ratings of guava leaf hard candy.

| GLE Conc. | LO Conc. (%) | Color | Aroma | Taste | Texture | Aftertaste | Overall |
|---|---|---|---|---|---|---|---|
| 2MIC | 0 | $5.2 \pm 0.9^{b}$ | $5.5 \pm 1.0^{a}$ | $5.3 \pm 1.2^{a}$ | $5.5 \pm 0.8^{a}$ | $5.0 \pm 1.3^{a}$ | $5.2 \pm 0.9^{a}$ |
| | 0.15 | $5.5 \pm 1.1^{b}$ | $5.7 \pm 1.1^{a}$ | $5.5 \pm 1.2^{b}$ | $5.5 \pm 0.9^{a}$ | $5.4 \pm 1.0^{a}$ | $5.7 \pm 0.8^{b}$ |
| | 0.3 | $5.2 \pm 1.0^{b}$ | $5.4 \pm 1.4^{a}$ | $5.6 \pm 1.3^{b}$ | $4.9 \pm 0.8^{a}$ | $5.4 \pm 1.4^{a}$ | $5.4 \pm 1.0^{b}$ |
| 3MIC | 0 | $4.8 \pm 0.8^{a}$ | $5.2 \pm 1.1^{a}$ | $4.5 \pm 1.6^{a}$ | $5.3 \pm 1.0^{a}$ | $4.9 \pm 1.0^{a}$ | $4.4 \pm 1.4^{a}$ |
| | 0.15 | $4.8 \pm 1.0^{a}$ | $5.0 \pm 1.3^{a}$ | $5.4 \pm 0.9^{b}$ | $5.5 \pm 0.8^{a}$ | $4.8 \pm 1.3^{a}$ | $5.7 \pm 0.8^{b}$ |
| | 0.3 | $4.8 \pm 0.6^{a}$ | $4.9 \pm 0.9^{a}$ | $4.3 \pm 1.4^{a}$ | $5.4 \pm 0.8^{a}$ | $4.8 \pm 1.2^{a}$ | $5.7 \pm 0.9^{b}$ |
| 4MIC | 0 | $5.2 \pm 1.1^{b}$ | $4.9 \pm 1.3^{a}$ | $3.8 \pm 1.4^{a}$ | $5.5 \pm 0.7^{a}$ | $4.9 \pm 0.8^{a}$ | $4.1 \pm 1.2^{a}$ |
| | 0.15 | $4.8 \pm 0.8^{a}$ | $4.8 \pm 1.1^{a}$ | $5.7 \pm 1.0^{b}$ | $5.5 \pm 0.7^{a}$ | $4.9 \pm 1.1^{a}$ | $5.5 \pm 0.9^{b}$ |
| | 0.3 | $5.0 \pm 1.0^{ab}$ | $5.3 \pm 1.4^{a}$ | $4.8 \pm 1.1^{a}$ | $5.3 \pm 0.9^{a}$ | $5.1 \pm 1.3^{a}$ | $5.2 \pm 1.0^{b}$ |
| 5MIC | 0 | $5.1 \pm 1.0^{b}$ | $4.7 \pm 1.4^{a}$ | $4.7 \pm 1.4^{a}$ | $5.7 \pm 1.0^{a}$ | $4.7 \pm 1.1^{a}$ | $4.6 \pm 1.2^{a}$ |
| | 0.15 | $4.7 \pm 1.1^{a}$ | $5.1 \pm 1.0^{a}$ | $5.6 \pm 1.0^{b}$ | $5.2 \pm 0.9^{a}$ | $5.2 \pm 1.1^{a}$ | $5.5 \pm 0.8^{b}$ |
| | 0.3 | $4.8 \pm 0.8^{a}$ | $5.1 \pm 1.1^{a}$ | $4.8 \pm 1.3^{a}$ | $5.2 \pm 0.9^{a}$ | $4.8 \pm 1.1^{a}$ | $4.8 \pm 0.8^{b}$ |

Note: - 1= dislike it extremely; 7 = like it extremely. - GLE = Guava Leaves Extract, LO = Lemongrass Oil. - data are presented in value ± SD. - different letter notations on the same column/parameter indicate significant difference ($p \leq 0.05$).

Table 5 shows a general increase in scoring value, *i.e.*, from 2.7 ± 1.0 to 5.6 ± 0.5 (which indicates a darker colour) with an increase in guava leaf extract concentration. This result is in accordance with the objective measurement using chromameter (Figure 2), which also shows that the lightness of hard candies significantly decreased as a higher concentration of guava leaf extract was added. In terms of acceptance (Table 6), hard candies formulated with 2 MIC guava leaf extract, regardless of lemongrass oil concentration, showed the highest acceptance score. However, it was not significantly different from hard candies formulated with 4 MIC guava leaf extract.

In terms of aroma, Table 5 shows that hard candies added with 5 MIC guava leaf extract had a significantly higher scoring value than 3 MIC and 4 MIC, but not with 2 MIC. However, the highest value of 3.6 ± 1.3 corresponds to "slightly undetectable", which means foreign aroma was completely to slightly undetectable regardless of guava leaf extract and lemongrass oil concentration. Furthermore, this phenomenon did not affect the acceptance of the panellists in terms of aroma (Table 6). This result also shows that the addition of lemongrass oil into hard candy was not sufficient to increase its sensory acceptance in terms of aroma, although Natisri et al. (2014) mentioned that lemongrass was effective as a flavoring agent due to its lemony characteristic, which can be attributed to citral and other volatile compounds such as limonene, citronellal, caryophyllene, linalool, and beta-mycrene. Altogether, these compounds contribute to the characteristic lemongrass aroma, which is generally thought to be fragrant and desirable in various confectioneries.

As shown in Table 6, hedonic ratings of taste range from 4.3 ± 1.4 to 5.7 ± 1.0, which correspond respectively to "neutral" and "like it slightly". In general, hard candy formulated with 0.15% lemongrass oil was found to have a significantly higher hedonic rating for taste, which indicated that the taste of guava leaf hard candies was more acceptable with the addition of lemongrass oil, but only up to a concentration of 0.15%.

The scoring values for texture ranged from 4.8 ± 1.0 to 5.2 ± 0.6, which correspond to "slightly hard" and "hard", respectively. The hedonic ratings of hardness range from 4.9 ± 0.8 to 5.7 ± 1.0, which correspond to "neutral" (neither like nor dislike) and "slightly like". These results are also in accordance with objective measurement (Table 4), in which the concentration of both lemongrass oil and guava leaf extract did not affect the hardness of hard candies produced.

In terms of aftertaste, a higher concentration of guava leaf extract and lemongrass oil resulted in a higher scoring value, from 1.6 ± 0.5 to 2.9 ± 1.1. However, this value still corresponds to "not bitter", and as can be seen from Table 6, there was no significant effect of acceptance in terms of aftertaste ($p > 0.05$), despite various concentrations of guava leaf extract and lemongrass oil added into hard candy.

Moreover, Table 6 shows that the concentration of lemongrass oil in the hard candy appeared to affect the overall acceptance. Hard candies added with 0.15% lemongrass oil showed the highest overall acceptance score despite the concentration of guava leaf extract added. The values ranged from 5.5 ± 0.8 to 5.7 ± 0.9, which correspond to "like it slightly".

### 3.4 Comparison of selected formulation of guava leaf hard candy to control

The selected concentration of guava leaf extract and lemongrass oil to be used in hard candy formulation was determined primarily by results from the hedonic test, which describes the degree of consumer acceptance towards the extract-infused hard candies. However, the concentration of guava leaf extract was found to have no significant effect on the hedonic ratings for overall acceptance, hence the selected candy formulated was determined primarily from antibacterial activity. Hard candies infused with guava leaf extract at a concentration of 4 MIC have exhibited significantly greater antibacterial activity and in terms of overall acceptance, the guava leaf hard candies received the highest acceptance rating when lemongrass oil was used at a concentration of 0.15%. Therefore, the selected concentrations of guava leaf extract and lemongrass oil to be used in the formulation of hard candy were 4 MIC and 0.15%, respectively.

In terms of physical parameters, the control had a higher lightness (L) value (47.18 ± 1.50), which indicates a lighter colour, compared to the selected hard candy (25.37 ± 0.83). which is due to the absence of pigmentation in its formulation. In terms of texture, the preferred formulation was found to have an average hardness value of 10585.4 g ± 255.35, while the control had an average hardness value of 10673.19 g ± 243.31. The similar hardness values indicated that the addition of guava leaf extract as well as lemongrass oil did not affect the hardness of the hard candy. Furthermore, a comparison of the organoleptic test results of the control hard candy to the selected formulation is shown in Table 7.

**Table 7.** Comparison of scoring and hedonic ratings for control and selected formulation.

| Parameters | Control | | Selected Formulation | |
|---|---|---|---|---|
| | Scoring | Hedonic | Scoring | Hedonic |
| Color | 1.1 ± 0.3 | 4.9 ± 1.7 | 4.9 ± 0.7 | 4.8 ± 0.8 |
| Aroma | 1.3 ± 0.5 | 5.2 ± 1.3 | 2.2 ± 0.6 | 4.8 ± 1.1 |
| Taste | 1.3 ± 0.4 | 4.9 ± 1.7 | 3.8 ± 1.0 | 5.7 ± 1.0 |
| Texture | 5.2 ± 0.8 | 5.8 ± 0.9 | 5.2 ± 0.6 | 5.5 ± 0.7 |
| Aftertaste | 1.1 ± 0.3 | 5.2 ± 1.8 | 2.9 ± 1.3 | 4.9 ± 1.1 |
| Overall | - | 4.7 ± 1.6 | - | 5.5 ± 0.9 |

Table 7 shows higher hedonic ratings for the control in terms of colour, aroma, texture, and aftertaste. The selected hard candy formulation, on the other hand, received higher ratings for taste and overall acceptance. Therefore, these organoleptic test results suggest the potential for the selected hard candy formulation to be developed as a commercial product as it received a higher average rating compared to the control in terms of overall acceptance.

## 4 Conclusions

Based on both hedonic test results and the antibacterial activity assay, hard candy added with 4 MIC guava leaf extract and 0.15% lemongrass oil was determined to be the selected formulation. At these concentrations, the hard candy had an average inhibition diameter of 11.92 ± 0.56 mm towards *S. pyogenes* and 10.77 ± 0.69 mm towards *P. aeruginosa*. The selected hard candy had darker colour, comparable texture in terms of hardness, and a higher acceptance level compared to the control. The findings in this research showed that a combination of guava leaf extract and lemongrass oil can be incorporated into hard candy making as natural antimicrobial agents against some URTI caused by bacteria. Although the number of panelists in this research

was limited, its higher acceptance level compared to control hard candy also indicated that guava leaf hard candy might be further developed into a commercial product.

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