

Original Paper

Antibacterial Activity of *Kaempferia galanga* L. Hard Candy Against *Streptococcus pyogenes* and *Staphylococcus aureus* Bacteria Growth

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Abstract — Kencur (*Kaempferia galanga* L.) is a spice plant that has a high volatile content with active compounds of *ethyl p-methoxy cinnamate* (23.65%) and *ethyl cinnamate* (5.98%). These two compounds have been reported to have antibacterial activity against *Streptococcus pyogenes* and *Staphylococcus aureus*, which are two types of bacteria that can cause sore throat. The application of Kencur essential oil in hard candy can potentially reduce inflammation in throat. This study aims to determine the effect of Kencur essential oil concentration in hard candy in inhibiting the growth of *Streptococcus pyogenes* and *Staphylococcus aureus* bacteria and to determine their acceptance by a group of panelists. This research was conducted with 6 treatments involving different concentrations of Kencur essential oil, including 0%, 0.2%, 0.4%, 0.6%, 0.8%, and 1%. The results showed that higher concentration of Kencur essential oil in hard candy would further inhibit the growth of *Streptococcus pyogenes* and *Staphylococcus aureus* bacteria. The highest inhibitory activity and the highest acceptance by panelists were found at 1% essential oil concentration with 3.29% moisture content, 0.12% ash content, and 7.13% reducing sugar. The use of Kencur essential oil in hard candy as a flavoring as well as inhibiting the growth of strep throat-causing bacteria (*Streptococcus pyogenes* and *Staphylococcus aureus*) has the potential to be developed.

Keywords — Antibakteri, hard candy, Kencur, minyak atsiri, *Streptococcus pyogenes*, *Staphylococcus aureus*

I. INTRODUCTION

Pharyngitis or sore throat is a disease that is categorized as an Upper Respiratory Tract Infection (URTI). According to the World Health Organization (WHO) in 2016, the number of patients with URTI in the world is 59,417 cases with the number of cases in developing countries ranging from 40 to 80 times higher than that in developed country. In the United States approximately 15 million patients suffer from this disease per year [1]. Sore throat is caused by bacterial infections, including *Streptococcus pyogenes* and *Staphylococcus aureus* [2].

Kencur (*Kaempferia galanga* L.) is a tropical plant which is traditionally known as a medicinal plant. Kencur, Rhizome, contains starch (4.14%), minerals (13.37%), and essential oils (2.4–3.9%) [3]. [4] report that Kencur essential oil produced from steam distillation contains the two highest compounds, *ethyl cinnamate* (5.98%) and *ethyl p-methoxy cinnamate* (23.65%) which have antimicrobial activity. The *ethyl p-methoxy cinnamate* compound is a derivative of phenol which is bacteriostatic. These compounds can cause damage to plasma membranes, inactivate enzymes and denature proteins, causing bacterial cells to lose their shape and trigger the occurrence of lysis [5]. Some researchers stated that Kencur essential oil has antibacterial activity against the growth of *Staphylococcus aureus* [6]; *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* [7].

Hard candy is a hard textured non-crystalline candy made from a mixture of sucrose, glucose, water and additives, namely flavors, dyes and acidifying substances [8]. Making hard candy with the addition of essential oils has been carried out in several studies, namely honey propolis hard candy [9]; ginger candy [10] and cajuputs candy [11]. The addition of essential oils to hard candy can add aroma and flavor as well as active compounds as antibacterial agents [12]. Consuming Kencur essential oil in hard candy offers several advantages, including practicality, active compound as antibacterial agent, and unique ability to dissolve and disintegrate slowly in the mouth so that it has the potential to kill microbes that cause sore throat. Studies employing the right concentration of Kencur essential oil with the highest antibacterial activity which meets acceptability criteria of a group of panelists have never been reported before. Therefore, this study was conducted to delve into that empirical gap for potential development.

II. MATERIALS AND METHOD

A. Ingredients

Kencur rhizome (*Kaempferia galanga* L.) in this study was obtained from Kencur plantation in Kemuning village, Jember, Indonesia. Species identification [7] was carried out

at the Botanical Laboratory of the Department of Biology, Faculty of Mathematics and Science (FMIPA), University of Jember.

The ingredients for making hard candy included white crystal sugar (Gulaku), glucose syrup, and water (Aqua) from the local market. The other ingredients included inoculum of *Streptococcus pyogenes* and *Staphylococcus aureus* from the Microbiology Laboratory of the Faculty of Medicine, University of Jember. Also, the study used NA (Nutrient Agar), MHA (Mueller Hinton Agar), 2%, DMSO (Dimethyl Sulfoxide) nelson solution, arsenol solution, CaCO_3 , Pb Acetate, Na Oxalate, Arsenomolybdate, BaCl_2 (Merck) and H_2SO_4 (Smartlab) for the manufacture of Mc Farland 0.5, disc paper (wathman 42 diameter 12.5) ordered from Ina Lab.

B. The Distillation of Essential Oil

The extraction of essential oil was carried out by employing steam distillation method [4]. Three Kg of Kencur rhizome were sorted, washed, cleaned of dirt, and then sliced into thin slices of $\pm 1-4$ mm thickness. Simplicia was placed in a distiller with a water ratio of 1: 2, which was then distilled for 5 hours. The essential oil distillate was separated in 15 ml amber vial.

C. The Production of Hard Candy [38]

White crystal sugar (120 g) was mixed in 20 ml of water and then heated to 100°C . Sixty g glucose was added and heated to the final temperature of hard crack heating (150°C), then cooled to 110°C . Afterwards, Kencur essential oil was added according to the treatments (0%, 0.2%, 0.4%, 0%, 8% and 1%), molded, and kept at room temperature until it became hardened. After hard candy was removed from the mold, it would be stored in closed packaging for further testing.

D. Antimicrobial Testing

The Production of Mc Farland 0.5 Solution

Mc Farland 0.5 solution was used as the standard for the turbidity of microbial suspensions to determine the number of microbes in the suspension [13]. 1.175% BaCl_2 0.05 ml and H_2SO_4 1% 9.95 ml were mixed until homogeneous. The turbidity of Mc Farland's solution was measured using a spectrophotometer at a wavelength of 625 nm, with absorbance values ranging from 0.08 to 0.1. The Mc Farland value of 0.5 indicates that the number of microbes is equivalent to a bacterial density of 3×10^8 / ml.

The Production of Bacterial Suspension

One ose of test bacteria was each suspended in 10 ml of sterile physiological solution and then homogenized using vortex, the absorbance which was measured at a wavelength of 625 nm. The turbidity of the suspension was adjusted to the Mc Farland absorbance value. One ml of bacterial suspension was put into a test tube containing 9 ml sterile distilled water. Dilution was carried out to a level of 10^{-6} .

Production of Test Solution

Three grams of hard candy was dissolved in 6 ml of DMSO 10% solution, which was sterilized by autoclaving (121°C , 2 atm for 15 minutes).

Testing Inhibition Zone Activity

Inhibition zone activity testing was performed using the agar diffusion method with disc paper (Kyrbi-Bauer method) and observed based on the resulting clear zone around the disc [14]. Sterile paper discs measuring 6 mm were immersed in the test for 30 minutes. MHA (Mueller Hinton Agar) media weighing 3.4 g was dissolved in 100 ml of distilled water and then heated until dissolved. The media was then sterilized using autoclave at 121°C for 15 minutes. Twelve ml of sterile MHA media was poured into a sterile petri dish with a diameter of 10 cm. After the media had been evenly distributed and solidified, 0.1 ml of bacterial suspension was inoculated into the petri dish and then flattened using a drigalsky spatula. Furthermore, the soaked paper discs were placed on the surface of the test media, which was then incubated at 37°C for 24 hours. The zone of inhibition was obtained from measuring the diameter of clear zone around the disc. The diameter of inhibition zone was classified into 4 stages of criteria in accordance with [15], namely weak activity (<5 mm), moderate (5-10 mm), strong (10-20 mm), very strong (>20 mm).

Minimum Inhibitory Concentration (MIC) and Concentration Inhibiting 50% (IC_{50}) [39]

The agar dilution method antibacterial test was carried out to determine MIC (Minimum Inhibitory Concentration) and IC_{50} against *Streptococcus pyogenes* and *Staphylococcus aureus* bacteria. The dilution test was carried out by pouring the solution. One ml of bacterial suspension was inoculated into the petri dish. Once evenly distributed, 1 ml of the test solution was inoculated into the petri dish. A total of 12 ml of sterile MHA media was poured into the petri dish and then flattened before it was kept to become solid. The concentration was put in incubation for 24 hours at 37°C . The number of colonies on the petri dish was counted using a colony counter (Stuart Scientific).

E. Preference Test

The preference test refers to a modified study [16]. The preference sensory assessment used a hedonic scale which aimed to evaluate panelists' acceptance of the color, aroma, taste and overall properties of hard candy. The test was conducted by 80 untrained male and female panelists aged 19-25 years. The test rating scale was based on the level of preference with 7 categories, ranging from 1 to 7, with 1 indicating strong disagreement and 7 corresponding to strong agreement. The threshold to accept a sample was at intervals ranging from 5 to 7.

F. Effectiveness Calculation [17]

The effectiveness test was carried out by giving a weighted value (variable value) to each variable with a relative number, namely 0-1. The effectiveness value can be calculated using the following formula:

$$\text{Effectiveness Value} = \frac{\text{Treatment score} - \text{the worst score}}{\text{The best score} - \text{the worst score}} \times \text{Normal Weight}$$

G. Testing Chemical Characteristics of The Best Sample

The best sample obtained from the effectiveness test was put into the chemical characteristic test. The chemical contents tested were moisture content, ash content, and reducing sugar content [18]. The test results were then adjusted to the quality requirements of the Indonesian National Standard 3547.1: 2008 for hard candy [8].

III. RESULTS AND DISCUSSION

A. Activity of Inhibition Zone of Hard Candy with Kencur Essential Oil

The inhibition zone of hard candy was investigated by observing the diameter of the clear zone around the disc as the potential determinant to the presence or absence of antibacterial compounds in inhibiting microbial growth [19]. The results of the antibacterial activity test showed that the increase in the concentration of Kencur essential oil in hard candy had a significant effect on the increased diameter of inhibition zone of *Streptococcus pyogenes* and *Staphylococcus aureus* ($p < 0.05$), as seen in Figure 1 and Figure 2.

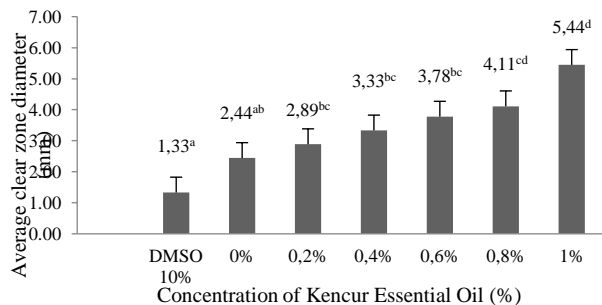


Figure 1. Inhibition Zone of Hard Candy with Kencur Essential Oil (0%: 0 ml, 0.2%: 0.4 ml, 0.4%: 0.8 ml, 0.6%: 1.2 ml, 0.8%: 1.6 ml, 1%: 2 ml) against the Growth of *Streptococcus pyogenes*

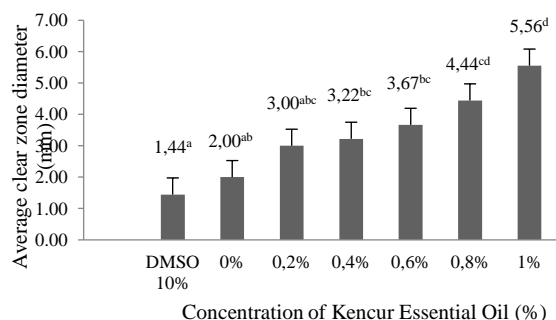


Figure 3. Inhibition Zone of Hard Candy with Kencur Essential Oil (0%: 0 ml, 0.2%: 0.4 ml, 0.4%: 0.8 ml, 0.6%: 1.2 ml, 0.8%: 1.6 ml, 1%: 2 ml) against the Growth of *Staphylococcus aureus*

Kencur essential oil in hard candy possesses the potential to defend against microbial growth of *Streptococcus pyogenes* and *Staphylococcus aureus*. The higher the concentration of essential oil added to hard candy, the larger the area of the inhibition zone for bacterial growth. Essential oil at a concentration of 1% produced the highest inhibition zone against bacterial growth with an inhibition zone diameter of 5.44 mm against *Streptococcus pyogenes* and 5.56 mm against *Staphylococcus aureus*. The inhibition ability was based on the diameter area, which in our study was classified as moderate. Figures 3 and 4 show that the use of Kencur essential oil has a better antibacterial activity, compared to the absence of essential oil. This was indicated by the smallest inhibition zone at 10% DMSO. DMSO is an organic solvent that can dissolve polar and non-polar compounds and is not bactericidal, so it does not affect the work of antibacterial compounds [20]. The compound content of Kencur rhizome essential oil is known to have antibacterial activity.

The active components contained in Kencur essential oil such as ethyl p-methoxycinnamate, methylsinamate and penta decane have the ability to inhibit bacterial growth [21].

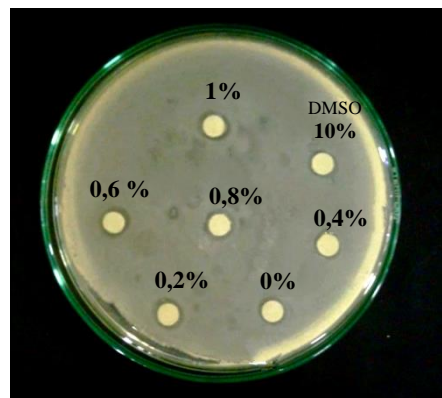


Figure 3. The Inhibition Zone against *Streptococcus pyogenes*

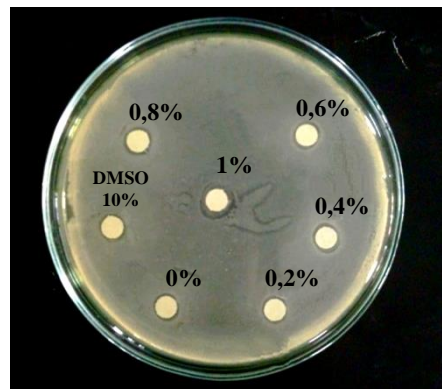


Figure 4. The Inhibition Zone against *Staphylococcus aureus*

The effectiveness of an antibacterial compound is determined by the concentration and type of active compound present in a material. To that point, the higher the

concentration of the active substance is, the greater the effectiveness will become [22]. Figures 3 and 4 show the increased diameter of the inhibition zone in proportion to the increase in the concentration of Kencur essential oil added to the sample. The inhibitory activity of Kencur essential oil was also reported by [7] who stated that Kencur essential oil formed a clear zone against the bacteria *Escherichia coli*, *Bacillus subtilis* and the fungus *Candida albicans*.

According to [23], Kencur essential oil showed strong inhibitory activity against *Salmonella typhimurium*, *Staphylococcus aureus* and *Escherichia coli*. Antimicrobial activity can occur due to membrane damage, cell wall damage and damage to electron transport [24]. The mechanism of changes that occur due to the administration of Kencur essential oil has been reported in study by [23], where the use of essential oils causes structural changes in bacterial cells as well as nucleic acids and causes proteins to undergo lysis, eventually leading to cell damage. Giving Kencur essential oil causes bacterial cells to stick together, cell membranes to undergo lysis and changes in bacterial morphology, including severe damage, defects, holes and wrinkles. The nucleic acids and proteins are released into the cell supernatant, and as a result the protein structure opens and decreases.

The integrity of the cell membrane is one of the main factors affecting bacterial growth and metabolism. The release of cytoplasmic components such as nucleic acids and proteins can affect the integrity of the cell membrane, because the intracellular components (small ions, ATP, nucleic acids and proteins) will be released if there is a change in the bacterial membrane after the exposure to antibacterial compounds [25]; [26]. The release of nucleic acids in the nucleus, proteins in cell membranes, cytoplasm and nucleus shows severe damage to cell walls and membranes, thereby affecting cell growth and inhibiting bacterial replication [27]. Longer and more intense concentration of antibacterial added increases the possibility of cell damages. According to studies [28] and [29], Kencur essential oil affects bacterial protein and decreases the amount of cellular protein by permeating and disrupting the cell membrane, resulting in bacterial death.

B. Inhibitory Concentration 50% (IC_{50}) and Minimum Inhibitory Concentration (MIC)

Antibacterial compounds are toxic compounds that can inhibit bacterial growth and activity. Antibacterial activity testing aims to determine the concentration of a substance containing antibacterial compounds so that it can be applied effectively and efficiently. IC_{50} is used to determine the minimum concentration of an antibacterial solution that can inhibit the growth of certain bacteria [30]. MIC aims to determine the minimum concentration of an antibacterial solution that can kill certain bacteria [31]. The determination of the IC_{50} and MIC values was calculated using a logarithmic curve equation. The logarithmic curve shows the log relationship between the number of *Streptococcus pyogenes*

colonies and the concentration of Kencur essential oil in hard candy, as seen in Figure 5 and Figure 6.

Based on Figure 5 and Figure 6, the equations $y = -0,152x + 8,067$ for the inhibition of *Streptococcus pyogenes* and $y = -0,155x + 7,824$ for the inhibition of *Staphylococcus aureus* are formulated. The logarithmic curve shows a linear line, where the greater the concentration of Kencur essential oil, while the log of the number of bacterial colonies decreases. The calculation results show that the IC_{50} value of hard candy on the growth of *Streptococcus pyogenes* is a concentration of 1.980 mg/ml, while the IC_{50} for the growth of *Staphylococcus aureus* is a concentration of 1.942 mg/ml. These data show that Kencur essential oil in hard candy at a concentration of 0.8% can inhibit bacterial growth.

The MIC value of Kencur in hard candy on the growth of *Streptococcus pyogenes* was a concentration of 6.58 mg/ml and 6.45 mg/ml on the growth of *Staphylococcus aureus*. These data show that Kencur essential oil in hard candy at concentrations higher than 1% can kill bacteria.

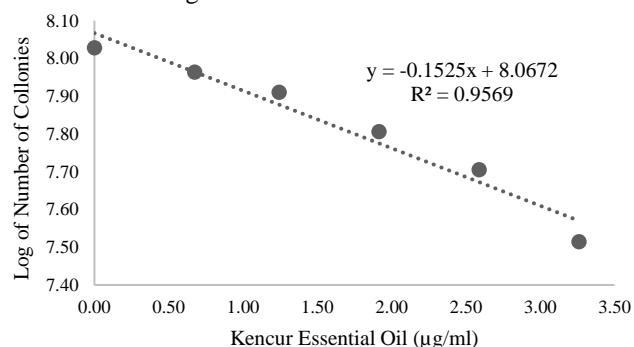


Figure 5. Logarithmic Curve on The Correlation between Inhibitory Property of Hard Candy with Kencur Essential Oil and The Growth of *Streptococcus pyogenes*

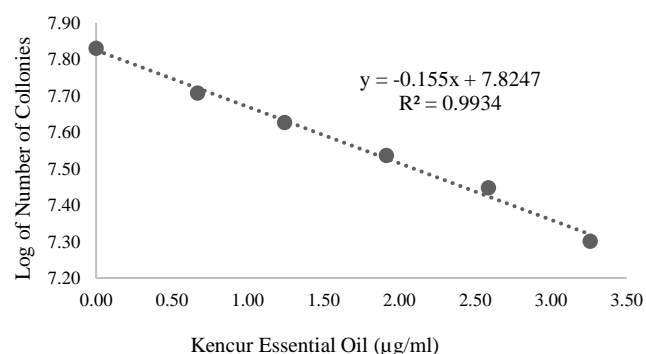


Figure 6. Logarithmic Curve on Inhibitory Activity of Hard Candy with Kencur Essential Oil against the Growth of *Staphylococcus aureus*

Kencur essential oil contains an antibacterial compound in the form of ethyl p-methoxy cinnamate which is classified as a phenolic compound group [32]. Phenol-based components can damage plasma membrane, and trigger enzyme inactivation and protein denaturation. According to [33] and [34], *Streptococcus pyogenes* and *Staphylococcus aureus* are gram-positive bacteria. Gram-positive bacteria are

prone to protein denaturation. This is because the cell walls of gram-positive bacteria contain peptidoglycan, teichoic acid and theikuronic acid which are mostly polysaccharides so they are easily denatured [35]. The denatured protein in the cell wall causes brittleness of the bacterial cell wall so that it is easily penetrated by other active substances that act as antimicrobials. The destruction of the cytoplasmic membrane due to phenol components causes bacteria to lose their pathogenicity and then die [32].

C. Preference

Color

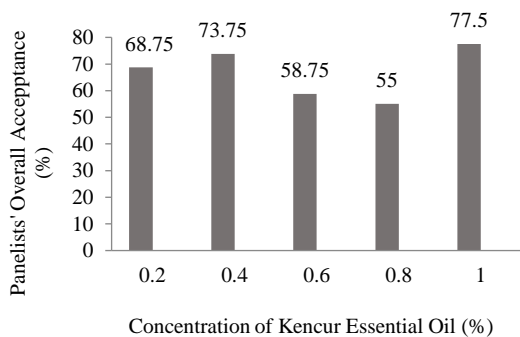


Figure 7. Acceptability Level of Hard Candy Color

Color is one of the determinants of food product quality because it can attract consumers. Majority of panelists, 77.5%, gave positive response to the color formed at a concentration of 1%. The lowest panelist acceptance, 55%, preferred the color at a concentration of 0.8%. Kencur hard candy with 1% concentration of essential oil is the product that shows the highest panelist acceptance. This is probably because Kencur gives an attractive color to the product. According to [36] the color of Kencur essential oil produced from the steam-water distillation process is yellow-red, so the addition of Kencur essential oil can cause the hard candy to appear transparent yellow.

Aroma

Aroma is an important indicator in food products because it can determine whether a product is accepted or not. The highest acceptance of aroma was 70% of the 80 panelists, as found in the essential oil concentration of 0.4%, while the lowest (51.25%) was at the concentration of 0.2% and 0.8%. Kencur hard candy with essential oil concentration of 0.4% is the product that shows the highest panelist acceptance.

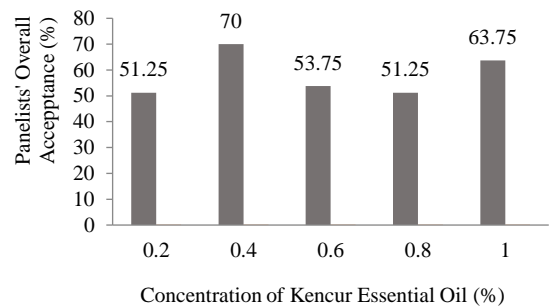


Figure 8. Panelists' Acceptance of Hard Candy Aroma

This may be due to the addition of Kencur essential oil. At this concentration, the amount is not too high, so that the scent of Kencur is not too strong, which is why it can still be accepted by the panelists. According to [4], Kencur *rhizome* essential oil has a distinctive fragrant and fresh aroma, like Kencur *rhizome*. The more essential oil is added, the stronger the distinctive scent becomes.

Flavor

Flavor is one of the factors that determine food quality. The highest acceptance of Kencur hard candy flavor was 70%, as found at 0.4% concentration of Kencur essential oil, while the lowest was at 0.2% essential oil concentration. The flavor of a product can be sensed by the taste buds, usually in the form of sweet, salty, sour, and bitter sensations. Hard candy with Kencur essential oil at concentration of 0.4% receives the highest panelists' acceptance. This is probably due to the fact that this concentration does not cause spicy Kencur taste, compared to flavor associated with higher concentration. [4] state that the essential oil of Kencur rhizome has a spicy and bitter flavor due to the presence of saponin compounds [37]. The more concentration of essential oils that is added, the stronger the spicy becomes.

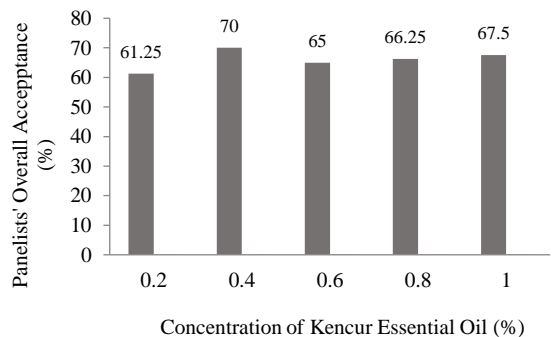


Figure 9. Acceptance of Hard Candy Flavor

Overall Evaluation

The highest percentage of overall acceptance was 73.5% of total panelists, as indicated in 0.4% and 1% essential oil concentrations in hard candy. The overall organoleptic characteristics were the panelists' acceptance to color, aroma, and taste of hard candy. Hard candies with Kencur essential oil concentrations at 0.4% and 1% are the

products that show the highest panelist acceptance. This finding concludes that the panelists give positive feedbacks to hard candy with the addition of Kencur essential oil, as indicated by its distinctive color, aroma, and flavor .

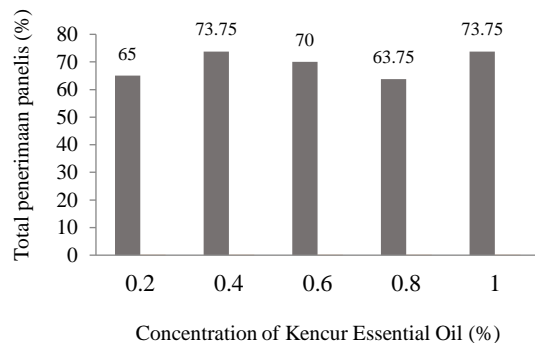


Figure 10. Overall Acceptance of Hard Candy

D. Effectiveness Value

An effectiveness test was carried out to determine the best sample based on the selected parameters [17]. Hard candies with various concentrations of Kencur essential oil were tested by the panelists. The best treatment was chosen based on the hedonic test parameters including color, aroma, flavor, overall acceptance, and inhibition zone test (disc method). Based on Table 1, the best treatment in hard candy sample is hard candy with a concentration of 1% essential oil, found to have an effectiveness value of 0.83.

Table 1. Effectiveness Value of Hard Candy with Kencur Essential Oil

Sample	Effectiveness Value
A1	0,22
A2	0,80
A3	0,48
A4	0,18
A5	0,83

E. The Characteristics of The Best Chemical Treatment

Based on Table 2, the test of the chemical characteristics of hard candy with Kencur essential oil corroborated the best treatment with 1% concentration of essential oil, 3.29% moisture content, 0.12% ash content, and 7.13% reducing sugar. The best treatment on chemical content has met the requirements of the Indonesian National Standard.

Table 2. Analysis Results on The Best Treatment for Chemical Characteristics

No.	Parameters	Analysis Results (%)	Standard (SNI in Hard Candy)
1.	Water content	3,29	maximum 3,5 %
2.	Ash content	0,12	maximum 2,0 %
3.	Reducing sugar	7,13	maximum 24 %

IV. CONCLUSION

The addition of Kencur essential oil results in increasing antibacterial activity and panelist preference. Hard candy with Kencur essential oil at a concentration of 1% has

the highest inhibition activity against *Streptococcus pyogenes*, namely 5.44 mm, and against *Staphylococcus aureus*, namely 5.56 mm. Also, the addition of essential oil has met the Indonesian National Standard for hard candy standards. The IC_{50} test on the growth of *Streptococcus pyogenes* and *Staphylococcus aureus* has acknowledged that the best concentration is 0.8% (2.59 mg/ml). The MIC against *Streptococcus pyogenes* and *Staphylococcus aureus* is marked at a concentration of >1%, namely 6.45-6.58 mg/ml. Hard candy with 1% concentration of Kencur essential oil induces satisfactory antibacterial activity and is presumed to gain positive consumer acceptance, as corroborated by the panelists' evaluation.

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