EFFECTIVENESS BETWEEN MANGO HONEY AND KESAMBI HONEY AS ANTIBACTERIAL AGAINST STREPTOCOCCUS PYOGENES

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ABSTRACT

Introduction: Infectious disease is one of the biggest health problems in developing countries, including Indonesia. The development of the discovery of antibiotics was not balanced with the development of resistance that occurred after that Group A streptococcus β hemolytic causes various events of a severe degree of infection. Now many people use natural ingredients as an alternative to antibiotics, one of which is honey.

Aim: Knowing the benefits of mango honey and kesambi honey as an antibacterial against *Streptococcus pyogenes* and knowing the difference in the effectiveness and potency to inhibit growth and kill *Streptococcus pyogenes* between the two honey.

Method: Using the macro dilution method in determining the value of MIC and the streaking method to determine the value of MBC.

Results: MIC mango honey at 15.625 μ L, MIC kesambi honey at 15.625 μ L, MBC mango honey at 15.625 μ L, and MBC kesambi honey at 31.25 μ L.

Conclusion: There is a difference of MBC between mango honey and kesambi honey against *Streptococcus pyogenes* where mango honey at 15.625 μ L and kesambi honey at 31.25 μ L, there is no difference in MIC between mango honey and kesambi honey, and the effectiveness and potency of mango honey as an antibacterial against Sp higher than kesambi honey which was observed through differences in the value of MBC.

Keywords: MIC, MBC, mango honey, kesambi honey, *Streptococcus pyogenes*.

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INTRODUCTION

Infectious disease is one of the biggest health problems in developing countries, including Indonesia. The 2007 Household Health Survey provides an overview of some of the highest causes of death, including 28.1% caused by infectious diseases, 18.9% caused by vascular disease, and 15.7% caused by respiratory diseases¹.

The discovery of penicillin as an antibiotic by Sir Alexander Fleming in 1928 was significant to fight bacterial infections. Since then, antibiotics have changed modern medicine and saved many lives. In the 1940s, antibiotics were first prescribed to treat serious infections². The development of antibiotics did not account for development of resistance that occurs in microorganisms that cause infectious diseases after that. resistance occurs due to excessive use of antibiotics and inappropriate prescribing. Some public health organizations declare the emergence and development of resistance events as "crises" or "nightmare scenarios" that can have "catastrophic consequences." The Centers for Disease Control and Prevention (CDC) states that in 2013 humanity is in the "post-antibiotic era." The World Health Organization (WHO) in 2014 stated that the incidence of antibiotic resistance crises became increasingly dire³.

Streptococcus pyogenes is a member of the *Streptococcus* group A bacteria, which is β hemolytic. This bacterium causes various events of severe infection. Infection that is often caused in the form of *streptococcal pharyngitis* or commonly called strep throat, impetigo, middle ear infection, mastoiditis, and also other infections that spread hematogenously such as *glomerulonephritis* and acute rheumatic fever⁴. All *Streptococcus* groups are sensitive to the penicillin group antibiotics, especially penicillin G. Penicillin is useful in more than 90% of cases and is generally used as the drug of choice. Tetracycline antibiotics and sulfonamide groups are generally not recommended because *Streptococcus* rapidly develops resistance to these drugs⁵. There is also a mention that *Streptococcus pyogenes* has been resistant to erythromycin and other macrolide groups⁶.

If resistance continues to occur and increases, it is feared that common treatment of infectious diseases will fail so that morbidity and mortality rates will rise. Today many people use natural ingredients as an alternative to antibiotics, one of which is honey. Honey is a natural substance produced by honey bees, produced from the nectar of flowers or plant secretions collected by honey bees and then converted and stored in beehives for maturation⁷. Honey has many uses for

health benefits, beauty purposes and can be used as food sweeteners, food seasonings, and beverage mixes. Honey can also be used to treat fevers, prevent thrush, treat coughs, heal wounds, and maintain immunity⁸. Honey can suppress the growth of certain bacteria through several mechanisms, including high sugar levels that can inhibit bacteria from living and developing. High acidity levels can inhibit the growth and survival rate of bacteria so that bacteria will die. Honey has a radical compound hydrogen peroxide (H₂O₂) that can kill microorganisms that cause disease. There are antibacterial organic compounds (for example, flavonoids), which are currently often used as a basis for modern antibacterial drugs⁹. Each type of honey has different chemical composition depending on the source of the nectar. This difference is thought to produce differences in antibacterial activity between honey¹⁰. For the reasons stated above, a study was carried out to determine the antibacterial activity and study the difference in effectiveness and antibacterial potential between mango honey and kesambi honey against *Streptococcus pyogenes*.

METHOD

This research was an experimental study using an actual experiment with a post-test-only control group design,

divided into two groups: the treatment group and the control group. This research was conducted at the Pharmacy Faculty Research Laboratory at Widya Mandala Catholic University Surabaya, and the Clinical Microbiology Laboratory at the Center for Health Laboratory of Surabaya. The population of this research was *Streptococcus pyogenes*. Samples taken were *Streptococcus pyogenes* ATCC 19615 obtained from the Clinical Microbiology Laboratory at the Center for Health Laboratory of Surabaya. The levels of mango honey and kesambi honey used were 15.625 µL, 31.25 µL, 62.5 µL, 125 µL, and 250 µL.

Honey Setup

The two honey samples used, namely mango honey and kesambi honey, were purchased from honey producers who took mango honey from beehives in the mango tree and kesambi honey from beehives in the kesambi tree in Atambua City area, Belu Regency, NTT. Honey was taken from beehives in the forest by honey farmers and then processed to get pure honey. Honey was tested for pH/acidity and flavonoid content as a differentiator between mango honey and kesambi honey in the Pharmacy Faculty Research Laboratory at Widya Mandala Catholic University Surabaya. Furthermore, both sterility of honey was tested at the Clinical Microbiology Laboratory at the Center for

Health Laboratory of Surabaya to determine whether there was bacterial contamination. After that, the two honey samples were used in the KHM and KBM testing procedures.

Making bacterial suspensions

bacterial culture that has been grown was taken with a sterile loop and then suspended in a tube containing 0.9% NaCl solution to obtain the same turbidity suspension bacterium with standard turbidity of 0.5 Mc Farland on Mc Farland densitometer which is equivalent to 1.5×10^8 CFU/ml¹¹.

Antibacterial Test

Testing for antibacterial activity was carried out using the macro dilution method. This study was divided into control groups and treatment groups.

$K_{x1-5}; y1-5$ = Negative control groups

K_{x1-5} = Negative control of mango honey

K_{x1} = Mango honey 15,625 microliter (μ l) + *Mueller Hinton Broth* (MHB)

K_{x2} = Mango honey 31,25 μ l + MHB

K_{x3} = Mango honey 62,5 μ l + MHB

K_{x4} = Mango honey 125 μ l + MHB

K_{x5} = Mango honey 250 μ l + MHB

K_{y1-5} = Negative control of kesambi honey

K_{y1} = Kesambi honey 15,625 μ l + MHB

K_{y2} = Kesambi honey 31,25 μ l + MHB

K_{y3} = Kesambi honey 62,5 μ l + MHB

K_{y4} = Kesambi honey 125 μ l + MHB

K_{y5} = Kesambi honey 250 μ l + MHB

K_z = Media control group

K_z = Bacterial suspension

Streptococcus pyogenes + MHB

K_a = Positive control group

K_a = Bacterial suspension

Streptococcus pyogenes + MHB +

Penicillin G

$P_{x1-5}; y1-5$ = Treatment groups

P_{x1-5} = Mango honey treatment

P_{x1} = Mango honey 15,625 μ l + MHB

+ Bacterial suspension *Streptococcus pyogenes*

P_{x2} = Mango honey 31,25 μ l + MHB +

Bacterial suspension *Streptococcus pyogenes*

P_{x3} = Mango honey 62,5 μ l + MHB +

Bacterial suspension Sp

P_{x4} = Mango honey 125 μ l + MHB +

Bacterial suspension Sp

P_{x5} = Mango honey 250 μ l + MHB +

Bacterial suspension *Streptococcus pyogenes*

P_{y1-5} = Kesambi honey treatment

P_{y1} = Kesambi honey 15,625 μ l +

MHB + Bacterial suspension

Streptococcus pyogenes

P_{y2} = Kesambi honey 31,25 μ l + MHB

+ Bacterial suspension *Streptococcus pyogenes*

P_{y3} = Kesambi honey 62,5 μ l + MHB

+ Bacterial suspension *Streptococcus pyogenes*

P_{y4} = Kesambi honey 125 μ l + MHB +
Bacterial suspension *Streptococcus*
pyogenes

P_{y5} = Kesambi honey 250 μ l + MHB +
Bacterial suspension *Streptococcus*
pyogenes

H = KHM dan KBM

Determination of MIC of mango honey and kesambi honey against bacteria *Streptococcus pyogenes* was through visual means of observing the lowest levels of honey in the treatment tube with the same clarity as a negative control tube the same levels and types of honey after incubated at 37°C for 24 hours. Visual observations were made in a room exposed to sunlight, carried out at the same time by three different people.

Determination KBM of mango honey and kesambi honey against *Streptococcus pyogenes* bacteria was done by calculating the number of colonies from the results of honey streaking with the lowest levels of the treatment tube determined as MIC to the highest concentration of honey compared with negative controls with the same levels and types of honey. No bacterial growth was obtained in the form of the number of colonies after incubation at 37°C for 24 hours on Blood Agar.

RESULTS

Visual Observation Results of KHM

Honey Mango Test Tube

Figure 1. Tubes containing treatment and control of MIC test of mango honey.

From the observations, it was found that 15.625 μ L (P_{x1}), 31.25 μ L (P_{x2}), 62.5 μ L (P_{x3}), 125 μ L (P_{x4}), and 250 μ L (P_{x5}) could inhibit the growth of *Streptococcus pyogenes*. Then the MIC value of mango honey against *Streptococcus pyogenes* is 15,625 μ L (P_{x1}) because the levels of mango honey are 15,625 μ L (P_{x1}) smaller than the levels of mango honey 31.25 μ L (P_{x2}), 62.5 μ L (P_{x3}), 125 μ L (P_{x4}), and 250 μ L (P_{x5}).

Visual Observation Results of Kesambi Honey KHM Test Tubes

Figure 2. Tubes containing treatment and control of MIC test of kesambi honey.

From the observation, it was found that honey porch 15.625 μL (Py1), 31.25 μL (Py2), 62.5 μL (Py3), 125 μL (Py4), and 250 μL (Py5) could inhibit the growth of *Streptococcus pyogenes*. Then the KHM value of kesambi honey against *Streptococcus pyogenes* was 15,625 μL (Py1) because the level of kesambi honey is 15,625 μL (Py1) smaller than the level of kesambi honey 31.25 μL (Py2), 62.5 μL (Py3), 125 μL (Py4), and 250 μL (Py5).

Streaking Observation Results of Mango Honey on Blood Agar

Figure 3. Plates contain treatment and control of MBC test of mango honey.

It can be concluded that carrying mango honey levels of 15,625 μL (Px1), 31.25 μL (Px2), 62.5 μL (Px3), 125 μL (Px4), and 250 μL (Px5) had the killing power against *Streptococcus pyogenes*, so the value of KBM mangoes honey against

Streptococcus pyogenes was levels of 15,625 μ L (Px1) because mango honey levels of 15,625 μ L (Px1) was lower than mango honey levels of 31.25 μ L (Px2), 62.5 μ L (Px3), 125 μ L (Px4), and 250 μ L (Px5)

Observation of Streaking Kesambi Honey on Blood Agar

Picture 4. Plates contain treatment and control of MBC test of kesambi honey.

It can be concluded that carrying honey from levels of 15,625 μL (Py1) had no killing power against *Streptococcus pyogenes*. In comparison, kesambi honey content of 31.25 μL (Py2), 62.5 μL (Py3), 125 μL (Py4), and 250 μL (Py5) had the killing power of *Streptococcus pyogenes*, so that the minimum kill rate of the kesambi honey against *Streptococcus pyogenes* was a level of 31.25 μL (Py2) because mango honey content of 31.25 μL (Py2) was lower than honey kesambi content of 62.5 μL (Py3), 125 μL (Py4), as well as 250 μL (Py5) and the lowest kesambi honey content was 15.625 μL (Py1) had no killing power against *Streptococcus pyogenes*.

DISCUSSION

The results obtained in this study were the KHM in mango honey was 15,625 μL , the KHM in kesambi honey was 15,625 μL , the KBM in mango honey was 15,625 μL , and the KBM in kesambi honey was 31.25 μL . It can be said that there were differences in the effectiveness and potential between mango honey and kesambi honey, which is indicated by the differences in the KBM of the two honey. Researchers suspect this difference was caused by various factors, including the content in both kinds of honey.

In general, four factors are supporting the antibacterial ability of honey, namely

high levels of sugar can inhibit the development and growth of bacteria; the acidic nature of honey (pH in the range 3-4) makes bacteria unable to survive; organic antibacterial compounds such as inhibine from the flavonoid group, glycosides, and polyphenols in honey; and honey contains radical compounds that can kill bacteria and other pathogenic microorganisms, namely hydrogen peroxide¹².

Total sugar levels are generally affected by water content and acidity¹³. High sugar content will cause honey to become concentrated or thick, making honey has hygroscopic properties. Hygroscopic properties of honey are determined by fructose because fructose is more soluble than glucose¹⁴. The composition of sugar in honey is influenced by geographical conditions such as climate, environment, soil type, plant vegetation composition, nectar quality, and storage conditions¹⁵. In general, high levels of honey sugar can inhibit the growth and development of bacteria due to osmotic pressure (water movement from low solute concentrations to high solute concentrations). The intense interaction between sugar molecules and water molecules leaves minimal amounts of water molecules for bacteria. When water drops to a minimum level for growth, bacteria can stay alive for a while. However, if the water drops dramatically,

the bacteria will lose its ability to live. Very high glucose and fructose content cause honey to be very hypertonic compared to the environment in the bacterial body; this trait will cause bacterial lysis due to severe dehydration due to the osmosis effect^{16,17}. In this study, sugar testing was not carried out on mango honey and kesambi honey. It could not be used as an evaluation material for differences in the antibacterial ability between mango honey and kesambi honey.

The pH value can determine the degree of acidity of the honey, the higher the acidity of honey the lower the pH of¹⁸. The pH value can be different due to differences in mineral and acid content in honey¹⁹. Several acids can affect the pH of honey, such as amino acids (0.05 to 0.1%) and organic acids (average 0.57%, ranging from 0.17 to 1.175). The dominant amino acid in honey is proline, and the dominant organic acid is gluconic. The enzyme glucose oxidase produces gluconic acid²⁰. High water content in honey can cause the fermentation process to occur more quickly, thereby affecting its acidity¹³. Acidity has a significant influence on the growth and survival of bacteria. Each species has an optimum acidity for growth. When the pH drops to the lowest level for bacterial growth, the bacteria will not only stop growing but also lose the ability to live²¹.

High amounts of acid can denature bacterial cell proteins by disrupting the salt bridge by an ionic charge²². The acidic pH of honey will inhibit bacterial metabolism so that it will easily undergo lysis, which can inhibit bacterial growth²³. Honey with high acidity causes the concentration of hydrogen ion to increase. This can disrupt the membrane proton gradient in bacterial cells^{16,17}. In this study, after checking the pH value, the mango honey had a pH of 3.33, and kesambi honey had a pH of 3.00. Following the theory that *Streptococcus pyogenes* is a bacterium that grows optimally in the pH range of 7.4-7.65, so that with high acidity, mango honey with a pH of 3.33 and kesambi honey with a pH of 3.00 which is lower than the optimum pH *Streptococcus pyogenes* (pH 7.4-7.6) enables mango honey and kesambi honey to inhibit growth and kill *Streptococcus pyogenes*. this pH difference may be one of the distinguishing factors in the inhibitory and killing ability between the two honey. according to this theory, kesambi honey with a lower pH of 3.00 should inhibit growth and kill *Streptococcus pyogenes* bacteria better than mango honey with a higher pH of 3.33. The results of the comparison of the MIC and KBM values showed mango honey can inhibit growth and kill *Streptococcus pyogenes* bacteria better than kesambi honey, therefore this is

thought to be influenced by other antibacterial honey content.

Honey contains organic compounds that are antibacterial such as inhibine from the flavonoid group, glycosides, and polyphenols. Flavonoids are one of the phytochemical contents most often found in honey. This compound is a reducing compound that can inhibit many oxidation reactions through enzymatic and non-enzymatic processes. The function of flavonoids is as a reservoir of hydroxy radicals and superoxide²⁴. The mechanism of action of these organic compounds as antibacterial through poisoning protoplasm, damaging and penetrating cell walls, and depositing microbial cell proteins. Honey is dark in color because it contains more phenolic components than bright honey²⁵. Mango honey and kesambi honey used in this study are dark in color, which means they contain many phenolic components. Abundant molecular phenolic compounds can activate essential enzymes in microbial cells, even at deficient concentrations. The phenol compound can break the peptidoglycan bond when it breaks through the cell wall. After breaking through the bacterial cell wall, phenol compounds will cause leakage of cell contents by damaging the hydrophobic bonds of cell membrane components (such as proteins and phospholipids) and dissolving the hydrophobic binding

components resulting in an increase in membrane permeability that causes the bacterial cell contents to come out. Damage to the cell membrane causes the activity and biosynthesis of specific enzymes needed in bacterial metabolic reactions to be inhibited²⁶. After examining the flavonoid content qualitatively to determine the presence of flavonoids in this study, the results showed that mango honey and kesambi honey contain positive flavonoid compounds, which affect the ability of mango honey and honey kesambi in inhibiting growth and killing bacteria. In this study, a quantitative examination of flavonoid content was not carried out to determine the levels of flavonoids in mango honey and kesambi honey. It could not be used as further consideration to support the difference in MIC and MBC values between the two honey.

Some critical enzymes in honey are the diastase, invertase, glucose oxidase, peroxidase, and lipase²⁷. The glucose oxidase enzyme can increase the antibacterial ability by converting glucose in honey to gluconic acid and hydrogen peroxide. Hydrogen peroxide compounds can kill bacteria. These compounds can reactively damage the functional groups of bacterial cell biomolecules. The mechanism of hydrogen peroxide action is to denature proteins and inhibit the synthesis or function of bacterial nucleic

acids, with damage to the bacterial cell wall and interference with nucleic acid synthesis, causing inhibited bacterial growth²⁸. The content of hydrogen peroxide is cytotoxic to bacterial cells. Antimicrobial hydrogen peroxidase process through the ability to oxidize and the formation of hydroxyl free radicals that are more toxic to facilitate bacterial cell damage²³. The catalase enzyme converts hydrogen peroxide into water and oxygen⁴. The catalase test results give a negative result, meaning that *Streptococcus pyogenes* used for this research do not have the catalase enzyme to hydrolyze hydrogen peroxide, which is one of the factors supporting the ability of honey as an antibacterial. In this study, there was no testing of hydrogen peroxide compounds both qualitatively and quantitatively so that it could not be used as a benchmark further in determining differences in the effectiveness of the antibacterial ability of the two honey.

The alternate hypothesis in this study was supported by the results obtained that mango honey can inhibit *Streptococcus pyogenes* as evidenced by the MIC value of mango honey which is 15,625 μL , kesambi honey can inhibit the growth of *Streptococcus pyogenes* as evidenced by the KHM value of honey kesambi which is 15,625 μL , mango honey can kill *Streptococcus pyogenes* which is proven

through KBM value of mango honey that is 15,625 μL , kesambi honey can kill *S. pyogenes* which is proven through KBM value of kesambi honey which is 31.25 μL , and there are differences in the effectiveness and potency between mango honey and kesambi honey which is seen through KBM value that is KBM Mango honey at a level of 15,625 μL is smaller than KBM honey kesambi at a level of 31,25 μL which indicates that mango honey is more effective and potent because with a smaller level it can kill *Streptococcus pyogenes*.

CONCLUSION

In this study, the following conclusions are obtained:

1. Mango honey has MIC at levels 15,625 μL and KBM at levels 15,625 μL against *Streptococcus pyogenes*.
2. Kesambi honey has a KHM at a level of 15,625 μL and a KBM at a level of 31,25 μL against *Streptococcus pyogenes*.
3. There is a difference in the value of KBM between mango honey and kesambi honey against *Streptococcus pyogenes*, namely mango honey at a level of 15,625 μL and kesambi honey at a level of 31,25 μL .
4. There is no difference in the MIC value between mango honey and kesambi honey.

5. The potential of mango honey as an antibacterial agent against *Streptococcus pyogenes* is higher than that of kesambi honey, which was observed through differences in the values of KBM.

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