

Antibacterial Activity of Kasturi Orange (*Citrus microcarpa* Bunge) to Inhibit *Propionibacterium acnes*

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ABSTRACT

Kasturi orange (*Citrus microcarpa* Bunge) is one of the plants that can be used as medicine, for example to treat coughs, itching or body odor removal. The purpose of this study was to determine whether Kasturi oranges were able to inhibit the growth of *Propionibacterium acnes* bacteria that causes acne. The research method used is experimental. The experiment consisted of 4 treatments and three repetitions. The results showed that the diameters of the inhibition zones obtained by lime juice at concentrations of 25%, 50%, 75%, 100% were 3.86 mm, 7.13 mm, 9 mm, 11.1 mm. Based on the results obtained, it can be concluded that Kasturi orange juice is able to inhibit the growth of the *Propionibacterium acnes* bacteria.

Keywords:

Kasturi Orange (*Citrus microcarpa* Bunge), *Propionibacterium acnes*

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1. INTRODUCTION

Indonesia has a fairly large tropical forest area with biodiversity, both flora and fauna, approximately 30,000 to 40,000 species of plants spread from Aceh to Papua which can be used as medicinal ingredients. [1]. One of the medicinal plants is Kasturi orange. This orange grows to a height of 3-4 meters with relatively very small fruit compared to other types of oranges, has a distinctive smell and is often used as a cooking spice. In Indonesia this type is commonly known as lime and is generally used as a flavoring dish and used as a cough medicine, itching or body odor remover[2]

A number of studies have been conducted on this kasturi citrus plant. Tested the activity of essential oil from kaffir lime fruit peels and leaves against several types of pathogenic bacteria such as: *Escherichia coli*, *Salmonella enteridis*, *Staphylococcus epidermis* and *Staphylococcus aureus*, where at a concentration of 25% the diameter of the inhibition zone (DDH) was produced which is equivalent to the DDH produced by 50 ppm chloramphenicol. Sebiomo, *et al* (2011) showed that chloramphenicol was able to inhibit *Staphylococcus aureus* with an inhibition zone diameter of 30 mm at a concentration of 10 g/disk for 18 hours [2]

Propionibacterium acnes a normal flora of the polysebaceous glands of human skin, these bacteria cause acne by producing lipases that break down free fatty acids from skin lipids. These fatty acids can cause tissue inflammation when it comes to the immune system and promote acne. This bacterium belongs to the type of gram-positive anaerobic bacteria that is tolerant to air. Based on the content and efficacy of kaffir lime fruit, the researchers were interested in testing the antibacterial activity of musk orange juice against *Propionibacterium acnes* bacteria.



2. RESEARCH METHODOLOGY

The research was conducted in August-November at the Microbiology Laboratory, Faculty of Pharmacy and Health Sciences, Sari Mutiara Indonesia University Medan Jl. Captain Muslim No.79 Medan 20123. The samples used were musk oranges obtained from the Jalan Metal market, Tanjung Mulia, Medan Deli District.

Research Tools and Materials

The tools used in this study include petri dishes, Erlenmeyer (pyrex), test tubes (pyrex), beakers (pyrex), measuring cups, funnels (pyrex), autoclaves, incubators, hot plates, analytical balances, refrigerators, binocular microscope, Bunsen, vortex mixer, object glass, cover glass, watch glass, loop needle, test tube rack, tweezers, glass stirrer, marker pen, mask, filter paper, caliper, and gloves.

The materials used in this study included kaffir lime fruit (*Citrus microcarpa* Bunge), pure culture of *Propionibacterium acnes*, Nutrient Agar (NA) media, Muller Hinton Agar (MHA) media, sterile distilled water, 96% alcohol, Crystal violet, iodine solution, safranin solution, immersion oil, paper disc, H₂SO₄ 1% and BaCl₂ 1%.

The tools used in this study were sterilized first. Glassware is sterilized in an oven at 170°C for 1 hour (dry sterilization), the media was sterilized in an autoclave at 121°C for 15 minutes (wet sterilization) [3]

Preparation and Sterilization of Nutrient Agar (NA) Media

2 grams of NA is dissolved in 100 ml of distilled water, then stirred accompanied by heating at a temperature of 70°C. This media was sterilized by autoclaving at 121°C for 15 minutes. Furthermore, as much as 3 ml of this medium, put into a test tube, placed at an angle of 30-45° and allowed to solidify, then stored in the refrigerator [4], [5].

Production and Sterilization of Muller Hinton Agar (MHA) Media

38 grams of MHA was dissolved in 1 liter of distilled water, then heated until completely dissolved. The agar solution was sterilized in an autoclave at 121°C for 15 minutes. When the agar temperature is 40-45°C, then poured into a petri dish as much as 20 ml [6].

Preparation of *Propionibacterium acnes* Bacteria

Pure cultures were obtained at the Microbiology Laboratory, FMIPA, University of North Sumatra. Pure cultures of *Propionibacterium acnes* were re-cultured to increase the population of these bacteria. One colony of *Propionibacterium acnes* was taken using a sterile wire loop, then planted on Nutrien Agar media tilted by scratching, after that it was incubated in an incubator at 37°C for 24 hours.[5].

Gram stain

Preparations made from the bacteria provided. Given crystal violet dye and left for 1 minute, rinse with distilled water, dry. Give 1-2 drops of iodine for 30 seconds. Rinsed with alcohol for 15 seconds, then rinsed with distilled water. Given 1 drop of safranin solution (matching dye) for 1 minute, rinsed with distilled water and dried. Observed under a microscope [7].

Making Kasturi Orange

Kasturi oranges are washed with running water until clean. 96% alcohol was sprayed all over the musk orange peel and then rinsed using sterile distilled water and drained. The musk orange is cut into 2 parts, then each musk orange is squeezed manually using gloved hands. Kasturi orange is accommodated in a beaker glass and transferred to an Erlenmeyer chili, filtered using a filter cloth 1 time and filter paper 2 times. The filter cloth and filter paper have previously been sterilized using an autoclave.

Antibacterial Activity Test

The antibacterial activity test was carried out using the paper disc diffusion method. Sterile paper discs with a diameter of 6 mm were immersed in different concentrations of lemon juice and musk orange juice for 2 hours. The bacterial suspension was taken as much as 0.1 ml and then poured into Muller Hinton Agar (MHA) media and leveled using a drigalsky rod. The paper disc was taken aseptically with tweezers by rubbing it against the wall of the test tube and then allowed to stand for a while. This is done so that the remaining solution does not drip and spread on the agar surface. The drained paper disc was taken using tweezers and placed on the Muller Hinton Agar (MHA) medium containing the test bacteria by gently pressing it with tweezers so that

it really sticks to the surface of the media that has been planted with the test bacteria. The antibacterial test was incubated for 24 hours at 37°C [8] and then the diameter of the inhibition zone was measured using a caliper.

3. RESEARCH RESULT

The identification of musk orange plants was carried out at the Herbarium Medanense (MEDA) University of North Sumatra which showed the musk citrus species. Bacterial morphology was carried out by Gram staining technique. Gram staining was carried out through several stages of staining, namely with crystal violet, iodine, 96% alcohol, and safranin. Observations were made under a microscope with an objective lens with a magnification of 40 times. In accordance with [9] the result is that *Propionibacterium acnes* bacteria will look purple in color and have a rod-shaped appearance.

Antibacterial activity was indicated by the formation of a clear zone around the paper disc. The clear zone formed is called the inhibition zone. The inhibition zone formed after being incubated for 24 hours at 37°C was measured every day using a caliper for 3 days and the results can be seen in Table 4 below:

Table 1 Results of Inhibition Zone of Kasturi Orange (*Citrus microcarpa* Bunge) against *Propionibacterium acnes* bacteria for 3x24 hours.

Time observation	Concentration	Inhibition zone diameter (mm)			Average
		Repetition			
		I	II	III	
1x24 hours	25%	2.5	3	3.1	2.86
	50%	4.85	4.1	5.25	4.73
	75%	7.4	6.65	7.8	7.28
	100%	9.75	10.35	9.7	9.93
2x24 hours	25%	2.5	3.05	3.1	2.88
	50%	5.5	4.4	6	5.3
	75%	8.7	8.65	7.8	8.38
	100%	10	10.4	10.5	10.3
3x24 hours	25%	3.05	4.15	4.4	3.86
	50%	6.1	8	7.3	7.13
	75%	9.45	9.5	8.05	9
	100%	10.35	11.4	11.55	11.1

Based on Table 1, it can be seen that the average inhibition zone formed in each treatment of Kasturi orange juice concentration for 3 consecutive days. At 1x24 hours a concentration of 25% of the diameter of the inhibition zone formed is 2.86 mm, the concentration of 50% of the diameter of the inhibition zone formed is 4.73 mm, a concentration of 75% of the diameter of the inhibition zone formed is 7.28 mm, and a concentration of 100% of the diameter of the inhibition zone formed is 9.93 mm. At 2x24 hours a concentration of 25% of the diameter of the inhibition zone formed was 2.88 mm, a concentration of

50% of the diameter of the inhibition zone formed was 5.3 mm, a concentration of 75% of the diameter of the inhibition zone formed was 8.38 mm, and a concentration of 100% of the diameter of the inhibition zone formed was 10.3 mm. At 3x24 hours a concentration of 25% of the diameter of the inhibition zone formed was 3.86 mm,

Based on the results of the study, it was known that musk orange juice at concentrations of 25%, 50%, 75%, and 100% had antibacterial activity against *Propionibacterium acnes* bacteria.

The results of the test of inhibition formed by musk juice on the growth of *P. acnes* at 1x24 hours were included in the medium category at 100% concentration treatment, the inhibitory power at 2x24 hours, and 3x24 hours was included in the strong category at 100% concentration treatment.

The clear zone formed around the paper disc indicated that the juice of musk orange (*Citrus microcarpa* Bunge) had antibacterial properties against the growth of *Propionibacterium acnes*. The clear zone is an area that is not overgrown with bacteria and looks clearer than the surrounding area. The clear zone is known as the inhibition zone. The ability of Kasturi orange juice to inhibit the growth of *Propionibacterium acnes* bacteria is due to the presence of antibacterial active compounds in Kasturi orange juice. The active compound diffuses throughout the surface of the agar medium, causing the active compound to spread out. The active compound was able to inhibit the growth of *Propionibacterium acnes* bacteria so as to form an inhibition zone around the paper disc. The larger the inhibition zone formed, the stronger the potential of a solution as an antibacterial. Citrus fruits basically contain flavonoid and limonoid compounds which are believed to be useful for fighting various diseases. The main flavonoid compound contained in citrus fruits is naringin and for limonoids is limonene. From a number of studies conducted by experts, it shows that the content of limonin compounds in citrus fruits is efficacious as an antimalarial, antimicrobial substance, and is able to lower cholesterol levels in the blood [10].

Kasturi orange juice has active compounds that have the potential as antibacterial substances. The main content of lime juice is citric acid. Citric acid is very soluble in water, easily soluble in ethanol, and poorly soluble in ether. Citric acid is the most abundant organic acid in musk orange juice. The content of vitamin C and citric acid makes the acidity (pH) of musk orange juice sour. Acidic pH can cause the internal pH of bacterial cells to decrease so that it can interfere with the activity of bacterial cells and inhibit bacterial growth (Molina, et al, 2009). Based on the results of this study, it was proven that the juice of musk lime with concentrations of 25%, 50%, 75%, and 100% had the potential as an antibacterial that was able to inhibit the growth of *Propionibacterium acnes* bacteria.

4. CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that Kasturi orange juice (*Citrus microcarpa* Bunge) with concentrations of 25%, 50%, 75%, and 100% has antibacterial activity that is able to inhibit the growth of *Propionibacterium acnes* bacteria characterized by the formation of a clear zone around paper disc.

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