

The Inhibitory Effect of Kaffir Lime Extract towards Staphylococcus Aureus Bacteria

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Abstract

Staphylococcus aureus is an anaerobic gram-positive bacterium that becomes one of the causes of infections in the oral cavity. The infections that occur in the oral cavity can be prevented by using a mouthwash. Kaffir lime (*Citrus hystrix*) contains flavonoid, saponin, tannin and essential oils that have the inhibitory effect on the bacteria by destroying and inhibiting the formation of the bacterial cell wall. This research aims to identify the effect of kaffir lime extracts at various concentration on the growth of Staphylococcus aureus. This in vitro study was conducted in an experimental laboratory. The findings of this study showed that kaffir lime extract at concentration of 12.5%; 25%; 50% and 100% can inhibit the growth of Staphylococcus aureus. It was concluded that the kaffir lime extract has an antibacterial activity.

Experimental article (J Int Dent Med Res 2020; 13(2): 539-542)

Keywords: Kaffir Lime, Staphylococcus aureus, Inhibitory effect.

Received date: 16 March 2020

Accept date: 28 April 2020

Introduction

Cases of allergy to antiseptic agents were found in several hospitals, including those reported by National Taiwan University Hospital. Su (1999) found out that 34% of patients were allergic to iodine that was used as an antiseptic in the hospital.^{1,2} In addition, as many as 75% of patients also complained about antiseptic made from iodine because it causes less pleasant aroma and causes brown marks in the oral cavity.¹

Staphylococcus aureus is one of the most common bacterial species of the Staphylococcus that causes infection in humans.^{2,3,4,5} The infections that often appear in the oral cavity include abscesses, angular cheilitis, parotitis, staphylococcal mucositis, denture stomatitis, staphylococcal osteomyelitis of the jawbone, epulis, stomatitis and dentoalveolar abscess.⁶ These infections may occur because Staphylococcus aureus produces toxins or direct invasion damaging body tissues.⁶ Infections that occur in the oral cavity can be prevented by

using mouthwash as an antiseptic. One of the common mouthwashes used is povidone iodine 1%.²

Povidone is capable of killing various microorganisms such as bacteria, fungi, viruses, protozoa, and spores, but povidone iodine can cause irritation toxic reaction from iodine, nasty smell and skin discoloration due to the dye.⁷ These have led to the creation of alternative antiseptic as substitutes of iodine.^{1,7}

Kaffir lime is one of the herbal ingredients that contain flavonoids, saponins, tannins and essential oils that are considered to be able to inhibit bacterial growth. In addition, the fruit also serves as antiseptic as the pharmacological effect so it is expected to be a basic ingredient for making antiseptics that are safe and do not cause side effects.⁸

Based on the content and benefits of kaffir lime, the researchers are interested to examine the inhibitory power of kaffir lime extract toward Staphylococcus aureus bacteria.

Materials and methods

This in vitro study was conducted at Molecular Medicine and Therapy Research Laboratory, Microbiology Laboratory Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, Indonesia. Staphylococcus aureus bacteria was employed as the subject of the study. The variables of this

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research were kaffir lime extract and growth of *Staphylococcus aureus*. Kaffir lime was extracted by maceration using 96% ethanol solvent. The growth of *Staphylococcus aureus* was measured by bacterial growth test after 24 hours treatment and incubation. The growth of *Staphylococcus aureus* was observed from clear zones that were not formed. The clear zone formed showed the growth of *Staphylococcus aureus* bacteria inhibited by the test material. The clear zone formed was measured by using a sliding range and was expressed in millimeters. Diffusion method used 8 petri dishes, and then placed 4 paper discs with a diameter of 6 mm on each petri dish. There were 6 petri dishes as treatment group used to test the kaffir lime extract which was spilled with a test solution of kaffir lime extract by 0.78%; 1.57%; 3.125%; 6.25%; 12.5%; 25%; 50%; 100% concentrations, whereas the next 2 Petri dishes were then given 1% povidone iodine solution and sterile aquadest as positive and negative control groups, then incubated at 37°C for 24 hours to see the growth of *Staphylococcus aureus* bacteria.⁴

The study has received ethical clearance by The Medical Research Ethics Committee of Health Universitas Muhammadiyah Yogyakarta. The data was analysed using One Way ANOVA to test whether there is an effect of kaffir lime extract in inhibiting the growth of *Staphylococcus aureus* at a significant level of $p < 0.05$. If the p -value was close to 0.01, the presence of a tendency was noted.

Results

No	Concentration	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean (mm)
1	Extract of 0.78%	0	0	0	0
2	Extract of 1.57%	0	0	0	0
3	Extract of 3.125%	0	0	0	0
4	Extract of 6.25%	0	0	0	0
5	Extract of 12.5%	1.7	2.1	1.3	1.7
6	Extract of 25%	2.4	2.4	1.4	2
7	Extract of 50%	2.2	4.5	1.1	2.6
8	Extract of 100%	10.8	11.6	8.9	10.4
9	Positive control	2.9	4.2	3.3	3.5
10	Negative control	0	0	0	0

Table 1. Zone Inhibition Measurement of *Staphylococcus aureus* Growth.

The inhibition zone was formed around the disc given the kaffir lime extract with a

concentration of 12.5%; 25%; 50%; 100%; and the positive control was povidone iodine 1% (Table 1). The average diameter of the inhibitory zone was measured by the kaffir lime extract with 100% concentration.

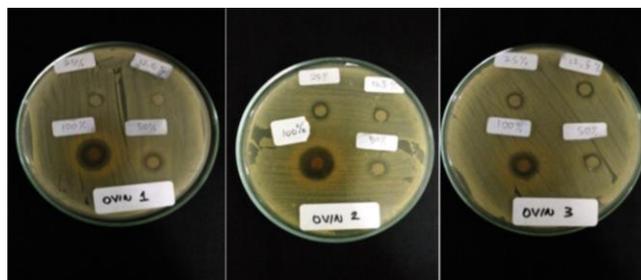


Figure 1. Inhibition zone of *Staphylococcus aureus* growth by kaffir lime extract with the concentration of 12.5%; 25%; 50% and 100%.

Measurement	Df	F	Sig.
Between Groups	4	33.632	.000*
Within Groups	10		
Total	14		

Table 2. The statistical test result on the effect of kaffir lime extract in inhibiting the growth of *Staphylococcus aureus*.

Description: (*) There is significant influence of kaffir lime extract in inhibiting the growth of *Staphylococcus aureus*.

There was a significant difference in average of inhibition zone on the five groups ($p < 0.05$). The finding proves that there was an effect of kaffir lime extract in inhibiting the growth of *Staphylococcus aureus*.

Discussion

The results showed that the extract of kaffir lime has antibacterial activity on *Staphylococcus aureus* bacteria. Aquadest as a negative control did not show any inhibition zone around the disc, whereas povidone iodine 1% as a positive control indicated the presence of antibacterial activity. Kaffir lime extract with the concentration of 12.5% was the minimum concentration of kaffir lime extract which is able to inhibit the growth of *Staphylococcus aureus* bacteria. Kaffir extract with the concentration of 100% was the most effective concentration to inhibit the growth of *Staphylococcus aureus* bacteria because it had the largest inhibitory

zone of all. The inhibitory zone diameter was measured by using the sliding caliper.

Antibacterial agent is the ability of a substance to kill or inhibit bacterial growth. The bacterial susceptibility test of a substance can be done *in vitro* to determine whether the substance has antibacterial activity. Jawetz et al., (2013) reported that the mechanism of action of most antimicrobial agents is done by inhibiting cell wall synthesis and cell membrane function, which can lead to cell lysis.⁹

The antibacterial activity of kaffir lime extract is consistent with those of Yuliani (2011) where the phytochemical content is mostly found in kaffir lime and the active ingredients such as flavonoids, saponins, tannins and essential oils are reported to have a good antibacterial activity. Flavonoids contained in kaffir lime extract can form complex compounds with extracellular proteins and dissolve with cell walls and have lipophilic properties.⁸ These activities cause the damaged cytoplasmic membranes so that important metabolites in the cell, amino acids and nucleotides leak out the cell wall causing death in these cells.¹⁰ Saponins in kaffir lime can increase the permeability or leakage of cells that result in the release of intracellular compounds. This is due to the decrease of surface tension on the cell wall.¹¹ Manali Cavalieri et al. (2005) stated that these compounds diffuse through the outer membrane and there is a bond between the cytoplasmic membrane and the cell wall that can disrupt the stability and have bactericidal properties.¹² Tannin is a chemical compound in water-soluble plants with molecular weight ranging from 500 to 3000 g/mol.¹² Tannin is able to decrease cell volume because these compounds affect the permeability changes of cell membranes. The cell will be perforated and shrink then lose metabolic functions that causes the cell to collapse.¹³ Essential oils contained in kaffir lime have a coumarin compound. Coumarin is a secondary metabolic compound of essential oils formed from nonvolatile glucose derivatives during aging or wounding. The mechanism of coumarin action is by destroying cells by forming the cell wall pores and changing the structure and function of the plasma membrane which causes an increase in transmembrane and leakage of amino acids and other cytoplasmic contents then the cells shrink and disintegrate.¹⁴ Yuliani (2011) reported that the essential oil content of kaffir lime extracted by distillation

techniques could inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria.⁸

Staphylococcus aureus is a gram-positive bacterium that can infect humans. The infections caused by these bacteria cause several diseases with distinctive signs such as necrosis, inflammation and abscess formation.^{4,5} Gram-positive bacteria have layer of cells consisting of two to three layers of cytoplasmic membrane, peptidoglycan layer and some other bacteria have an outer layer of the capsule. The first layer is a cytoplasm membrane composed of phospholipids and proteins while the second layer is a cell wall composed of peptidoglycan and teichoic acid.^{9,15}

The mechanism of cell wall action and the cytoplasmic membrane of *Staphylococcus aureus* bacteria can be disrupted by flavonoid compounds, essential oils, saponins and tannins contained in kaffir lime, resulting in inhibition of bacterial growth leading to cell death. An antibacterial agent lead to changes in the metabolism of cells that lead to damage so that cell growth can be disrupted and cause the death of these cells.^{10,15,16} the action mechanism of flavonoids, saponins, tannins and essential oils found in kaffir lime extract has a role as an antibacterial agent to *Staphylococcus aureus* bacteria.¹⁷ The active substances of kaffir lime can inhibit cell wall synthesis and damage cell membrane function.¹⁷ Based on the result of the study, kaffir lime extract at the concentration of 12.5% is the minimum concentration of kaffir lime extract that can inhibit the growth of *Staphylococcus aureus* and the concentration of 100% is the most effective concentration to inhibit bacterial growth of *Staphylococcus aureus*.

Conclusions

Kaffir lime extract at concentration of 12.5%; 25%; 50% and 100% is able to inhibit the growth of *Staphylococcus aureus* bacteria. The concentration of 100% is the most effective concentration in inhibiting the growth of *Staphylococcus aureus* bacteria because it has the largest inhibition zone (10.4 mm) compared to other concentrations.

Declaration of Interest

The authors report no conflict of interest.

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