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Antimicrobial potential of Kaffir Lime (*Citrus hystrix D.C*) peel extract against *Staphylococcus aureus*

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ABSTRACT

Staphylococcus aureus is a normal bacterial flora that can cause skin infections such as boils, acne, impetigo and is also a major cause of nosocomial infections. This study examined the effect of kaffir lime (*Citrus hystrix D.C*) peel extract on *Staphylococcus aureus* . This research was a true experimental study with a post test only control group design. Kaffir lime peel was extracted using maceration method with 70% ethanol solvent, then diluted using 2ml ethanol to a concentration of 25mg/ml, 20mg/ml, 15mg/ml, 10mg/ml and 5mg/ml. *MinimumInhibitory Concentration* (MIC) and *MinimumBactericidal Concentration* (MBC) were conducted to assess the kaffir lime peel extract against *Staphylococcus aureus* bacteria. The MIC test was measured using a spectrophotometer in the liquid dilution method and MBC test was carried out using solid dilution on Mueller Hinton media. *MinimumInhibitory Concentration*of kaffir lime peel extract against *Staphylococcus aureus* bacteria at a concentration of 20mg/ mland MBC has not been determined at maximum concentration of 25mg/ ml.

Keywords :*Citrus hystrix*, kaffir lime, *Staphylococcus aureus* , MIC, MBC.

INTRODUCTION

Infectious disease is a process of damage due to microbial interactions in host body that causes various clinical symptoms and signs (Fenty, 2013). Microorganisms that cause disease in humans are called pathogenic microorganisms. One of the bacteria that often causes infection is *Staphylococcus aureus* (Fatimah, 2016). *Staphylococcus aureus* is a normal microflora that was commonly found on healthy human skin and nose (Widhianto, 2017), is coagulase positive and pathogenic (Toelle, 2014). Skin infections that are usually caused by *Staphylococcus aureus* include impetigo, cellulitis, folliculitis, and abscesses (Razak, 2013). Almost everyone has experienced *Staphylococcus aureus* infection in their life (Toelle, 2014). Treatment of *Staphylococcus aureus* infection using Penicillin G class antibiotics (Razak, 2013).

Excessive/irrational use of antibiotics can cause changes in the ecology of bacteria and lead to resistance. The existence of resistance to antibiotics encourages researchers to look for alternative treatments as antibiotics, one of which can use plants as traditional medicine (Setiawati, 2015). The use of plants as traditional medicine has been widely used as alternative medicine by the community, one of which is the kaffir lime plant. One of the citrus species that is also often used is kaffir lime (*Citrus hystrix D.C*) (Tanjaya, 2019). Some parts of the kaffir lime plant have several functions, one of which is as an antimicrobial against several pathogenic bacteria. The skin of the fruit contains flavonoids, tannins, terpenoids, and saponins which have antibacterial properties (Widyastuti, 2017).

Several previous studies have tested the use of kaffir lime leaf extract (Astriani, 2021; Maimunah, 2020; Saifuddin, 2018), kaffir lime leaf essential oil (Saptarini, 2021; Yuliani, 2011), kaffir lime juice (Putra, 2017), and extracts of kaffir lime leaves. ethoanol of kaffir lime fruit (Dandy, 2021) against *Staphylococcus aureus* . Other studies have also used kaffir lime leaf essential oil against *Staphylococcus epidermidis* (Rosmalawati, 2022) and kaffir lime leaf extract against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* (Dhavesia 2017).Other studies have also tested kaffir lime leaf extract as an antifungal on *Candida albicans* (Sophia, 2021).

Several previous studies have discussed kaffir lime peel as a natural insecticide against *Culex sp*. larvae (Novera, 2017), *Aedes sp* (Daswi, 2019), mosquito larvicide (Rachmawati, 2019), and as a mosquito repellent *Aedes sp*. (Santya, 2013). In addition to testing as a larvicide, kaffir lime peel extract has been tested on *Staphylococcus aureus* using the disk diffusion agar method (Hantantris, 2015)

The potential for antibiotic resistance due to irrational use, efforts to develop alternative antibiotics using herbal ingredients, and the potential content of kaffir lime as an anti-microbial, as well as the limited studies that reveal the antimicrobial potential of kaffir lime peel, the authors wanted to know the effectiveness of kaffir lime peel extract on *Staphylococcus aureus* bacteria were measured based on MIC and MCB

METHODS

The research design used in this study was a laboratory experimental design with a post test only control group design. The manufacture of kaffir lime peel extract was conducted at FMIPA Laboratory,STIFARSemarang and extract testing was conducted at Biomedical Laboratory,Medical Faculty Universitas Muhammadiyah Semarang. Sample calculation used the frederer formula.This study used 5 groups (25mg/ml, 20mg/ml, 15mg/ml, 10mg/ml, and 5mg/ml) with 5 repetitions added 2 tubes for the extract sterility control and bacterial control. Subject of this study was a pure culture of *Staphylococcus aureus* bacteria with initial inoculum was a suspension of *Staphylococcus aureus* bacteria according to 0.5 Mc Farland standard (1x108 CF/mL).

Kaffir lime raw materials are collected, washed under running water, and drained. Then only the skin is taken and dried in the sun to dry, sorting done. The resulting orange peel powder is ready to be continued for the extraction process. Making kaffir lime peel extract using the maceration method. Orange peel powder was soaked in 70% ethanol for 24 hours, then filtered to obtain the filtrate. Filtrate is evaporated using a Rotary Evaporator to produce a thick extract. The thick extract was then diluted using aquadest and 2ml ethanol to a concentration of 25mg/ml, 20mg/ml, 15mg/ml, 10mg/ml and 5mg/ml.

Minimum Inhibitory Concentration testing used the dilution method, measurement was assessed by looking at the results of MIC was determined by absorbance assay measured by spectrophotometer at pre and post incubation at 625nm wavelengthand MBCtesting used the streak plate method, measurement was assessed by looking at lowest concentration that did not show the growth of bacterial colonies on solid media (*Mueller Hinton Agar*/ MHA) after being incubated for 16-20 hours at 37°C. This research was conducted based ethical clearance from the Medical Research Ethics Commission of Medical faculty, Universitas Muhammadiyah Semarang published number: 040/EC/FK/2020.

RESULTS AND DISCUSSION

The extract of kafir lime peel powder was taken as much as 0.5 kg mixed with 90% ethanol as much as 5L to produce a thick extract of 32 grams. After qualitative testing, it was found that the kaffir lime peel extract contained flavonoids, phenolics, tannins, saponins, alkaloids, and terpenoids. Based on the data obtained from 5 groups of research sample repetitionswas carried out the results were (table 1):

Table 1. Absorbance value pre and post incubation of Kaffir Lime Peel extract *(Citrus hystrix D.C)* against *Staphylococcus aureus* bacteria

Group	*Pre*- Incubation					Mean	*Post*-Incubation					Mean	Description
	1	2	3	4	5		1	2	3	4	5		
25mg/ml	2,65	2,67	2,64	2,65	2,67	2,66	2,36	2,40	2,36	2,43	2,64	2,44	Decrease
20mg/ml	2,01	2,13	2,23	2,03	2,15	2,11	1,96	2,03	1,90	2,01	2,03	1,98	Decrease
15mg/ml	1,72	1,88	1,70	1,83	1,88	1,80	1,87	1,70	1,87	1,84	1,75	1,81	Increase
10mg/ml	1,17	1,09	1,06	1,05	1,42	1,16	1,45	1,14	1,51	1,45	1,16	1,34	Increase
5mg/ml	0,54	0,55	0,96	0,90	0,93	0,78	1,02	1,05	1,06	1,01	1,07	1,04	Increase
Extract control	2,88					2,88	1,80					1,80	Decrease
Bacteria control	0,11					0,11	0,39					0,39	Increase

Note: "Increase" indicates the absorbance value of pre-incubation > post-incubation which means that there is bacterial growth. "Decrease" indicates the absorbance value of pre-incubation < post-incubation which means that bacterial growth is inhibited.

After obtaining all the absorbance values of the MIC test of kaffir lime peel extract against *Staphylococcus aureus*, then the average value of the absorbance of the MIC was calculated. This average value is then subtracted between the average pre-incubation and post-incubation absorbance values, the difference is used to determine the MIC. Based on table 1, MIC of kaffir lime peel extract against *Staphylococcus aureus* bacteria at a concentration of 20mg/ml.

Table 2. The results of the *Minimum Bactericidal Concentration* (MBC) Test of Kaffir Lime Peel Extract (*Citrus hystrix D.C*) against *Staphylococcus aureus* bacteria.

Concentration	Repetitions	Colony (n x10^8)	Log*
5 mg/ml	1	288	10,46
	2	201	10,30
	3	54	9,73
	4	87	9,94
	5	151	10,18
10 mg/ml	1	173	10,24
	2	94	9,97
	3	76	9,88
	4	49	9,69
	5	39	9,59
15 mg/ml	1	115	10,06
	2	135	10,13
	3	40	9,60
	4	85	9,93
	5	282	10,45
20 mg/ml	1	90	9,95
	2	110	10,04
	3	140	10,15
	4	272	10,43
	5	88	9,94
25 mg/ml	1	244	10,39
	2	71	9,85
	3	-	-
	4	-	-
	5	-	-

**Log: simplification of the number of colonies in the form of an algorithm

Minimum Bactericidal Concentration was obtained if no growing colonies were found. The concentration of extract with colony growth >300 was diluted with distilled water 8 times. The results of the colony growth of *Staphylococcus aureus* at each concentration of the extract are shown in table 2. From5 repetitions were carried out, the results of bacterial growth colonies were obtained MBC in this study could not be determined because at a concentration of 25% there was still colony growth in repetitions 1 and 2.

Table 3. Absorbance Value of *Staphylococcus aureus* bacteria agains Kaffir Lime Peel extract *Bonferroni* Method

Concentration	5mg/ml	10mg/ml	15mg/ml	20mg/ml	25mg/ml
5mg/ml	-	1.000	0.386	0.034*	0.006*
10mg/ml	1.000	-	1.000	0.160	0.028*
15mg/ml	0.386	1.000	-	1.000	0.749
20mg/ml	0.034*	0.160	1.000	-	1.000
25mg/ml	0.006*	0.028*	0.749	1.000	-

* significant difference between extract concentrations

Based on the table 3, the *Bonferroni* method of *Post Hoc* test on the results of the MIC test data includes a significant difference if p-value < 0.05. The groups that differed significantly included concentrations of 5mg/ml with 20mg/ml (p=0.034), 5mg/ml with 25mg/ml (p=0.006), and between 10 mg/ml and 25mg/ml (0.028); the other groups were not significantly different (p-value > 0.05).

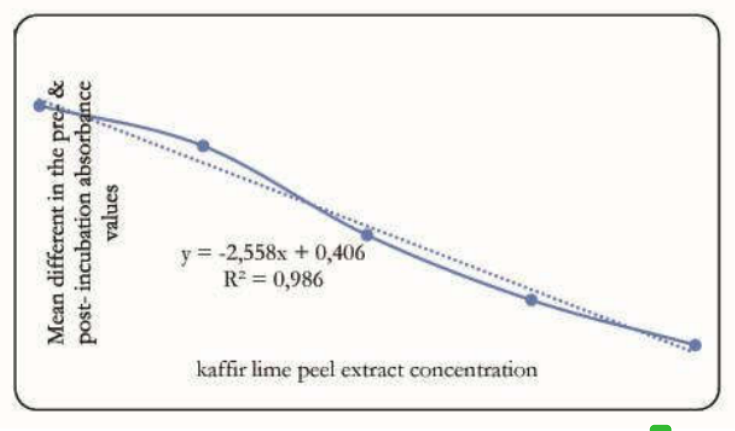

Figure 1. Liner regression graph of the relationship between the concentration of kaffir lime (*Citrus hystrix D.C)* peel extract and the mean *Staphylococcus aureus*

In Figure 1. Graph of the difference in the absorbance values of pre- and post-incubation MIC tests on concentration of kaffir lime peel extract. This graph shows a decrease in absorbance difference pre- and post-incubation along with the increase in concentration of kaffir lime peel extract. The results of data correlation test obtained a correlation coefficient value of 0.993, which

means that there is a very strong relationship between difference in absorbance value of pre- and post-incubation and concentration of kaffir lime peel extract.

DISCUSSION

Minimum Inhibitory Concentration of kaffir lime peel extract against the growth of *Staphylococcus aureus* was observed from the difference in pre- and post-incubation absorbance. From the results of data analysis, it was found that MIC of kaffir lime peel extract against the growth of *Staphylococcus aureus*bacteria was shown at a concentration of 20mg/ml and MBC has not been determined at maximum concentration of 25mg/ ml.

Results of previous studies stated that kaffir lime peel extract inhibited the growth of *Staphylococcus aureus*in vitro at a concentration of 25% (Hantantris, 2015). There is a difference in concentration. In this study, *Staphylococcus aureus* was inhibited at a concentration of 20%, while in the Hantantris study at a concentration of 25%. This may be due to differences in methods used. This study used the dilution method to determine MIC whereas previous studies used the disk diffusion agar method.

Several previous studies have tested antibacterial activity of *Staphylococcus aureus* on kaffir lime leaves. Study stated that MIC of kaffir lime leaf extract was obtained at a concentration of 5% (Astriani, 2021), 20% (Maimunah, 2020), 10% which was tested on milkfish (Saifuddin, 2018). Previous studies have also discussed that kaffir lime juice has an inhibitory effect on the growth of *Staphylococcus aureus* bacteria found in the oral cavity (Putra, 2017).

From the results of the phytochemical test of kaffir lime peel extract, it was found that the content of alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids. The results of phytochemical test support the results that kaffir lime peel extract can inhibit the growth of *Staphylococcus aureus*.Like kaffir lime peel, kaffir lime leaves contain polyphenolic compounds, quinones, monoterpenoids, sesquiterpenoids (Arfania M, 2017), essential oils, saponins, terpenoids (Lestari, 2016), and flavonoids (Arfania, 2017; Lestari, 2016; Astriani, 2021), alkaloids (Arfania, 2017; Astriani, 2021), tannins (Astriani, 2021).

Flavonoids are secondary metabolites of polyphenols. Flavones and flavonols are the most abundant flavonoids. Flavonoids are chemical compounds with strong anti-viral/bacterial activity. The flavonoid methoxylation reaction has an effect on increasing the fluidity of the membrane so that it is correlated with a decrease in the pathogenesis of several viruses/bacteria. Apart from being antiviral/bacterial, flavonoids also have an anti-inflammatory role through molecular planarity pathways, hydroxylation patterns, methoxylation reactions, and glycoside pathways with lipophilicity (Wang, 2017). Flavonoids are the most abundant secondary metabolites contained in citrus species (Vikram, 2010).The antibacterial activity of polyphenols is seen through the

mechanism of damage/modification of bacterial membrane structure (Adnan, 2017), through inhibition of energy metabolism (Li, 2017), toxin production or secretion (Tang, 2019), as well as by preventing the formation of biofilms (Lin, 2011; Trentin, 2013).

Taninns identified (condensed and hydrolyzable) are able to inhibit biofilm formation via bacteriostatic properties, damaging the bacterial membrane and hindering matrix production (Trentin, 2013). While mechanism of action of saponins as antibacterial is to decrease the surface tension of bacterial cells so that cell leakage occurs. Saponins will diffuse through the outer membrane and vulnerable cell walls, then bind to the cytoplasmic membrane and disrupt and reduce the stability of bacterial cells. This causes bacterial death (Bintoro, 2017).

The mechanism of terpenoids as antibacterial is through reactions with porins (transmembrane proteins). The outer membrane of the bacterial cell wall forms a strong polymeric bond, resulting in the destruction of the porin. Damage to the porin reduces the permeability of the bacterial cell wall which results in the bacterial cell being deprived of nutrients and inhibiting bacterial growth (Agustina, 2017).

CONCLUSION

Based on the results and discussion, MIC of kaffir lime peel extract against *Staphylococcus aureus* bacteria at a concentration of 20mg/ml. The value ofMBC in this study could not be determined because at a concentration of 25% there was still colony growth in repetitions 1 and 2. It can be concluded that there is an effect of giving kaffir lime peel extract (*Citrus hystrix D.C*) on the growth of *Staphylococcus aureus* bacteria.It is necessary to carry out further studies to use of kaffir lime peel as an anti-microbial *Staphylococcus aureus* with higher dosage variations and test the antibacterial activity of kaffir lime peel using different methods.

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