Antibacterial Activity of Kecombrang Flower (Etlingera elatior (Jack) R.M. Sm) Extract against Staphylococcus epidermidis and Propionibacterium acnes

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Antibacterial Activity of Kecombrang Flower Extract (*Etlingera elatior* (Jack) R.M. Sm) Against *Staphylococcus epidermidis* and *Propionibacterium acnes*

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Abstract

Staphylococcus epidermidis and *Propionibacterium acnes* are commensal bacteria which can be opportunistic causes acne vulgaris. Acne treatment in skin clinics usually uses antibiotics, but it causes problems such as antibiotic resistance. To overcome this problem, the discovery of new antibacterial agents from natural resources such as plants are needed. Kecombrang (*Etlingera elatior* (Jack) R.M.Sm) is one of medicinal plant used empirically to treat various diseases in Indonesia. Kecombrang flower contains secondary metabolites such as flavonoids, tannins, saponins, and terpenoid known as antibacterial. The purpose of this study was to determine the antibacterial activity from ethanol extract of kecombrang flowers against *S. epidermidis* and *P. Acnes*. The kecombrang flowers obtained from kecombrang plantation in Lubuk Begalung, Padang city, West Sumatra. The extract was made by maceration method with 70% ethanol as a solvent. Antibacterial activity test was carried out by disk diffusion method on Mueller Hinton Agar (MHA) media with a concentration of 10%, 20%, 40% and 80%. The value of Minimum Inhibitory Concentration (MIC) was done at concentrations of 10%, 8%, 6%, 4% and 2%. The results showed that the ethanol extract of kecombrang flowers had antibacterial activity against *S. epidermidis* at concentrations of 10%, 20%, 40% and 80%

with Inhibition Zone (IZ) respectively 10.61 mm, 11.41 mm, 12.44 mm, and 14.41 mm, while against *P. acnes* the IZ were about 11.24 mm, 11.46 mm, 14.51 mm, and 19.37 mm. The MIC value for *S. epidermidis* is at a concentration of 4%, while in *P.acnes* cannot determine yet.

Keywords: antibacterial, ethanol, kecombrang flower, *Propionibacterium acnes, Staphylococcus epidermidis*

1. Introduction

Staphylococcus epidermidis and *Propionibacterium acnes* are known as commensals bacteria in human skin which can change into opportunistic (Nakase et al. 2014; Chessa et al. 2015). *Staphylococcus epidermidis* colonizes various areas of the skin, while *P. acnes* resides mainly in the pilosebaceous skin follicles. This microbial interplay, for instance, mediated through molecules involved in intercellular competition or communication, may have an impact on the fine balance of the skin ecosystem. A disturbed balance (dysbiosis) can impact skin health and might initiate or support the events that lead to skin disorders. One of such disorders is acne vulgaris, a multifactorial disease of pilosebaceous units of the skin that affects adolescents (Christensen et al. 2016).

Propionibacterium acnes can be related to the initial stage of acne because it causes an increase in the lipogenesis originated in sebaceous glands. It induces inflammation and pustules on the skin (Neves et al. 2015; Blaskovich et al. 2019). *Staphylococcus epidermidis* besides can cause acne vulgaris inflammatory, it also can be opportunistic when it enters the bloodstream (Nakase et al. 2014; Tabri 2019).

Acne treatment in skin clinics usually uses antibiotics that can overcome inflammation and kill bacteria such as tetracycline, erythromycin, doxycycline and clindamycin (Nakatsuji, 2009; Dogan et al. 2017). However, these drugs have side effects such as irritation and allergic, while long-term use of antibiotics can cause resistance, organ damage, and immune-hypersensitivity (Adawiyah et al. 2010; Tan et al. 2018; Dikicier 2019). These problems have led many researchers to discover and develop new sources for antimicrobial agents from natural products, e.g. medicinal plants (Abdallah 2011). Sadeek & Abdallah (2019) stated that some phytochemical compounds extracted from medicinal plants showed effective antibacterial potential against multi-drug-resistant pathogens and these compounds could be exploited as antibacterial drugs.

Indonesia is known as one of the countries that has many medicinal plants. One of them is kecombrang (*Etlingera elatior* (Jack) R.M.Smith). Kecombrang is a spice plant that belongs to the Zingiberaceae Family and has been used in making medicine as well as flavour enhancers. This plant contains secondary metabolites such as phenols, flavonoids, glycosides, saponins, tannins, steroids, terpenoids (Silalahi 2017; Juwita et al. 2018; Effendi et al. 2019). Those compounds are known as potential sources for antibacterial agents (Abdallah 2011; Sadeek & Abdallah 2019). Based on Farida & Maruzy (2016) report, kecombrang flower have more antibacterial compounds compare to its rhizome, leaves or fruit. Kecombrang fruit is contain flavonoid only. Kecombrang leaves contain of saponin and flavonoid. The rhizome of kecombrang contain saponin, tannin, sterol, and terpenoid. Meanwhile, kecombrang flower contains flavonoid, saponin, tannin, and terpenoid.

Some studies reported that kecombrang flower extract has antibacterial activity to some bacteria. Mackeen et al. (1997) reported that ethanol extract of kecombrang flower can inhibit the growth of *Pseudomonas aeruginosa, Escherichia coli, Bacillus megaterium*, and *Cryptococcus neoformans*. Wijekoon et al. (2013) reported that kecombrang flower extract with various solvents (water, 50% ethanol, and 96% ethanol) can inhibit the growth of *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Listeria monocytogenes*, and *Klebsiella pneumoniae*. Another research by Ghasemzadeh et al. (2015), showed that the ethanol and water extract of kecombrang flower can inhibit *S. aureus, B. subtilis, E. coli, Salmonella sp., Micrococcus sp.,* and *Proteus mirabilis*. Naufalin & Rukmini (2018) reported that the ethanol extract of kecombrang flower has better antibacterial activity than the ethyl acetate's against *B. cereus* and *E. coli*.

Based on that background, this study was done to determine the antibacterial activity from the ethanol extract of kecombrang flower against *Staphylococcus epidermidis* and *Propionibacterium acnes*. Since kecombrang flower is high containing polyphenol compounds, so it is best to use ethanolic solvents (Farida & Maruzy 2016). Ethanol can attract more polyphenol compounds than others (Tiwari et al. 2011). The outcome of the study is expected to show that ethanol extract of kecombrang flower can be used as an alternative for acne treatment.

2. Materials and methods

2.1. Materials

Chemicals and Reagents. Mueller Hinton Agar (Oxoid), Mueller Hinton Broth (Oxoid), 70%Ethanol (Brataco), Aquadest (Brataco), FeCl3 (Merck), Bouchardat reagent, Mayer reagent, Dragendorff reagent, Ammoniak (Merck), Acetic acid anhydride (Merck), NaNO2 (Merck), $AlCl_3$ (Merck), HCl (Merck), Chloroform (Merck), H_2SO_4 (Merck), DMSO, immersion oil (Gargille), Crystal violet (Merck), Safranin, Lugol's iodine, 0,9% NaCl, *Blank disc* (Oxoid), the antibiotic disk of ciprofloxacin 5 μg (Oxoid) and clindamycin 10 μg (Oxoid).

Bacteria. *Staphylococcus epidermidis* obtained from Parasitology Department, Faculty of Medicine, UI; and *Propionibacterium acnes* obtained from Microbiology Laboratory, Faculty of Pharmacy, ISTN.

2.2. Methods

Plant source and preparation. Kecombrang flowers were obtained from the kecombrang plantation in Lubuk Begalung, Padang, West Sumatra. About 8 kg of the flowers was washed with clean water and cut into small pieces, then placed in a container and spread evenly for the drying process. It was dried in the oven with a temperature of 40-50 ºC for 3 x 24 hours. Furthermore, the flowers have been dried, then ground into a homogeneous powder.

2.2.1. Extraction and Phytochemical Screening

Extraction. The extraction was using a maceration method. Kecombrang flower powder as much as 500 g were macerated using 70% ethanol as a solvent with a concentration 1:10. The maceration was done 1 x 24 hours with occasional stirring. Remaceration was done for 2 x 24 hours using the same solvent. The filtrate was evaporated using rotary evaporator into a thick extract.

Phytochemical screening. Phytochemical screening was carried out based on Materia Medika Indonesia (Depkes RI 1989) and Pandey & Tripathi (2014). Screening includes testing for alkaloids, flavonoids, tannins, saponins, steroids and triterpenoids.

2.2.2. Antibacterial test

Bacterial suspension. Preparation of bacterial suspensions was carried out by taking several ose the bacteria aged 24 hours. It was inoculated into a tube containing 5 ml of sterile 0.9% NaCl solution, then homogeneous using vortex. The turbidity of the suspension was equated with Mc Farland 3 which is equivalent to a concentration of 9×10^8 CFU/ml. After that, the dilution was carried out about 1 ml of the suspension and then put in a test tube containing 9 ml of NaCl 0.9% (9×10^7 CFU / ml) (Pratiwi, 2008).

Antibacterial Test. Antibacterial activity test was carried out using the disk diffusion method. The suspension of bacteria was pipetted about 1 ml onto a petri dish containing Mueller Hinton Agar (MHA), and then spread evenly. After the media and bacterial suspension dried, sterile disk paper was inserted into a petri dish and 20 μl of the extract was dropped. The concentration of the extract was using 10%, 20%, 40%, and 80%. The positive control using two antibiotic discs, which were ciprofloxacin for *Staphylococcus epidermidis* and clindamycin for Propionibacterium acnes, while the negative control used was DMSO 10%. The tested discs then incubated for 24 hours at 37ºC. The diameter of the inhibition zone was measured using a calliper (Hudzicki, 2016).

8 **Minimum Inhibitory Concentration (MIC) Test.** The Minimum Inhibition Concentration Test was carried out by the method of solid dilution and liquid dilution, which is by observing the growth of test bacteria from the lowest extract concentration produced from the Inhibition Zone (IZ). The concentrations used are 10%, 8%, 6%, 4%, and 2%. Preparation for solid dilution using a petri dish where MHA media was added 1 mL of kecombrang flower extract and mixed with 1 mL of bacterial suspension test. The control used was negative control containing only the media and positive control containing media that had been inoculated for 1 mL suspension of the test bacteria. Furthermore, incubate at 37ºC for 24 hours. 20 The lowest concentration of the extract that still poses a bacterial growth is 9 determined as the Minimum Inhibitory Concentration (MIC).

3. Results and Discussion

3.1. Preparation and Extraction Samples

The fresh flower of kecombrang was dried using an oven. The purpose of drying is to get simplicia that is not easily damaged and not overgrown with fungus in long-term storage (Sa'adah & Nurhasnawati, 2015). The oven was chosen because it can keep at controlled temperature and gave faster drying (Singh & Laishram, 2010).

The drying flower then mashed up into a homogenous powder to expand the contact between the solvent and the simplicial. This texture can speed up the extraction process because it enlarging the contact between the powder and the solvent (Depkes RI 1989).

The extraction was done by maceration method. It is a very simple method and could be used for the extraction of thermolabile (Zhang et al. 2018). The yield extract was about 17.6%. The calculation of the yield was shown in Table 1.

Table 1. Calculation yield of kecombrang powder and extract

Sample	Flower powder (g)	Extract (g)	Yield (%)
Kecombrang flower	500	88	17.60

3.2. Phytochemical Screening

Phytochemical screening was carried out to determine the content of secondary metabolites which include alkaloid, flavonoid, saponin, tannin, steroid/triterpenoid tests. The results of the phytochemical screening were shown in Table 2.

Table 2. Phytochemical screening of Kecombrang Extract

Metabolites	Phytochemical screening	
	Powder	extract
Alkaloid	-	-
Flavonoid	+	+
Saponin	+	+
Tannin	+	+
Steroid/ Triterpenoid	-	-

(+) : contain tested metabolite; (-) : do not contain tested metabolites

Table 2 showed that kecombrang flower extract contains flavonoid, saponin, and tannin. While for alkaloid and steroid/triterpenoid it shows negative results. This results in accordance with Silalahi's (2017), which stated that kecombrang contains many secondary metabolites from terpenoids and phenolic groups, whereas alkaloid groups have not been reported. The flavonoid, phenolic, and terpenoid compounds are highly potential as antimicrobial agents found from *E. elatior* (Juwita et al. 2018).

3.3. Antibacterial Test

The antibacterial test was done by the disk diffusion method. The clear zone appears around the disk was measured as inhibition zone. The measurements of inhibition zone showed in Table 3.

Table 3. Inhibition Zone (IZ) of Kecombrang Extract Against *Staphylococcus epidermidis* and *Propionibacterium acnes*

Bacteria	Inhibition Zone (IZ) (mm)				Positive control (mm)	Negative control (mm)
	10%	20%	40%	80%		
Staphylococcus epidermidis	10.61	11.41	12.44	14.41	28.67*	-
Propionibacterium acnes	11.24	11.46	14.51	19.37	26.31#	-

*: Ciprofloxacin 5 mg; #: Clindamycin 10 mg; Negative control: DMSO 10%; -: no inhibition zone

The data in Table 3 showed that the ethanol extract of kecombrang flower has the activity to inhibit the growth of *S. epidermidis* and *P. acnes*. The results showed that the greater concentration of the extract showed a greater inhibition against those two bacteria. The difference in diameter of inhibition zones at each concentration possibly was due to differences in the magnitude of active substances contained in the concentration. The active compounds in higher concentration are more than the opposite (Lingga et al. 2016). Besides that, the size of the inhibition zone was also influenced by the level of sensitivity of the organism, the culture medium, the incubation conditions, and the diffusion rate of the antibacterial compound (Fitriah et al. 2017).

Antibacterial differences are based on their mechanism of action inhibiting the growth of cell walls, resulting in changes in cell membrane permeability and inhibiting protein synthesis, and nucleic acids (Brooks et al. 2005). According to Fitriah et al. (2017), that each group of compounds can have different effects in inhibiting bacterial growth. The difference in the activity that occurs is caused by secondary metabolites contained having synergistic energy that is different depending on the nature and morphology of bacteria.

Flavonoid compounds in kecombrang flower ethanol extract (*Etlingera elatior* (Jack) R.M.Smith) have antibacterial activity by binding to neophilic amino acids in protein and enzyme inactivation (Mulyani et al. 2017). The mechanism of flavonoid inhibition of bacterial growth is thought to be due to the ability of these compounds to form complexes with extracellular proteins, activate enzymes, and damage cell membranes. In general, flavonoid compounds can inhibit the growth of Gram-positive and Gram-negative bacteria and act as antimicrobial agents by forming complex bonds with cell walls and damaging membranes (Marselia et al. 2015). Flavonoids play a role in inhibiting the synthesis of bacterial cell nucleic acids. The mechanism of action of flavonoids functions as an antibacterial by forming complex compounds against extracellular proteins that interfere with the integrity of bacterial cell membranes by denaturing bacterial cell proteins and damaging cell membranes beyond repair (Juliantina, 2008).

Tannins are a group of polyphenol compounds that have antibacterial activity, the mechanism of action of tannins as an antibacterial is thought to be able to shrink the cell wall so that it interferes with the permeability of bacterial cells, due to disturbed permeability, bacterial cells cannot carry out living activities so that their growth is inhibited or even dies. Tannins also have antibacterial power by precipitating proteins, because tannins are suspected to have the same effect as phenolic compounds. The antibacterial effects of tannins include, among others, reactions with cell membranes, enzyme inactivation, and inactivation or inactivation of genetic material functions (Ibrahim & Kuncoro, 2012).

Other metabolite compounds contained in kecombrang flowers are saponins. According to Marselia et al. (2015), saponin acts as an antiseptic on surface wounds, works as a bacteriostatic which is usually used for infections of the skin, mucosa and fight infections in wounds. Saponin compounds are detergents that work by forming a complex with sterols found in the membrane. Saponin compounds also interact with cell phospholipid membranes that are impermeable to lipophilic compounds, causing membrane integrity to decrease, cell membrane morphology to change and ultimately can cause brittle cell membranes and lysis. Damage to the bacterial cell membrane results in ruptured plasma membranes, cell loss of cytoplasm, impaired substance transport and inhibited metabolism so that bacteria experience growth retardation and even death causing bacterial cell lysis.

3.4. Minimum Inhibitory Concentration (MIC)

The data of MIC test was showed inTable 4.

Table 4. Minimum Inhibitory Concentration (MIC) Test Against *Staphylococcus epidermidis* and *Propionibacterium acnes*

Extract Concentrations	Bacteria	
	Staphylococcus epidermidis	*Propionibacterium acnes*
10 %	-	-

8 %	-	-
6%	-	-
4 %	-	-
2 %	+	-
Negative control	-	-
Positive control	+	+

-: no growth; +: growth

MIC test results indicate that the MIC value in *S. epidermidis* is at a concentration of 4% because in a concentration of 2% the bacteria showed growth. Meanwhile, the MIC value of *P. acnes* cannot determine yet. It's due to at the lowest concentration of the test (2%), the *P. acnes* still showed no growth. Based on that data, the MIC test should be conducted at concentrations below 2% to ascertain at what concentration bacteria can grow despite the effect of extracts in the media.

4. Conclusions

The ethanol extract of kecombrang flower can inhibit the growth of *Staphylococcus epidermidis* and *Propionibacterium acnes*. The MIC for *S. epidermidis* was at concentration 2%, while for *P. acnes* it can't determine yet.

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