Short Communication

Antibacterial Activity of Kecombrang Flower Extract (*Etlingera elatior* (Jack) R.M. Sm) Against *Staphylococcus epidermidis* and *Propionibacterium acnes*[a1]

Abstract

Kecombrang flower contains several secondary metabolites that can act as antibacterial. [a2]This study aims to determine the antibacterial activity of kecombrang flower [a3]extract against *Staphylococcus epidermidis* and *Propionibacterium acnes*. [a4]Antibacterial activity test was carried out by disk diffusion method with a concentration of 10%, 20%, 40% and 80%[a5]; while the Minimum Inhibitory Concentration (MIC)[a6] was done at concentrations of 10%, 8%, 6%, 4% and 2%.[a7] The results showed that the kecombrang flower [a8]extract had antibacterial activity against *S. epidermidis* and *P. acnes*[a9]. The MIC for *S. epidermidis* is at a concentration of 4%, while in *P.acnes* cannot determine yet.[a10]

Keywords:

antibacterial, ethanol[a11], kecombrang flower, *Propionibacterium acnes, Staphylococcus epidermidis*

"[a12]

Staphylococcus epidermidis and *Propionibacterium acnes* [a13]are commensal bacteria which can be opportunistic causes acne vulgaris (Nakase et al. 2014; Chessa et al. 2015). Acne treatment in skin clinics usually uses antibiotics, but it causes problems such as antibiotic resistance[a14] (Dogan et al. 2017; Tan et al. 2018; Dikicier 2019). To overcome this problem, the discovery of new antibacterial agents from natural resources such as plants are needed[a15] (Abdallah 2011).

Kecombrang (*Etlingera elatior* (Jack) R.M.Sm) is one of a medicinal plant used empirically to treat various diseases in Indonesia (Juwita et al. 2018). Kecombrang flower contains secondary metabolites such as flavonoids, tannins, saponins, and terpenoid known as antibacterial (Farida & Maruzy 2016). [a16]The purpose of this study was to determine the antibacterial activity from the ethanol extract of kecombrang flower against *S. epidermidis* and *P. acnes.*[a17]

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The kecombrang flowers obtained from kecombrang plantation in Lubuk Begalung, Padang City, West Sumatra. The flowers were dried in the oven with a temperature of 40-50 ºC for 3 x 24 hours. The purpose of drying is to get the simplicia of flower 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).[a20]

"[a21]

The extract was made by the maceration method with 70% ethanol[a22] as a solvent. Ethanol was used because it can attract more polyphenol compounds than others (Tiwari et al. 2011). Kecombrang flower is high containing polyphenol compounds, so it is best to use ethanolic solvents (Farida & Maruzy 2016). Maceration was chosen because it is a very simple method and could be used for the extraction of thermolabile compounds (Zhang et al. 2018). The filtrate from maceration then evaporated using rotary evaporator into a thick extract (Depkes RI 1995). The extract then screened for phytochemical contents based on Materia Medika Indonesia (Depkes RI 1989) and Pandey & Tripathi (2014). Screening

includes testing for alkaloids, flavonoids, tannins, saponins, steroids and triterpenoids.

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The yield extract in this research was about 17.6%. The yield of an extract shows the number of active compounds extracted from the sample. The higher of the yield extract, then the active compounds contained in the extract also bigger (Harborne 1984; Hasnaeni et al. 2019). The calculation of the yield extract was shown in Table 1.

Table 1. Calculation Yield of Kecombrang Flower Powder and Extract

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

“[a26]

The results of the phytochemical screening 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 (2017) and Juwita et al. (2018), which stated that kecombrang contains many secondary metabolites from terpenoids and phenolic groups, whereas alkaloid groups have not been reported. The phenolic compounds found in kecombrang are flavonoid, saponin, tannin, and polyphenol. The result of the phytochemical screening was shown in Table 2.

Table 2. Phytochemical Screening of Kecombrang Flower Extract

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

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

The presence of flavonoid, saponin, and tannin in this research indicates that kecombrang flower extract has the potential as an antimicrobial. This is as stated by Juwita et al. (2018) that the high potential anti-microbial activity possessed by kecombrang is due to the presence of flavonoid, phenolic, and terpenoid contents.

The 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 clear zone appears around the disk was measured as the inhibition zone (IZ) (Hudzicki, 2016). The positive control used in this research is two antibiotics, named ciprofloxacin for *S. epidermidis* and clindamycin for *P. acnes*. Antibiotics are known as a substance that can inhibit the growth of bacteria (Pratiwi 2008). The use of different antibiotics in this research was due to in preliminary study ciprofloxacin gave a very wide inhibition zone in the dish, so it can create ambiguous results. The negative control used in this test is DMSO 10%. DMSO is a surfactant that can dissolve polar and nonpolar materials, it also showed no antibacterial activity (de Brito et al. 2017). The measurements of inhibition zone showed in Table 3.

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

Bacteria	Inhibition Zone (IZ) (mm)				Positive control (mm)	Negative control (mm)
	10%	20%	40%	80%		
Staphylococcus epidermidis	10.62±0.06	11.41±0.06	12.44±0.12	14.41± 0.02	28.67*± 0.17	-
Propionibacterium acnes	11.25± 0.05	11.46± 0.02	14.51±0.06	19.37± 0.11	26.31#±0.06	-

*: Ciprofloxacin 5 µg; #: Clindamycin 10 µg; 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*[a27]. 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[a28] (Fitriah et al. 2017).

"[a29]

Antibacterial agents have four mechanisms to inhibit the growth of bacteria. First, by inhibiting the growth of cell walls[a30]. Secondly, changing the cell membrane permeability[a31], then inhibit the protein synthesis, and also the nucleic acids [a32](Hogg 2005). According to Fitriah et al. (2017), 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.

The kecombrang flower extract in this research contains flavonoid, tannin, and saponin which has different mechanisms to inhibit the growth of bacteria. According to Juwita *et al.* (2018), flavonoid compounds in kecombrang (*Etlingera elatior* (Jack) R.M.Smith) have antibacterial activity by targeting of membrane cell wall due to its capability to composite with extracellular and soluble proteins[a33]. This mechanism is similar with saponin which also attack bacterial cell membrane[a34]. Saponin can dissolve lipids in bacterial cell membranes (lipoprotein), which causes the bacterial cell become lysis and death

(Syafriana *et al.*, 2019). Meanwhile, tannins act by disturbed the DNA gyrase [a35]which is an enzyme that plays a role in DNA replication (Khameneh *et al.*, 2019). However, we cannot determine yet which compounds have a significant effect in inhibiting the bacterial growth because this research has only a qualitative phytochemical data, not a quantitative one. So, we cannot find out whether these compounds are equal in quantity or one is higher than another.

The data in Table 3 also showed us that at a minimum concentration (10%) can inhibit the two bacteria. Because of that, the antibacterial test continued by determined the MIC to find out the minimum concentration of extract which can inhibit the two bacteria. The test was carried out by the solid dilution method which is observing the growth of the bacteria at a petri dish (Hudzicki 2016). The data of MIC was showed in Table 4.

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

Extract Concentrations	Bacteria	
	Staphylococcus epidermidis	*Propionibacterium acnes*
10 %	-	-
8 %	-	-
6%	-	-
4 %	-	-
2 %	+	-

-: no growth; +: growth [a36]

Data at Table 4 showed that MIC 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 to *P. acnes* should be

conducted at concentrations below 2% to ascertain at what concentration bacteria can grow despite the effect of extracts in the media.

This research is a preliminary study which shows a potency of kecombrang flower extract as an antibacterial agent against *S. epidermidis* and *P. acnes* that causes acne vulgaris. To ensure a more valid antibacterial activity, further research is needed such as determining levels of flavonoid, tannin, and saponin. Besides that, it is also necessary to study the mechanism of inhibition or cell damage caused by the secondary metabolites to prove that the mechanisms mention above are suitable.

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Short Communication

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

Abstract

Kecombrang flower contains several secondary metabolites that can act as antibacterial. This study aims to determine the antibacterial activity of kecombrang flower extract against *Staphylococcus epidermidis* and[K.1] *Propionibacterium acnes*. Antibacterial activity test was carried out by disk diffusion method with a concentration of 10%, 20%, 40% and 80%; [K.2]while the Minimum Inhibitory Concentration (MIC) was done at concentrations of 10%, 8%, 6%, 4% and[K.3] 2%. The results showed that the kecombrang flower extract had antibacterial activity against *S. epidermidis* and *P. acnes*. The MIC 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*

Staphylococcus epidermidis and *Propionibacterium acnes* are commensal bacteria ~~which~~[K.4] can be opportunistic ~~causes~~[K.5] acne vulgaris (Nakase et al., 2014; Chessa et al., 2015). Acne treatment in skin clinics usually uses antibiotics, but it causes problems such as antibiotic resistance (Dogan et al. [K.6], Tan et al., 2018; Dikicier, 2019). To overcome this problem, ~~the discovery~~ [K.7]of new antibacterial agents from natural resources such as plants ~~are~~[K.8] needed (Abdallah, 2011).

Kecombrang (*Etlingera elatior* (Jack) R.M.Sm) is one of a medicinal plant used empirically to treat various diseases in Indonesia (Juwita et al. 2018). Kecombrang flower contains secondary metabolites such as flavonoids, tannins, saponins, and terpenoid known as antibacterial (Farida & Maruzy[K.9], 2016). The purpose of this study was to determine the antibacterial activity from the ethanol extract of kecombrang flower against *S. epidermidis* and *P. acnes.*

The kecombrang flowers obtained from kecombrang plantation in Lubuk Begalung, Padang City, West Sumatra. The flowers were dried in the oven with a temperature of 40-50 [K.10] ºC for 3 x 24 hours. The purpose of drying is to get the simplicia of flower 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[K.11] 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[K.12] enlarging the contact between the powder and the solvent (Depkes RI,[K.13] 1989).

The extract was made by the maceration method with [K.14]70% ethanol as a solvent. Ethanol was used because it can attract more polyphenol compounds than others (Tiwari et al., 2011). Kecombrang flower is high containing [K.15]polyphenol compounds, so it is best to use ethanolic solvents (Farida & Maruzy 2016). Maceration was chosen[K.16] because it is a very simple method and could be used for the extraction of thermolabile compounds (Zhang et al. 2018[K.17]). The filtrate from maceration then evaporated using rotary evaporator into a thick extract (Depkes RI 1995[K.18]). The extract then [K.19]screened for phytochemical contents based on Materia Medika Indonesia (Depkes RI 1989[K.20]) and Pandey & Tripathi (2014). Screening includes testing for alkaloids, flavonoids, tannins, saponins, steroids and [K.21]triterpenoids.

The yield extract in this research was about 17.6%. The yield of an extract shows the number of active compounds extracted from the sample. The higher ~~of~~ the yield extract, then the active compounds contained in the extract also bigger (Harborne 1984; Hasnaeni et al. 2019). The calculation of the yield extract was shown [K.22]in Table 1.

Table 1. Calculation Yield of Kecombrang Flower Powder and Extract

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

The results of the phytochemical screening showed that kecombrang flower extract contains flavonoid, saponin, and tannin. While for alkaloid and steroid/triterpenoid it[K.23] shows negative results. This results in accordance with [K.24]Silalahi (2017) and Juwita et al. (2018), which stated that kecombrang contains many secondary metabolites from terpenoids and phenolic groups, whereas alkaloid groups have not been reported. The phenolic compounds found in kecombrang are flavonoid, saponin, tannin, and polyphenol. The result of the phytochemical screening was shown [K.25]in Table 2.

Table 2. Phytochemical Screening of Kecombrang Flower Extract

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

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

The presence of flavonoid, saponin, and tannin in this research indicates that kecombrang flower extract has the potential as an antimicrobial. This is as stated by Juwita et. al. (2018) that the high potential anti-microbial activity possessed by kecombrang is due to the presence of flavonoid, phenolic, and terpenoid contents.

The 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 clear zone appears around the disk was measured as the inhibition zone (IZ) (Hudzicki, 2016). The positive control used in this research is two antibiotics, named ciprofloxacin for *S. epidermidis* and clindamycin for *P. acnes*. Antibiotics are known as a substance that can inhibit the growth of bacteria (Pratiwi 2008). The use of different antibiotics in this research was due to in preliminary study ciprofloxacin gave a very wide inhibition zone in the dish, so it can create ambiguous results. The negative control used in this test is DMSO 10%. DMSO is a surfactant that can dissolve polar and nonpolar materials, it also showed no antibacterial activity (de Brito et al. 2017). The measurements of inhibition zone showed in Table 3.

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

Bacteria	Inhibition Zone (IZ) (mm)				Positive control (mm)	Negative control (mm)
	10%	20%	40%	80%[K.37]		
Staphylococcus epidermidis	10.62±0.06	11.41±0.06	12.44±0.12	14.41± 0.02	28.67*± 0.17	-
Propionibacterium acnes	11.25± 0.05	11.46± 0.02	14.51±0.06	19.37± 0.11	26.31#±0.06	-

*: Ciprofloxacin 5 µg; #: Clindamycin 10 µg[K.38]; Negative control: DMSO 10%; [K.39]-: 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 [K.40]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 level of sensitivity of the also influenced the size of the inhibition zone organism, the culture medium, the incubation conditions, and the diffusion rate of the antibacterial compound (Fitriah et al. 2017).

Antibacterial agents have four mechanisms to inhibit the growth of bacteria. First, by inhibiting the growth of cell walls. Secondly, changing the cell membrane permeability, [K.41]then inhibit[K.42] the protein synthesis,[K.43] and also[K.44] the nucleic acids (Hogg 2005[K.45]). According to Fitriah et al. (2017), 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.

The kecombrang flower extract in this research contains flavonoid, tannin, and saponin which [K.46]has different mechanisms to inhibit the growth of bacteria. According to Juwita *et al.* (2018), flavonoid compounds in kecombrang (*Etlingera elatior* (Jack) R.M.Smith) have antibacterial activity by targeting of membrane cell wall due to its capability to composite with extracellular and soluble proteins. This mechanism is similar ~~with~~ [K.47]saponin which[K.48] also attack[K.49] bacterial cell membrane. Saponin can dissolve lipids in bacterial cell membranes (lipoprotein), which causes the bacterial cell become[K.50] lysis and death

(Syafriana *et al.*, 2019). Meanwhile, tannins act by disturbed the DNA gyrase which[K.51] is an enzyme that plays a role in DNA replication (Khameneh *et al.*, 2019). However, we cannot determine yet which compounds have a significant effect in inhibiting the bacterial growth because this research has only a [K.52]qualitative phytochemical data, not a [K.53]quantitative one. So, we cannot find out whether these compounds are equal in quantity or one is higher than another.

The data in Table 3 also showed us that at[K.54] a minimum concentration (10%) can inhibit the two bacteria. Because of that, the antibacterial test continued by determined the MIC to find out the minimum concentration of extract which[K.55] can inhibit the two bacteria. The test was carried out by the solid dilution method which is observing the growth of the bacteria at a petri dish (Hudzicki 2016). The data of MIC was showed in Table 4.

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

Extract Concentrations	Bacteria	
	Staphylococcus epidermidis	*Propionibacterium acnes*
10 %	-	-
8 %	-	-
6%	-	-
4 %	-	-
2 %	+	-[K.56]

-: no growth; +: growth

Data at Table 4 showed that MIC in *S. epidermidis* is at a concentration of 4%[K.57] because in a concentration of 2%[K.58] 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 to *P. acnes* should be

conducted at concentrations below 2% to ascertain at what concentration bacteria can grow despite the effect of extracts in the media.[K.59]

This research is a [K.60]preliminary study which shows a potency of kecombrang flower extract as an antibacterial agent against *S. epidermidis* and *P. acnes* that causes acne vulgaris. To ensure a more valid antibacterial activity, further research is needed such [K.61]as determining levels of flavonoid, tannin, and saponin. Besides that, it is also necessary to study the mechanism of inhibition or cell damage caused by the secondary metabolites to prove that the mechanisms mention[K.62] above are suitable.

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Short Communication

RECOMMENDATION:
This article does not meet the standard of a scientific article. This manuscript requires major revision in order to get accepted. I suggest to decline this article.

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

Abstract

Kecombrang flower contains several secondary metabolites that can act as antibacterial. This study aims to determine the antibacterial activity of kecombrang flower extract against *Staphylococcus epidermidis* and *Propionibacterium acnes*. Antibacterial activity test was carried out by disk diffusion method with a concentration of 10%, 20%, 40% and 80%; while the Minimum Inhibitory Concentration (MIC) was done at concentrations of 10%, 8%, 6%, 4% and 2%. The results showed that the kecombrang flower extract had antibacterial activity against *S. epidermidis* and *P. acnes*. The MIC for *S. epidermidis* is at a concentration of 4%, while in *P.acnes* cannot determine yet.

- Grammar errors on abstract and entire parts of manuscript.
- Lack of description regarding gap between our current state of the art of this topic and an expected understanding on such core problem to be resolved by this study. It will be considerable to add it in introduction part.
- There have been many similar reports using this topic. The author should figure out to provide the novelty of this research.

Keywords:

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

Staphylococcus epidermidis and *Propionibacterium acnes* are commensal bacteria which can be opportunistic causes acne vulgaris (Nakase et al. 2014; Chessa et al. 2015). Acne treatment in skin clinics usually uses antibiotics, but it causes problems such as antibiotic resistance (Dogan et al. 2017; Tan et al. 2018; Dikicier 2019). To overcome this problem, the discovery of new antibacterial agents from natural resources such as plants are

needed (Abdallah 2011).

Kecombrang (*Etlingera elatior* (Jack) R.M.Sm) is one of a medicinal plant used empirically to treat various diseases in Indonesia (Juwita et al. 2018). Please add stronger the argument why choosing this plant . Kecombrang flower contains secondary metabolites such as flavonoids, tannins, saponins, and terpenoid known as antibacterial (Farida & Maruzy 2016). The purpose of this study was to determine the antibacterial activity from the ethanol extract of kecombrang flower against *S. epidermidis* and *P. acnes.*

The kecombrang flowers obtained from kecombrang plantation in Lubuk Begalung, Padang City, West Sumatra. The flowers were dried in the oven with a temperature of 40-50 ºC for 3 x 24 hours. The purpose of drying is to get the simplicia of flower 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 extract was made by the maceration method with 70% ethanol as a solvent. Ethanol was used because it can attract more polyphenol compounds than others (Tiwari et al. 2011). Kecombrang flower is high containing polyphenol compounds, so it is best to use ethanolic solvents (Farida & Maruzy 2016). Maceration was chosen because it is a very simple method and could be used for the extraction of thermolabile compounds (Zhang et al. 2018). The filtrate from maceration then evaporated using rotary evaporator into a thick extract (Depkes RI 1995). The extract then screened for phytochemical contents based on Materia Medika Indonesia (Depkes RI 1989) and Pandey & Tripathi (2014). Screening includes testing for alkaloids, flavonoids, tannins, saponins, steroids and triterpenoids.

The yield extract in this research was about 17.6%. The yield of an extract shows the number of active compounds extracted from the sample. The higher of the yield extract, then the active compounds contained in the extract also bigger (Harborne 1984; Hasnaeni et al. 2019). **This statement is debatable, this extraction method used is simple crude extraction. It cannot be concluded that the extraction yield is the amount of the active component of the plant**.

The calculation of the yield extract was shown in Table 1.

Table 1. Calculation Yield of Kecombrang Flower Powder and Extract

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

The results of the phytochemical screening 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 (2017) and Juwita et al. (2018), which stated that kecombrang contains many secondary metabolites from terpenoids and phenolic groups, whereas alkaloid groups have not been reported. The phenolic compounds found in kecombrang are flavonoid, saponin, tannin, and polyphenol. The result of the phytochemical screening was shown in Table 2.

Table 2. Phytochemical Screening of Kecombrang Flower Extract

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

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

The presence of flavonoid, saponin, and tannin in this research indicates that kecombrang flower extract has the potential as an antimicrobial. This is as stated by Juwita et al. (2018) that the high potential anti-microbial activity possessed by kecombrang is due to the presence of flavonoid, phenolic, and terpenoid contents.

W

The 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 authors mentioned that the concentration of tested extract was ranged from 10%-80%. What is the reason to consider such doses of treatment? Any other preliminary studies or previous reports suggesting the effective dose for this extract? If so, please then describe it clearly. The authors did not mention what the standard of isolates were?

The clear zone appears around the disk was measured as the inhibition zone (IZ) (Hudzicki, 2016). The positive control used in this research is two antibiotics, named ciprofloxacin for *S. epidermidis* and clindamycin for *P. acnes*. Antibiotics are known as a substance that can inhibit the growth of bacteria (Pratiwi 2008). The use of different antibiotics in this research was due to in preliminary study ciprofloxacin gave a very wide inhibition zone in the dish, so it can create ambiguous results. The negative control used in this test is DMSO 10%. DMSO is a surfactant that can dissolve polar and nonpolar materials, it also showed no antibacterial activity (de Brito et al. 2017). The measurements of inhibition zone showed in Table 3.

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

Bacteria	Inhibition Zone (IZ) (mm)				Positive control (mm)	Negative control (mm)
	10%	20%	40%	80%		
Staphylococcus Epidermidis	10.62±0.06	11.41±0.06	12.44±0.12	14.41± 0.02	28.67*± 0.17	-
Propionibacteriu	11.25±	11.46± 0.02	14.51±0.06	19.37±	26.31#±0.0	-

m acnes	0.05		0.11	6

*: Ciprofloxacin 5 µg; #: Clindamycin 10 µg; Negative control: DMSO 10%; -: no inhibition Zone

- The data will be more readable by presenting it in a graph instead of confused table as depicted by Table 3.
- There was no statistical notation in the data indicating the significance of difference. It should be provided clearly on the graph.
- Please describe the extraction procedure clearly.

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 agents have four mechanisms to inhibit the growth of bacteria. First, by inhibiting the growth of cell walls. Secondly, changing the cell membrane permeability, then inhibit the protein synthesis, and also the nucleic acids (Hogg 2005). According to Fitriah et al. (2017), 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.

The kecombrang flower extract in this research contains flavonoid, tannin, and saponin which has different mechanisms to inhibit the growth of bacteria. According to Juwita *et al.* (2018), flavonoid compounds in kecombrang (*Etlingera elatior* (Jack) R.M.Smith) have antibacterial activity by targeting of membrane cell wall due to its capability to composite with extracellular and soluble proteins. This mechanism is similar

105 with saponin which also attack bacterial cell membrane. Saponin can dissolve lipids in
106 bacterial cell membranes (lipoprotein), which causes the bacterial cell become lysis and death.

(Syafriana *et al.*, 2019). Meanwhile, tannins act by disturbed the DNA gyrase which is an enzyme that plays a role in DNA replication (Khameneh *et al.*, 2019). However, we cannot determine yet which compounds have a significant effect in inhibiting the bacterial growth because this research has only a qualitative phytochemical data, not a quantitative one. So, we cannot find out whether these compounds are equal in quantity or one is higher than another.

The data in Table 3 also showed us that at a minimum concentration (10%) can inhibit the two bacteria. Because of that, the antibacterial test continued by determined the MIC to find out the minimum concentration of extract which can inhibit the two bacteria. The test was carried out by the solid dilution method which is observing the growth of the bacteria at a petri dish (Hudzicki 2016). The data of MIC was showed in Table 4.

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

Extract Concentrations	Bacteria	
	Staphylococcus epidermidis	*Propionibacterium acnes*
10 %	-	-
8 %	-	-
6%	-	-
4 %	-	-
2 %	+	-

-: no growth; +: growth

- The reason for using this concentration (10%) is unclear.
- The discussion part was not focus on the result.
- Provide information from previous studies that demonstrated similar result to your finding.
- Instead of discussing about cellular mechanism of extract, which was not conducted in this study, the author had better discuss regarding MIC result which can be associated with the of bacteria tested.

Data at Table 4 showed that MIC 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 to *P. acnes* should be

conducted at concentrations below 2% to ascertain at what concentration bacteria can grow despite the effect of extracts in the media.

This research is a preliminary study which shows a potency of kecombrang flower extract as an antibacterial agent against *S. epidermidis* and *P. acnes* that causes acne vulgaris. To ensure a more valid antibacterial activity, further research is needed such as determining levels of flavonoid, tannin, and saponin. Besides that, it is also necessary to study the mechanism of inhibition or cell damage caused by the secondary metabolites to prove that the mechanisms mention above are suitable.

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