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Research Article

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Antibacterial actifity of kecombrang (*Etlingera elatior*) leaf ethanol extract to against *Klebsiella pneumoniae*

Eka Puspitasari[1*], Chalies Diah Pratiwi[1], Dede Rival Novian[2], Dora Dayu Rahma Turista[3]

[1]Department of Medical Analysis, STIKES Hutama Abdi Husada, Tulungagung, Indonesia
[2]Department of Veterinary Medicine, Nusa Cendana University, Kupang, Indonesia
[3]Department of Biology Education, Mulawarman University, Samarinda, Indonesia
Email: ekanikusetunggal@gmail.com*, diahchalies@gmail.com, dede.rival.novian@staf.undana.ac.id, doraturistaofficial@gmail.com

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ABSTRACT

Infectious diseases caused by bacteria in generals can be cured using antibacterial. The use of antibacterials tends to cause local hypersensitivity either on the skin or mucous membranes so that their use is starting to be reduced. Therefore, the development of natural antibacterials made from plants to reduce resistance is very necessary. kecombrang (*Etlingera elatior*) is one of the plants that has been shown to have activity in inhibiting bacterial growth. This research is an experimental study that aims to determine the effectiveness of the ethanol extract of the leaves of kecombrang in inhibiting the growth of *Klebsiella pneumoniae* bacteria in vitro. The method used is the well method with a completely randomized design. The sample came from the *Klebsiella pneumoniae* bacteria culture. Kecombrang leaf ethanol extract was made in concentrations of 20%, 40%, 60%, 80% and 100%. The extract was tested on *Klebsiella pneumoniae* bacteria and observed for the formation of inhibition zones. Inhibition the results of the zone measuremens were then analyzed descriptively. The results showed kecombrang leaf ethanol extract 20%, 40%, 60%, 80%, and 100% produce inhibition zone diameter 1.9 mm; 2.6 mm; 7.7 mm; 12.1 mm; and 14.7 mm, 0.4 mm for the negative control and positive control 22.0 mm. Concentration 100% has better antibacterial ability due to higher compound content. The test results show the class of secondary metabolites kecombrang leaf ethanol extract contains flavonoids, tannin, and saponin. The conclusion of this study that the ethanol extract of kecombrang leaves has potential to inhibit the growth of *Klebsiella pneumoniae* bacteria.

Keywords: Antibacterial; kecombrang; *Klebsiella pneumoniae*

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Biology Education Department
IKIP Budi Utomo, Malang, Indonesia

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INTRODUCTION

Bacteria are the microorganisms that have the most number and widespread on Earth when compared to other microorganisms. Bacteria are unicellular organisms (single-celled), prokaryotic, do not have chlorophyll, with a size so small that it can only be seen using a microscope. The proliferation of bacteria is influenced by several factors such as the source of nutrients, pH, temperature, chemical substances, salt content, and metabolic waste. In general, bacteria are divided into 2 types, namely pathogenis and non-pathogenic bacteria. Pathogenic bacteria are bacteria that are capable of causing disease by invading directly or contaminating food, while non-pathogenic bacteria are bacteria that do not have the potential to cause disease (Kusuma et al., 2014).

Bacteria that have pathogenic properties and are able to cause infection, one of them is *Klebsiella pneumoniae*. The bacterium *Klebsiella pneumoniae* often causes infections of the respiratory tract, urinary tract, and sepsis (Dewi et al., 2019). Pneumonia is an acute infection that occurs in the lung tissue (alveoli). Pneumonia caused by *Klebsiella pneumonia* is included in the Community Acquired Pneumonia (CAP) or commonly called community pneumonia. The bacterium *Klebsiella pneumoniae* has the ability to spread very quickly, especially among people who are infected and the immune condition is declining. Pneumonia is usually characterized by the occurrence of bleeding and thickening of the mucous layer of the organ (Elfidasari et al., 2013). Where there are 2.1% data indicate that pneumoniae cases are caused by *Klebsiella pneumoniae* bacteria (Manuaba et al., 2021).

Infectious diseases caused by bacteria in general can be cured using antibacterials, as well as infections caused by the bacteria *Klebsiella pneumoniae*. The use of antibacterials tends to cause a hypersensitivity effect locally either on the skin area or mucous membranes, so their use begins to be reduced. *Klebsiella pneumoniae* is also resistant to certain types of antibacterials. *Klebsiella pneumoniae* is also resistant to certain types of antibacterials, including ampicillin, cefuroxime, cefuroxime-aksetil, cefotaxime, ceftazidim, and trimethoprim-sulfamethoxazole (Sudarwati, 2016).

The development of antibacterials with natural ingredients made from plants to reduce resistance is necessary. Medicinal plants that can be used as antibacterial therapy naturally in the treatment of infectious diseases are of a wide variety. However, one of the plants that can be used is the plant Kecombrang (*Etlingera elatior*) (Kusumawati, 2016). Research that has been conducted by Kusumawati (2016), shows that ethanol extract from kecombrang leaves has an influence on the growth of *Salmonella typhi* bacteria. Ratnah et al. (2018) also proved that the activity of kecombrang leaf extract compounds in addition to being able to be used as an antibacterial can also be used as an anti-fungal. One of the fungi that can be inhibited growth is the fungus *Candida albicans*.

Kecombrang leaf extract is known to inhibit the growth of *Salmonella typhi* bacteria and *Candida albicans* fungi, the content of compounds that can inhibit the growth of fungi and bacteria is tannin. However, it is not yet known its benefits in inhibiting the growth of *Klebsiella pneumoniae* bacteria. Therefore, it is necessary to conduct a study with the title"the effectiveness of ethanol extract of kecombrang leaves (*Etlingera elatior*) in inhibiting the growth of *Klebsiella pneumoniae* bacteria in Vitro".

Based on the background that has been described previously, the content of compounds owned by kecombrang leaves and the harmful effect of *Klebsiella pneumoniae* bacteria.The purpose of this study was to determine the effectiveness of the ethanolic extract of kecombrang (*Etlingera elatior*) in inhibiting the growth of *Klebsiella pneumoniae* bacteria in vitro, and to determine the most effective concentration of ethanol extract of kecombrang leaves (*Etlingera elatior*) in inhibiting the growth of *Klebsiella pneumoniae* bacteria in vitro.

RESEARCH METHODS

This study is an experimental study with the aim to determine the effectiveness of ethanol extract of leaves kecombrang (*Etlingera elatior*) in inhibiting the growth of bacteria *Klebsiella pneumoniae* in vitro. This study uses the method of pitting and the design used is a complete random design (RAL) with 5 concentration treatment (20%, 40%, 60%, 80%, and 100%). Each treatment was repeated three times (Rusli, 2017). The study was conducted at the Microbiology Laboratory of STIKes Hutama Abdi Husada Tulungagung in March 2021.

Data collection in this study was conducted by measuring the diameter of the clear zone formed around the disk that has been soaked with ethanol extract of kecombrang leaves with various concentrations. Kecombrang leaves washed, cut and dried in the sun. Previously kecombrang leaves obtained from cultivation in the garden of researchers. 200 grams of dried kecombrang leaves are macerated with 96% ethanol as much as 2,000 mL. Then the kecombrang leaf extract solution is filtered and the filtrate is concentrated using a rotary evaporator. From this extraction process obtained extract with a concentration of 100%.

Klebsiella pneumonia used in this study is a culture of ATCC 13883 purchased. 0.1 mL bacterial suspension is inserted into a test tube containing 10 mL of Mueller Hinton Agar (MHA) homogenized media, then poured into petrisdish and allowed to solidify. After solidifying, a pit hole is made in the agar media with a drip pipette. Next are marked and each hole is filled with ethanol extract of kecombrang leaves with various concentrations (20%, 40%, 60%, 80%, and 100%) and positive control is the antibiotic chloramfenicol. The last stage of the medium was incubated at a temperature of 37^{o}C for 24 hours and measured the diameter of the formed clear zone. This treatment was repeated 3 times (Rusli, 2017). The data obtained then performed normality test using Kolmogorov-Smirnov test K-S) one sample. Because the normality test result are not normally distributed data and homogeneity test is not homogeneous distributed data, then the next test used is Kruskal-Wallis.

FINDING AND DISCUSSION

The results of the effectiveness test of the ethanol extract of kecombrang (*Etlingera elatior*) leaves in inhibiting the growth of *Klebsiella pneumoniae* bacteria in vitro are shown in Table 1 and Table 2, while the antibacterial activity of kecombrang leaves against *Klebsiella pneumoniae* bacteria is shown in Figure 1.

Table 1. The Results of The Effectiveness Test of Kecombrang Leaf Ethanol Extract in Inhibiting The Growth of *Klebsiella pneumoniae*

Extract Concentration	Result (mm)			Average
	1	2	3	
100%	15.0	14,3	14,7	14,7
80%	11,0	11,7	13,7	12,1
60%	7,0	8,7	7,3	7,7
40%	1,3	3,3	3.0	2,6
20%	1,0	3,0	1,7	1,9
Control -	0,7	0,0	0,7	0,4
Control +	22,7	22,0	21,3	22,0

Table 2. Wallis-Kruskal Test Results of The Effectiveness Test of Kecombrang Leaf Ethanol Extract in Inhibiting The Growth of *Klebsiella pneumoniae*

Variable	P_{value}	Description
Obstacle zone Diameter	0,027	There is influence

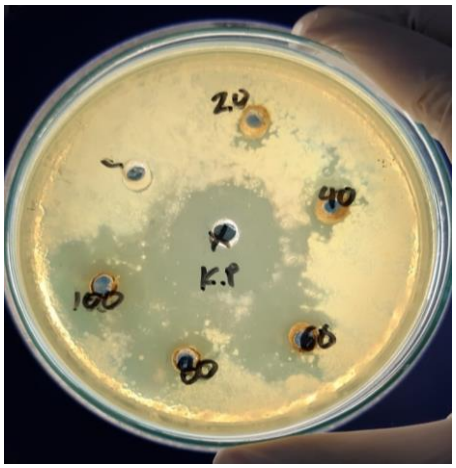

Figure 1. Antibacterial Activity of Kecombrang Leaves Against *Klebsiella pneumoniae*
(Source: Personal documentation, 2021)

Kecombrang leaves contain several chemical compounds. Among them are tannins, flavonoids and saponins. The presence of these chemicals plays a major role in inhibiting the growth of bacteria. Saponin chemicals work as antibacterial substances by disrupting the stability of cell membranes and causing bacterial cell lysis. Flavonoids have the ability to form complexes with extracellular proteins that can dissolve bacterial cell walls. Tannin compounds work as metabolites that can inhibit bacterial growth even in low concentrations (Kusumawati et al., 2016).

96% ethanol was chosen because it has non-toxic properties and compounds such as flavonoids, saponins, tannins, phenolics, alkaloids, terpenoids and steroids contained in kecobrang leaves can be dissolved in polar solvents so that active compounds that can provide antibacterial activity can be dissolved. Ethanol has no toxic properties, does not explode when mixed with air, is non-corrosive and easy to obtain (Damanik et al., 2014).

Antibacterial activity test of ethanol extract of Kecombrang leaves (*Etlingera elatior*) against the growth of *Klebsiella pneumoniae* bacteria in vitro was performed using the method of pitting on Mueller Hinton Agar (MHA) media. Antibacterial activity is characterized by the formation of a clear zone around the pit measured using a caliper. In this study chose Mueller Hinton Agar media, because this media has been recommended by the FDA and WHO in antibacterial tests, especially aerobic bacteria and facultative anaerobic bacteria with food samples and clinical materials (Nurhayati et al., 2020).

The positive control used in this study was the antibiotic Chloramphenicol. The antibiotic Chloramphenicol was chosen because it has a broad spectrum, which is effective against Gram-positive and Gram-negative bacteria and other microorganisms (Fredella & Rahman, 2022). The mechanism of action of the antibiotic Chloramphenicol occurs by inhibiting protein synthesis, preventing the aminoacyl end of t-RNA from joining the enzyme peptidyl tranferase (an enzyme that links amino acids with peptide chains during the protein synthesis process) (Qurrota et al., 2020).In addition, based on researh conducted by Makmum et al. (2020) stated that the use of chloramphenicol antibiotics are able to inhibit the syntheris of bacteria that are potent and the results of research with positive control of chloramphenicol antibiotics form an inhibition zone of 30 mm. Where the value of the inhibition zone is included in the sensitive class. Then the selection of chloramphenicol antibiotics use due to *Klebsiella pneumoniae* bacteria is sensitive to these antibiotics (Narlis et al., 2019).

Based on the data presented in figure 1, the average diameter of the largest inhibitory zone is located at a concentration of 100%. Determination of the concentration of ethanol extract leaves kecombarang very influential on the formation of inhibitory zone produced. According to Ningtyas that the

higher the concentration of the extract used, the higher the inhibitory power is formed. This is because the higher the concentration of the extract, the more the content of antibacterial active ingredients contained in it. The degree of effectiveness of an antibacterial substance in inhibiting bacteria growth depends on the nature of the tested microorganism, its concentration and length of contact time, and biostatistical properties may increase due to the high concentration added (Andries et al., 2014). Based on previous research conducted by Mufti et al. (2017) on the inhibitory power test of Sapodilla leaf extract against *Escherichia coli* bacteria, 100% concentration is the most effective concentration in inhibiting the growth of *Escherichia coli* bacteria. Meanwhile, based on research conducted by Pratama et al. (2018) on antibacterial test of raw banana peel extract against the growth of *Staphylococcus aureus* bacteria showed that the concentration of 100% is the most effective concentration in inhibiting the growth of *Staphylococcus aureus* bacteria.

Based on table 1, the antibacterial inhibition of ethanol extract of Kecombrang leaves at a concentration of 100% resulted in a inhibition zone of 14.7 mm which is included in the category of medium-acting antibacterials. Then based on Table 2, from the Kruskal-Wallis test, P_{value} 0.027 < α (0.05) was obtained, where the value indicates that there is a significant influence on the antibacterial ability of ethanol extract of kecombrang leaves on *Klebsiella pneumoniae* bacteria. The antimicrobial power of ethanol extract of kecombrang leaves is caused by the active ingredients contained in the ethanol extract of kecombrang leaves that play a role in inhibiting growth and killing *Klebsiella pneumoniae* bacteria. The active ingredients include saponins, flavonoids, and tannins (Kusumawati et al., 2017).

Saponin is a compound that has a function as a strong surface tension reducer and will cause foam when shaken in water. The properties of saponins resemble soap (latin sapo means soap). The mechanism of action of saponins as antimicrobial is by disrupting the stability of bacterial cell membranes so that bacterial cells become lysis. Flavonoids have antimicrobial abilities based on their ability to form complexes with extracellular proteins and soluble proteins as well as with bacterial cell walls (Pangestu et al., 2017). Tannins are secondary metabolites that are classified as condensed phenol compounds and are found in angiosperm plants. Tannins with a low concentration can inhibit the growth of bacteria and at high concentrations can kill bacteria. Phenolic compounds work as antimicrobials by coagulating or coagulating bacterial potoplasma to form a stable bond with bacterial proteins and in the digestive system, known tannins can eliminate toxins (Ngazizah et al., 2016). The results of this study are in line with the research conducted by Kurama et al. (2020) on the antibacterial activity test of ethanol extract of benalu langsat leaves (*Dendropthoe sp*) against *Klebsiella pneumoniae* bacteria, which shows that secondary metabolites of saponins, flavonoids and tannins have the ability to damage bacterial cells.

CONCLUSION

The research on the antibacterial activity of the ethanolic extract of kecombrang leaves against *Klebsiella pneumoniae* bacteria, it was concluded that the kecombrang leaf extract could inhibit the growth of *Klebsiella pneumoniae* bacteria. The greater concentration of the kecombrang leaf ethanol extract, the greater the resulting inhibition zone. The best concentration of kecombrang leaf ethanol extract that can be used as an antibacterial is a concentration of 100% and based on the Kruskal-Wallis test obtained P_{value} 0.027 < α (0.05), where the value indicates there is a significant effect on antibacterial ethanol extract of kecombrang leaves on bacteria *Klebsiella pneumoniae*.

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