ANTIBACTERIAL ACTIVITY TEST COMBINATION OF KENCUR (*Kaempferia galanga* L) RHIZOME AND SAPODILLA (*Manilkara zapota* L) LEAF EXTRACT AGAINST *Escherichia coli*

Amanda Fadilah Ilhani & *Ismedsyah

Department of Pharmacy, Polytechnic Health Ministry of Medan, Medan, Indonesia

*e-mail: ismedsyah@gmail.com

ABSTRACT

Kencur (Kaempferia galanga L) rhizome and sapodilla (Manilkara zapota L) leaf are one of the plants that have been empirically proven to treat infections. Kencur rhizome contains chemical compounds such as saponins, polyphenols, flavonoids and essential oil. Sapodilla leaf contains chemical compounds such as saponins, tannins, and flavonoids which function as antibacterial. The aim of this study was to determine the antibacterial activity of ethanol extract of kencur rhizome and sapodilla leaf ethanol extract combination against Escherichia coli bacteria. This study used an experimental method by looking at the antibacterial activity of ethanol extract of kencur rhizome and sawo leaf combination at a concentration ratio of 60%: 40%, 65%: 35%, 70%: 30%, 30 µg of chloramphenicol as a positive control and 70 % of alcohol as negative control. The study result showed that the combination of ethanol extract of Kencur rhizome and sapodilla leaf compared to 60%: 40%, 65%: 35% and 70%: 30%, each had an inhibitory zone against Escherichia coli of 15 mm, 13.17 mm, and 14.17 mm, chloramphenicol has an inhibitory zone of 19.83 mm and 70% alcohol does not have an inhibitory zone. This study concludes that the combination of Ethanol Extracts of Kencur Rhizome (Kaempferia galanga L) and Sapodilla leaf (Manilkara zapota L) which inhibited the growth of Escherichia coli and were more effective at a 60%: 40% comparison concentration because they were in the range of 14-16 based on Indonesian pharmacopoeia standard of IV edition.

Keywords: Antibacterial, Kencur, Sawo, Escherichia coli

INTRODUCTION

One of ingredient used to improve antibody and for health keep is Kencur rhizome. Kencur (*Kaempferia galanga* L) rhizome is one of the herb that has been known as medicinal ingredient. Kencur rhizome can heal coughs, heal swelling caused by collisions, boils, diarrhea, reduce nausea and as anti-toxins of Indonesia traditional food that is Tempe bongkrek and mushroom poisoning. Another ingredients leaf is Sapodilla (*Manilkara zapota* L). Sapodilla is a plant originally growing in Central America, Mexico and the West Indies alliance. Traditionally, society uses Sapodilla as an anti-diarrhea medicine. It is because tannin compounds contained in Sapodilla leaves can inhibit and kill bacteria such as *Shigella, Salmonella typhi, and Escherichia coli*.

According to Fajeriyati and Andika research results, kencur extract has a response to *Bacillus subtilis* and *E. coli*, but in this study showed that kencur extract has a stronger inhibitory activity against *B. subtilis* than *E. coli*. At 100% concentration, the kencur rhizome has inhibition zone of 29 mm in *B. subtilis*, while at a concentration of 100% the kencur rhizome has an inhibition zone of 27 mm in *E. coli*. In another study conducted by Mufti *et al*, state that sapodilla leaf extract can inhibit the growth of *E. coli*. At a concentration of 100% sapodilla has an inhibition zone of 14 mm.

E. coli is facultative anaerobic bacterium and has fermentation and respiration metabolism but most of it is grow under anaerobic conditions. *E. coli* is an opportunistic microbe found in human colon as a normal flora. It is a unique bacterium because it can cause primary infection of the intestine such as diarrhea in children and traveler diarrhea, as well as its ability to cause body infection in other the tissues outside intestine. *E. coli* are short rod-shaped microbes (coccobacillus), negative Gram, having a size of 0.4-0.7 µm x 1.4 µm, mostly positive motion and some strains have capsules. The Escherichia genus consists of 2 species: *E. coli* and *E. hermani*.

Kencur rhizome component contains are saponins, flavonoids, polyphenols and essential oil. Flavonoids is one of the largest natural phenol groups. Because it has a number of irreducible hydroxyl groups, flavonoids are polar compounds, so generally flavonoids are dissolve in polar solvents such as ethanol, methanol, butanol, water and others (Fajeriyati & Andika, 2017).

Flavonoids is a group of phenolic compounds that are widely found in plant tissues. Flavonoids is actually found in all parts of the plant including leaf, root, wood, skin, flower, buni leaf and seed. Previous studies have suggested that flavonoids not only function as antioxidants but also has some functions such as protecting cell structures, increasing the effectiveness of vitamin C, anti-inflammatory,

preventing bone loss, anti-diarrhea, anti-diabetic and even antibiotics (Yulianingtyas & Kusmartono, 2016). Sapodilla contains sodium, potassium, calcium, magnesium and phosphorus. Other sapodilla elements and compounds are selenium, zinc, copper, saponins, flavonoids, and vitamin C (Kariman, 2014). It is suspected that saponins can inhibit bacterial growth by inhibiting protein synthesis and lowering the surface tension of the cell so that the leakage occurs. Seeing the inhibitory ability of Escherichia coli from each extract at a concentration of 100% is different, the researcher is interested in conducting a combination of each research.

MATERIAL AND METHODS

Tools and Materials

The materials used in this study were kencur rhizome, sapodilla leaf, *E. coli*, 96% ethanol, and nutrient agar. The equipment used includes oven (Jico), autoclave (Jico), incubator (Memmert), freeze-dryer (Memmert), refrigerator (Sanyo), aseptic cabinet (ESCO), electricity (ACIS), rough configuration (Sigma), blender (National), microscope (SWIFT), rotary evaporator (Buchi), Bunsen lamp, laboratory glassware, aluminum foil, shovel runner, ose needle, cotton, tube clamp, picket, Petri dish, hot plate, flannelette, parchment paper, rope or thread, and paper disc.

Methods

The fresh samples are cleaned from dirt with clean water that flowing and then it draining. Then it is dried by aerated in the open air and protected from direct sun. The sample considers dry if crumpled to be destroyed. The sample is pollinated using a blender and stored in a dry place.

500 grams of dried simplicia powder were put into a tight close jar then macerated using ethanol solvent until the powder was completely submerged, it closed tightly and left for 5 days at room temperature and it protected from sunlight while being stirred frequently. Then it squeezed with flannelette. The pulp is re-macerated with 96% ethanol, it done twice, then the obtained mass is combined. The macerate obtained is left for 2 days in the refrigerator and then poured. The solvent is evaporated using a rotary evaporator at a temperature of not more than 50 °C until a thick extract is obtained. The next process is it dried using a freeze dryer. Weighed 6, 6.5, and 7 grams of thick kencur rhizome extract and then supplemented with 70% alcohol as much as 10 ml.

E. coli taken with sterile ose needle from the stock of culture that has grown and then suspended in a tube containing 10 ml of 0.9% NaCl then incubated at a temperature of 30-35 ° C until the turbidity of the bacterial suspension is equal to the standard turbidity of Mc. Farland, means that the bacterial suspension concentration is 108 CPU / ml. Then dilution was done by 0.1 ml pipette bacteria suspension, put into a sterile tube and added 0.9% NaCl solution as much as 9.9 ml homogeneously shaken, obtained bacterial concentration to 106 CPU / ml.

Pipette 0.1 ml of bacterial suspension with a concentration of 10^6 colony/ml into 100 ml MHA media (temperature 45 °C, do homogeneous, then pour as soon as 15 ml into each sterile petri dish, then let it solidify. Make 5 marks with the bottom of the petri dish as a place for laying disc paper. Soak each disc paper into a mixture of kencur rhizome extract and sawo leaf extract with a ratio of 65 : 35, 60 : 40, 70 : 30, chloramphenicol and 70% alcohol respectively. Leave it for 2 minutes.

Gently lift using tweezers, place the disc paper into a petri dish that already contains the MHA and bacterial suspension aseptically according to the mark that has been made first. Do incubation in an incubator for 24 hours at a temperature of 37 °C. Read the inhibition zone result using calipers in the form of clear-looking areas or the areas that are not overgrown with Escherichia coli bacteria. Record the result in millimeters. This experiment was carried out in triple.

RESULTS AND DISCUSSION

From the result of the measurement of *E. coli* growth inhibition obtained an average inhibition zone at a concentration of 60% : 40% by 15 mm, 65% : 35% by 13.17 mm, and 70% : 30% by 14.17 mm. It shows that of the three average inhibition zones obtained can be categorized as meeting the IV edition of the Indonesian Pharmacopoeia requirements which states that the antibacterial inhibition zone is satisfactory at 14-16 mm. While the measurement results of Chloramphenicol inhibition area on the growth of *E. coli* bacteria obtained at 19.83 mm showed no significant difference between chloramphenicol control with the average yield of the combination inhibitory zone of 15 mm, 13.17 mm and 14.17 mm,

From the results of the average combination inhibition zone extract at a comparison concentration of 60% : 40% at 15 mm, 65% : 35% at 13.17 mm, and 70% : 30% at 14.17 mm, indicating that the effective comparison concentration is at a comparison concentration of 60% : 40% because the antibacterial inhibition zone obtained is 15 mm larger than the results of the ratio of 65% : 35% obtained antibacterial

inhibition zone of 13.17 mm, and 70% : 30% obtained antibacterial inhibition zone 14.17 mm.

With the study result fulfilling of the IV edition of the Indonesian Pharmacopoeia requirement, it can be concluded for the illustration of the chemical compounds contained in the combination of extracts that can inhibit the activity of *E. coli* bacteria, and scientifically prove that ethanol extract combination of kencur rhizome and sapodilla leaf ethanol extract can be used as a traditional medicine for cases of *E. coli* bacteria.

The study of ethanol extract of kencur rhizome that was conducted by Fajeriyati and Andika (2017) concluded that 75% ethanol extract concentration of kencur rhizome was effective against *E. coli* with an average inhibitory zone 24.67 mm. But in this study, the combination concentration of 70% ethanol extract of kencur rhizome and 30% ethanol extract of sapodilla leaf obtained an average inhibition zone of 14.17 mm that still fulfill the requirements of IV edition of Pharmacopoeia.

In the study of ethanol extract sapodilla leaf inhibitory test towards *E. coli* conducted by Mufti *et al* (2017) concluded that at 45% concentration of sapodilla leaf ethanol extract against *E. coli* has average inhibition zone that is 9.08 mm. However, in this study the combination concentration of 60% kencur rhizome and 40% ethanol extract of sapodilla leaf obtained an average inhibition zone of 15 mm that still fulfill the requirement of IV edition of Pharmacopoeia. The larger of the average inhibition zone of the combination kencur rhizome and sapodilla ethanol extract, there is a possibility of potentiation effect from the extract combination.

According to Davis and Stout (1971) state that the provisions of antibacterial power strength as follows: inhibition zone of 20 mm or more is included in very strong category, 10-20 mm inhibition zone included in the strong category, 5-10 mm inhibition zone included in the medium category, inhibition zone 5 mm or less is included in the weak category. If we follow the provisions of Davis and Stout (1971), then the results of this study for all types of combinations including strong category inhibitory zones.

The combination of kencur (Kaempferia galanga L) rhizome ethanol extract and Sapodilla (Manilkara zapota L) ethanol extract can inhibit the growth of Escherichia coli bacteria because each extract contains both flavonoids and saponins. This is evidenced by phytochemical screening conducted by Rahman and Ganguly (2015) that ethanol extract of sapodilla leaves contains alkaloids, flavonoids, tannins, and saponins.

Then it is known that saponins have an active aglycone component which is membranolytic that can cause a decrease in the surface tension of bacterial cell walls. After the surface tension of the bacterial cell wall decrease, saponins form a complex with sterols which causes the formation of a single ion channel. The single ion channel causes instability of cell membranes which inhibits enzyme activity in ion transport which plays a role in bacterial life. The decreased surface tension of the bacterial cell wall can also cause cell leakage so that the intracellular compound exits. This is cause inhibition of bacterial cell growth.

In a study conducted by Latifah (2015) on the ethanolic extract of kencur rhizome which has positive results containing flavonoid. Flavonoid compounds are good reducing compound, inhibit many oxidation reactions, both enzymes and non-enzymes. Flavonoids are the largest group of phenol compound. The function mechanism of flavonoid action is as an antibacterial by forming complex compounds against extracellular proteins which interfere with the integrity of bacterial cell membranes, disrupt cell function of microorganisms and inhibit microbial cell cycle. The action mechanism of denaturing bacterial cell proteins and damaging cell membranes can be irreparably repaired (Juliantina *et al*, 2009).

Table 1. The result of inhibitory zone measurement of kencur rhizome and sapodilla leaf ethanol extract against *E. coli* growth.

Combination (%)	Inhibition Zone (mm)				
	Petri I	Petri II	Petri III	Average	According to 4th FI
60 : 40	20	12	13	15	
65 : 35	11.5	15	13	13.17	
70 : 30	14.5	13	15	14.17	14-16
Chloramphenicol	13	29	17.5	19.83	
Ethanol 70%	0	0	0	0	

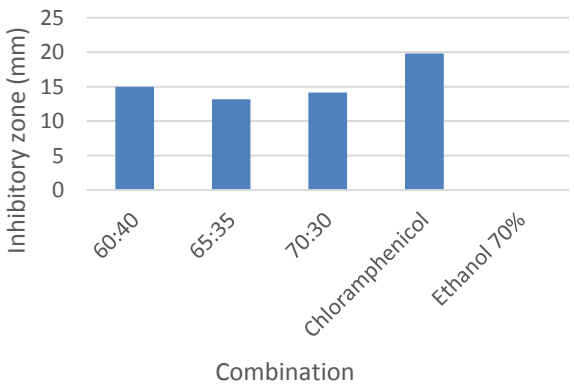

Figure 1. Average inhibitory zone of combination of kencur rhizome and sapodilla leaves on *E. coli*

CONCLUSION

The combination of kencur and sapodilla leaf ethanol extract effectively inhibits the growth of *E. coli*. The highest activity was shown by combination of kencur rhizome and sapodilla leaf ethanol extract at 60% : 40% comparison concentration, which effectively against *E. coli* at largest inhibition zone. However, the combination of kencur rhizome and sapodilla leaf ethanol extract did not approach the inhibition zone of chloramphenicol.

REFERENCES

Davis, W.W., Stout, T.R. 1971. Disc Plate Method of Microbiological Antibiotic Essay. *Applied Microbiology*. 22(4):659-665.

Fajeriyati, N., Andika. 2017. Uji Aktivitas Antibakteri Ekstrak Etanol Rimpang Kencur (Kaempferia galanga L) pada Bakteri Bacillus subtilis dan Escherichia coli. *Journal of Current Pharmaceutical Sciences*. 1(1):36-41.

Juliantina, F., Citra, D.A., Nirwani, B., Nurmasitoh, T., Bowo, E.T. 2009. Manfaat Sirih Merah (Piper crocatum) sebagai Agen Antibakterial terhadap Bakteri Gram Positif dan Bakteri Gram Negatif. *Jurnal Kedokteran dan Kesehatan Indonesia*. 1(1):12-20.

Latifah. 2015. *Identifikasi Golongan Senyawa Flavonoid dan Uji Aktivitas Antioksidan pada Ekstrak Rimpang Kencur (Kaemferia galanga L) dengan metode DPPH (1,1-difenil-2-pikrilhidrazil).* Undergraduate thesis, Universitas Islam Negeri Maulana Malik Ibrahim.

Mufti, N., Bahar, E., Arisanti, D. 2017. Uji Daya Hambat Ekstrak Daun Sawo terhadap Bakteri Escherichia coli secara In Vitro. *Jurnal Kesehatan Andalas*. 6(2):289-294.

Pelczar, M.J., Chan, E.C.S. 1986. *Dasar-dasar Mikrobiologi*. UI Press.

Yulianingtyas, A., Kusmartono, B. 2016. Optimasi volume pelarut dan waktu maserasi pengambilan flavonoid daun belimbing wuluh (Averrhoa bilimbi L). *Jurnal Teknik Kimia*. 10(2):58-64.