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ABSTRACT

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The antibacterial activity test galanga (*Alpinia galangal*) on the growth of becteria *Bacillus subtilis* and *Escherichia coli*

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Abstract. This research was conducted to test the effectiveness of antibacterial inhibition of galangal (Alpinia galanga L.) on the growth of *Escherichia coli* and *Bacillus subtilis* bacteria with concentration 0%, 15%, 30%, 45%, 60%, 75%, 90%, 100 %. The method used in this study with the Kirby-bauer disc diffusion method with 0% concentration as negative control. The results showed that galangal (*Alpinia galanga* L.) at 100% concentration had the highest inhibition area in *Bacillus subtilis* (1,13 mm) and *E. coli* (0,8 mm). Galangal has more effective antibacterial activity in *Bacillus subtilis* bacteria than *E. coli.*

1. Introduction

One of the reasons the occurrence of infectious digestion disease is caused by pathogenic microorganism. Continuous use of antibiotics with excessive dose can lead pathogenic microorganisms to become resistant. Currently, alternative medicines are being developed using plants and herbs as antimicrobial medicine to fight this problem [1]. Indonesia is a country rich with natural resources, especially in agricultural output, as well as plants and herbs. These plants and herbs contain necessary antimicrobial chemical compound from the secondary metabolites. Those chemical compounds are able to induce physiological effects when consumed by humans. Therefore this phenomenom needs to be further researched to study more about herbal remedies for humans [2]. Galangal, or greater galangal, belongs to *Zingiberaceae* family that contains chemical compounds like alkaloids, *saponins*, *glycocids*, terpenoids*, phenols, flavonoids, phytosterols*, and essenstial oils [3]. *E. coli* bacterias and *Bacillus subtilis* are bacterial pathogens belong to enterobacteriaceae family which inhabit in the humans' gastrointestinal tracst and cause diarrheal disease. According to Wardana (2010) from the result of galangal rhizome extract (*Alpinia galanga* L.) shows that the average result of growth inhibition of *Shigella dysentriae* bacteria is 0,5672 cm, *Salmonella typhi* is 0,8169 cm, *Bacillus subtilis* is 0,6717 cm, and *Escherichia coli* is 0,7028 cm [4]. According to Lely *et al* (2017) that the average inhibition diameter of essential oils in red galangal rhizome to *Bacillus cereus* and *E. coli* are at its peak, with 50% concentration, in 19,1 mm and 18,8 mm [5].

According to the analysis above, this needs to be further researched to study the effectivity of antibacteria found in galangal (*Alpinia galanga* L.) to *Escherichia coli* and *Bacillus subtilis* bacterial growth with disc diffusion method.

2. Experimental Method

The galangal used in this study is the rhizome obtained at traditional market in Sidoarjo. Galangal, are then cleaned thoroughly, drained, and dried for about a week, to ensure that galangal are fully dried. Next, dried galangal are weighted to 15 g to obtained concentration

15% b/v. For concentration level to be at 30%, 45%, 60%, 75%, 90%, 100% , the preparation processes are the same as preparing 15% b/v concentrated infused galangal. Weighted dried-galangal are heated with panic infusa contained 1,000ml of Aquades for 15 minutes at 90^0C. These boiled galangal are then drained using layered filter papers. These processes to be repeated 3 times each. Neutral breeding of *Bacillus subtilis* and *E. coli* is renewed at NA medium and is incubated for 24 hours, taken 1oz each and inoquate to sterilized aquades until reaching turbidity of 0.5 *McFarland* (1% $BaCl_2$ 9,95 ml dan 1% H_2SO_4 0,05 ml). Other concentration level used are 0%, 15%, 30%, 45%, 60%, 75%, 90%, 100%. To determine free-microbe zone, we are using difusion method.Sterilized MHA medium are poured in petri dish for about 15ml in aceptic and let it solidified. Sterilized cotton bud is dipped into standard 0.5 *McFarland*, and spread over medium's surface thoroughly. Next, aseptic dish soaked in galangal extract to the surface of the medium, and incubated for 24 hours in 37^0C. Inhibition zone that formed at around paper filter in the dish is being measured in vertical and horizontal diameter by using caliper and formula below [6]:

$$\frac{(Dv-Dc) + (Dh-Dc)}{2}$$

3. Result and Discussion

The average diameter of inhibition zone of galangal extract to Diameter *Bacillus subtilis* and *E.coli* bacteria showed in table 1.

Table 1. Average diamater of inhibition zone of galangal extract *Bacillus subtilis* and *E.coli* bacteria

Bacteria	Concentration (%)							
	0	**15**	**30**	**45**	**60**	**75**	**90**	**100**
Escherihia coli	0	0,46 mm	0,4 mm	0,5 mm	0,7 mm	0,73 mm	0,76 mm	0,8 mm
Bacillus subtilis	0	0,46 mm	0,63 mm	0,56 mm	0,8 mm	0,9 mm	1.03 mm	1,13 mm

The table shown that galangal extracts are able to inhibit the growth *Bacillus subtilis* and *E.coli* bacterias by forming various diameter of inhibition zone. Based on the research of galangal extract concentration to the inhibitation of bacterial growth, the higher the concentration of the extract, the less bacterial growth. This is because the higher the concentration of the extract, the higher the antibacteria found in those extract. In conttrolling using *Aquades* there is no clear zone in the ring, this is because *Aquades* is diluted solution , therefore it does not affect as an antimicrobia. However, this is apply differently to galangal extract to the growth of *E.coli* bacteria. It has the highest average of inhibition zone diameter with 100% concentration, which is 0,8 mm, and for the growth of *Bacillus subtilis* bacteria, the highest average diameter is 1.13mm with 100% concentration.

Bacillus subtilis are positive Gram bakteria which are more sensitive to antibacteria compared to Gram negative (*E. coli)*. This is because bacteria have Gram positive cell wall structure is simple so that the antibacterial compounds easier into cell and found the target to work [7]. It can be seen from the data based on the inhitory zones produced extracts galangal more influential against *Bacillus subtilis* compared with *E. coli*. *Escherichia coli* cell wall consist of more complex lipid (non polar), Phospholipids, lipopolysaccharide polypeptide and so difficult polar compounds contained in the extract to pass through. Galangal contains compounds that polar alkaloids that would more easily penetrate cell wall composed of

peptidoglycan layer which is owned by the bacteria *Bacillus subtilis* than cell walls bacteria *Escherichia coli* is composed of lipid layer that is non polar [8].

Extract galangal against *Bacillus subtilis* happened increase and decrease drag zone on concentration of 30% and 45% this is due to the chemical content in extracts is effected by chemical and biological factors. Biological factors include the location of the original plant of galangal, time of harvesting, storage and galangal rhizome age were used. Chemical factors include the size of the filter material, processing made extraction, content of pesticides on crops, the average levels of the active compounds contain in galangal [9]. Drag zone formed around the paper disc due to the active substances contained in galangal that is alkaloids, saponins, tannins, flavonoids, atsiri oils that as antibacterial [10]. But it is known which substances influential inhibits the growth of bacterial *Bacillus subtilis* and *Escherichia coli.*

Atsiri oils include phenolic is present on the galangal are bacterisidal with denaturation proteins and cytoplasmic membrane damaging cells. The instability of cell wall and cytoplasmic membrane causing selective permeability function, the function active transport, cell protein make up of control distracted. Disruption on cytoplasmic macromolecule and ions this sign may result from bacterial cells. Cells lose form so lisis [11, 12]. Lestari *et al* (2005) proved that drag zone average atsiri oil in the white galangal highest on a 15% concentration of 2,879 mm on growth *Stahphylococcus aureus* [13]. As well as research Parwata and dewi (2008) that the atsiri oil of white galangal rhizome on concentration 100 ppm can hibit growth bacteria *E. coli* drag zone diameters 7 mm [14].

The tannin compound contained in the galangal has spatmolytic effect that is shrinking the cell so that permeability of bacterial cell is disrupted due to permeability disturbed the cell cannot do life activity so that the growth of bacteria is hampered [15]. Tannins also have antibacterial ability by precipitating proteins inactivating proteins and the function of genetic material [16].

Alkaloid compounds also act as antibacterial. Alkaloids can disrupt the formation of bridges across a cross-sectional component of the peptidoglycan of bacterial cells so that the cell wall layer is not completely bogged down which can cause cell death [17].

Flavonoids contained in galangal function inhibit nucleic acid synthesis, inhibit cell membrane permeability and inhibit the enzyme bond of ATPase and Pospolipase [18], microsom and lysosome as the result of interaction between flavonoids with bacterial DNA [10].

Saponin as an antibacterial can cause leakage of proteins and enzymes from within cells [19]. Saponins have an active substance whose surface is similar to detergent as a result can decrease the surface tension of the bacterial cell wall and impair the membrane permeability by diffusing through the outer membrane and cell wall then binding the cytoplasmic membrane so as to disrupt and reduce the stability of the cell membrane causing the cytoplasm out of the cell to result in cell death [20].

4. Conclusion

Overall it can be conclude that the extract of galangal (*Alpinia galangal* L.) has antibacterial activity against bacteria on concentration 100%. The highest zone area on *Bacillus subtilis* (1,13 mm) and *E. coli* (0,8 mm).

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