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Abstract. This research was conducted to determine the antibacterial activity of BSF extract *in vitro* on the growth of *Salmonella typhimurium, E. coli* and *Pseudomonas aureginosa*. The experiment was carried out according to the Completely Randomized Design (CRD) consisting of six treatments and three replications for each treatment. The treatments were different concentration levels of BSF extract, i.e. 75, 125, 175, 225, 275 and 325 $mg.ml^{-1}$. *Chloramphenicol* with concentration of 30 µg.discs $paper^{-1}$ was used as a positive control and *dimethyl sulfoxide* (DMSO) as a negative control. BSF extract was made using maceration extraction method. The results of this study indicated that the antibacterial activity of BSF extract increased ($P<0.05$) in line with the increase level of BSF extract concentration. The average diameter of the inhibition zone for *Salmonella typhimurium, E. coli* and *Pseudomonas aureginosa* was 11.77 ± 0.03 mm, 11.15 ± 0.05 mm, and 11.15 ± 0.23 mm respectively, which was categorized as strong inhibition zone. In conclusion, the concentration of BSF extract of 325 $mg.ml^{-1}$ is an effective concentration to inhibit the growth of the bacteria *Salmonella typhimurium, E. coli* and *Pseudomonas aureginosa*.

1. Introduction

BSF (*Hermetia illucen*. L) is one of the insects that has good potential for use in rations, especially in poultry rations. This can be seen by starting the number of researchers who study the characteristics and nutritional content. According to [1], BSF have 44.9% crude protein content, 29.1% crude fat, 16.4% crude fiber, and 8.1% ash. BSF are also reported to have an amino acid composition that resembles the amino acid composition of soybean meal or fish meal [2]. Besides having a high protein content, BSF are also reported to have antibacterial activity in the form of *Antimicrobial peptides* (AMP) which are bacteriocidal [3].

In addition, BSF larvae are also known to have a high content of lauric acid, a type of fatty acid that can function as a natural antimicrobial agent [4] as well as the content of chitin, polysaccharides that could play a role in increasing the animal's immune response [5]. Research [6] shows that BSF larvae extract has inhibitory activity against *E. coli* and *Salmonella sp*. that included to the group of Gram negative bacteria. The antibacterial activity is very important role in the health and development of digestive tract organs, especially poultry, in the absorption of nutrients.

Prohibition of the use of antibiotics in feed as an optimal growth promoter known as AGP (Antibiotic *growth promotors*) is due to the danger of antibiotic residues in food products of animal origin and its potential to cause resistance both to their own animals and to consumers. Increased bacterial resistance to antibiotics provides a great opportunity to obtain antibacterial compounds. This has made researchers in recent years conduct a lot of research to find alternative ingredients that are natural as a substitute for AGP in feed both from plants and animals, so as to improve the health and immunity status of animal. This study aims to determine the existence of activities antibacterial extract of BSF towards the growth of *Salmonella typhimurium, E. coli* and *Pseudomonas aeruginosa in vitro*.

2. Methods

2.1. Sample collection and preparation

This research was conducted in July to August 2019 in the Biochemistry Laboratory of the Faculty of Mathematics and Natural Sciences and Microbiology Laboratory of the Faculty of General Medicine, Hasanuddin University. The insects used in this study were BSF maggot obtained from cultivation farmers in Depok, West Java. Shortly after collection, insect material is stored in the freezer (–20 °C). Making BSF meal is done by thawing the previously frozen BSF, then washing thoroughly. After that, it is dried in an oven at 60 °C for 24 – 48 hours. After drying, the BSF maggot is gring using a blender until smooth.

2.2. BSF meal extraction

BSF meal extraction is done by referring to the [7] method with a slight modification. The extraction method used is maceration with methanol solvent carried out at room temperature for 24 hours with occasional stirring. Comparison of sample and methanol of 1:10 (w / v). Then the methanol filtrate is separated from the residue by filtering with filter paper. The filtrate was evaporated with a reduced pressure rotary evaporator at 40 °C to obtain concentrated extracts

2.3. Antibacterial activity testing

Antibacterial activity testing was carried out *in vitro* against of the bacteria *Salmonella typhimurium, Escherichia coli,* and *Pseudomonas aeruginosa*. which is a collection of Unhas medical microbiology laboratories. Testing antibacterial activity using agar diffusion by using paper discs [8]. The bacteria to be tested were subcultured first on the *Triptic Soya Agar* (TSA) medium and incubated at 35 ± 1°C for 24 hours. Furthermore, bacteria that have been subcultured that have grown on TSA medium are made suspensions until a population of 10^7 cfu is obtained. A total of 0.1 ml of suspension was taken using a volumetric pipette and put into MHA medium (*Muller Hilton Agar*). The suspension is flattened on the surface of the MHA medium with the help of bent glass rods. The MHA medium was then allowed to stand for 15 minutes at room temperature. Previously, BSF extract was dissolved first with *dimethyl sulfoxide* (DMSO) solution according to the desired concentration (75 mg ml^{-1}, 125 mg ml^{-1}, 175 mg ml^{-1}, 225 $mgml^{-1}$, 275 mg ml^{-1} and 325 mg ml^{-1}) with three replications at each concentration. The *chloramphenicol* antibiotic was used as a positive control with a concentration of 30 µg. discs $paper^{-1}$. Petri dishes containing *Muller Hinton Agar* (MHA) are subjected to a bacterial suspension of about 1.5 x 10^8 to the entire surface so that they are evenly allowed to dry for about 1 hour. 60 µl BSF extract was dripped on each discs paper with a micropipette for each concentration and placed in a petri dish containing sterile MHA, then the petri dish was incubated at 37 °C for 24 hours.

2.4. Observation and measurement

Observations were made after 24 hours of incubation period. Clear zone is an indication of bacterial sensitivity to antibacterial material used as a test material expressed by the wide diameter of inhibitory zone [9]. Inhibitory zone diameters are measured using digital calipers in millimeters (mm).

2.5. Statistical analysis

In vitro antibacterial activity test data were analyzed using Analysis of Variance (ANOVA) with a 95% confidence level or α = 0.05. If the treatment has a significant differences, followed by *Duncan* test

3. Results and discussion

We know that insects have a well-developed *innate immune system*. This immune system consists of cellular immune system and humoral immune system. The production of *Antimicrobial peptides* (AMP) is related to the humoral immune system which is synthesized in fat organs and then secreted into hemolymph fluid. It has been reported by previous studies that there is antimicrobial activity both from BSF extract and hemolymph fluid [10].

The results of the *in vitro* antibacterial activity test using the diffusion method of BSF methanol extract discs paper on the growth of *Salmonella typhimurium, E. Coli* and *Pseudomonas aeruginosa* are presented in (table 1) and (figure 1).

Table 1. Results of measurement of *in vitro* antibacterial activity of BSF extract towards the growth of *Salmonella typhimurium, E. Coli* and *Pseudomonas aeruginosa* after incubation for 24 hours at 37°C.

Concentration	Bacterial Growth Inhibition Zone Diameter (mm)		
	Salmonella typhimurium	*E. Coli*	*Pseudomonas aeruginosa*
75 mg.ml^{-1}	7.25 ± 0.05^{a}	6.78 ± 0.06^{a}	6.81 ± 0.07^{a}
125 mg.ml^{-1}	7.83 ± 0.07^{b}	7.02 ± 0.03^{b}	7.58 ± 0.07^{b}
175 mg.ml^{-1}	8.87 ± 0.03^{c}	7.67 ± 0.07^{c}	8.73 ± 0.06^{c}
225 mg.ml^{-1}	10,15 ± 0.26^{d}	8.55 ± 0.05^{d}	9.56 ± 0.03^{d}
275 mg.ml^{-1}	11,47 ± 0.07^{e}	9.35 ± 0.0^{e}	10.23 ± 0.14^{e}
325 mg.ml^{-1}	11.77 ± 0.03^{f}	10.15 ± 0.05^{f}	11.15 ± 0.35^{f}
Control (+)	14.74 ± 0.38	27.61 ± 0.35	16.47 ± 0.09
Control (-)	0	0	0

The numbers followed by different letters (a,b,c,d,e,f) in the same column mean significantly different (P<0.05) BSF extract based on the *Duncan* test.

(a) (b) (c)

Figure 1. Test result of antibacterial activity of methanol extract BSF. a = *Salmonella typhimurium,* b = *E. coli*, c = *Pseudomonas aeruginosa.*

Variance analysis data (ANOVA) showed that the diameter of inhibitory zones of *Salmonella typhimurium, E. coli* and *Pseudomonas aeruginosa* showed a significant value of 0.000 (P <0.05), which means there was a significant difference between the treatments given to test bacteria (Gram negative). This shows that the six concentration and positive control of methanol extract of BSF both concentrations of 75, 125, 175, 225, 275 and 325 mg.ml^{-1} have provided activities that inhibit the growth of bacteria *Salmonella typhimurium, E. coli* and *Pseudomonas aeruginosa*. Inhibition zone diameter of *Salmonella typhimurium, E. coli* and *Pseudomonas aeruginosa* bacteria for negative control, showed significant differences in positive control and several levels of extract concentration. Where the negative control shows the absence of inhibitory zones. This gives an indication that the control used has no effect on the antibacterial test while the positive control shows the antibacterial activity against the test bacteria.

Based on the results of *in vitro* antibacterial activity testing of BSF extract showed that BSF extract has inhibitory activity against *Salmonella typhimurium, E. coli* and *Pseudomonas aeruginosa* bacteria which are included in the Gram negative bacteria group. It has been reported by previous studies that methanol extract from BSF shows a higher level of sensitivity to Gram-negative bacteria compared to Gram-positive bacteria. This difference may be due to differences in the interaction of bacterial cell wall components with active compounds from BSF [7].

Duncan test results showed that the inhibition zone diameter for extract concentration 325 $mg.ml^{-1}$, for *Salmonella typhimurium* (11.77 ± 0.03 mm), *E. Coli* (10.15 ± 0.05 mm), and *Pseudomonas aeruginosa* (11.15 ± 0.35 mm) showed differences significant ($P<0.05$) to each of the other extract concentrations, namely concentrations of 75, 125, 175, 225, and 275 $mg.ml^{-1}$. This means the concentration of the extract has shown different activities in inhibiting the growth of the three bacteria. The best antibacterial activity is at the concentration of extract 325 $mg.ml^{-1}$, even at the lowest concentration of 75 $mg.ml^{-1}$ can still inhibit the growth of the third the bacteria with a diameter of 7.25 ± 0.05 mm, 6.78 ± 0.06 mm, and 6.81 ± 0.07 mm.

Based on the results of this test, it was seen that the antibacterial activity of BSF extract increased with increasing concentration. The antibacterial activity was first seen at a concentration of 75 mg ml^{-1} and this activity increased with increasing concentrations to 325 mg ml-1 for the three types of bacteria tested. According to [11] the antibacterial growth inhibition response is divided into several categories, namely the inhibition zone diameter of 5 mm or less is categorized as weak, the inhibition zone of 5-10 mm is categorized as medium, the inhibition zone of 10-20 mm is categorized as strong and the inhibitory zone is 20 mm or more is categorized as very strong.

Based on the growth inhibition response, the antibacterial inhibition of BSF extract on *Salmonella typhimurium* bacteria with extract concentrations of 75 $mg.ml^{-1}$ (7.25 ± 0.05 mm), 125 $mg.ml^{-1}$ (7.83 ± 0.07 mm) and 175 $mg.ml^{-1}$ (8.87 ± 0.03 mm) including moderate and concentration of extract 225 $mg.ml^{-1}$ (10.15 ± 0.26 mm), 275 $mg.ml^{-1}$ (11.47 ± 0.07 mm) and 325 $mg.ml^{-1}$ (11.77 ± 0.03 mm) are strong. The antibacterial inhibition of *E. coli* at extract concentration of 75 $mg.ml^{-1}$ (6.78 ± 0.06 mm), 125 $mg.ml^{-1}$ (7.02 ± 0.03 mm) and 175 $mg.ml^{-1}$ (7.67 ± 0.07 mm) 225 mg. ml^{-1} (8.55 ± 0.05 mm), 275 $mg.ml^{-1}$ (9.35 ± 0.00 mm) including moderate and 325 $mg.ml^{-1}$ (10.15 ± 0.05 mm) including strong. *Pseudomonas aureginosa* antibacterial inhibition at extract concentration of 75 $mg.ml^{-1}$ (6.81 ± 0.07 mm), 125 $mg.ml^{-1}$ (7.58 ± 0.07 mm), 175 $mg.ml^{-1}$ (8.73 ± 0.06 mm), 225 mg. ml^{-1} (9.56 ± 0.03 mm) included moderate criteria while extract concentration was 275 mg.ml-1 (10.23 ± 0.14 mm) and 325 $mg.ml^{-1}$ (11.15 ± 0.35 mm) was strong. Based on the results obtained, it is known that the extract concentration of 325 $mg.ml^{-1}$ is an effective concentration to inhibit the bacteria *Salmonella typhimurium, E. coli* and *Pseudomonas aureginosa* and specifically, the antibacterial activity of BSF extract is higher in *Salmonella typhimurium* than in *E. coli* and *Pseudomonas aureginosa* (11.77 ± 0.03 mm, 10.15 ± 0.05 mm and 11.15 ± 0.35 mm), so that the antibacterial inhibition in the concentration is categorized strong to cause a large inhibitory zone.

Positive control showed the greatest antibacterial inhibition against test bacteria compared with negative control and various extract concentrations. The antibiotic used as a comparison or positive control is *chloramphenicol*. The negative control used is DMSO which indicates no inhibition zone. This indicates that the control used has no effect on the antibacterial test. The relationship of concentration and diameter of the antibacterial inhibitory zone of BSF extract against pathogenic bacteria *Salmonella typhimurium, E.coli* and *Pseudomonas aureginosa* is presented in figure 2.

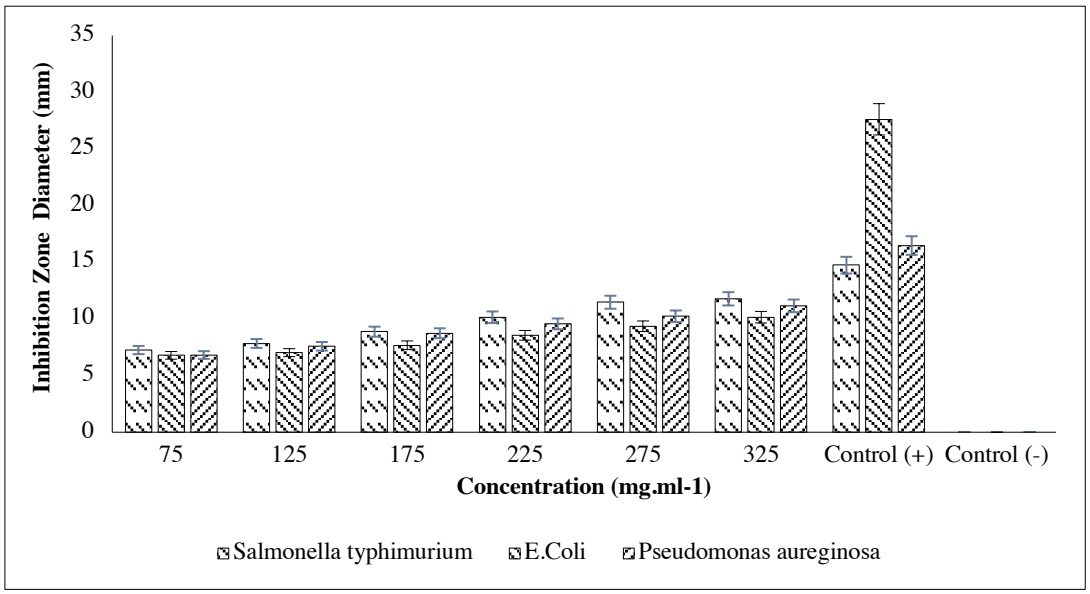

Figure 2. Relationship of concentration and diameter of inhibition zone of BSF extract towards the growth of *Salmonella typhimurium, E. coli* and *Pseudomonas aureginosa* Bacteria.

Methanol extract showed an antibacterial effect on the proliferation of Gram negative bacteria. These results indicate a significant difference in the effect of methanol extract on Gram positive and negative bacteria. The antibacterial effect of metanol extract increases with increasing concentration, but its activity decreases after 24 hours. The difference in sensitivity between the extract and bacteria can occur due to differences in interaction between the cell nucleus components and the bacterial cell wall with the active compound contained in the extract [7].

BSF can live and adapt to various adverse environmental conditions such as cow dung, compost and even on carcasses, which are inhabited by various types of bacteria and fungi so that they are termed scavengers. Livestock manure and dead plants can be decomposed and recycled by BSF or larvae. It is assumed that such environmental conditions will affect the development of insect innate immune systems. This biological characteristics make the BSF have various types of AMP (*Antimicrobial peptides*) and other compounds in the formation process that have inhibitory properties against various types of pathogenic microorganisms [3].

One type of AMP that is present in insects and has been marked as defensins. The mechanism of action of insects in general is to form channels on the bacterial cytoplasmic membrane. *Defensins* have a high attachment to *cardiolipin*, the main type of *phospholipids* in bacteria. This interaction between *defensins* and *phospholipids* can induce microheterogeneity in the lipid membrane, which is likely related to the formation of channels that are responsible for defensin's biological activity [12].

AMP is an *innate immune system*, BSF maggot, but it is also known to have a high content of lauric acid and is one type of saturated fatty acids (*medium chain fatty acids* / MCFA) that function as natural antimicrobial agent. The main target of MCFA as an antimicrobial is cell membrane, which causes membrane damage so that it can accelerate the entry of antimicrobial compounds into the cytoplasm which accelerates bacterial death. In addition, it is known that hydrogen ions are powerful bacterial killing agents in the cytoplasm, but these ions cannot enter the cell through the membrane due to polarity differences. Therefore, damage to cell membranes due to MCFA can accelerate uptake of hydrogen ions from extracellular fluid into cells [4].

4. Conclusion

BSF extract has antibacterial activity against *Salmonella thypimurium, E. Coli* and *Pseudomonas aeruginosa*. Increasing the concentration of BSF extract resulted in a wider diameter of inhibition. BSF extract concentration 325 $mg.ml^{-1}$ is an effective concentration to inhibit the growth of *Salmonella typhimurium, E. coli* and *Pseudomonas* aureginosa bacteria.

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