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Antibacterial Activity of *Hermetia illucens* (Black Soldier Fly) Maggot Extract Against *Salmonella typhi*

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Abstract: *Typhoid fever is an acute febrile illness caused by infection with Salmonella typhi. This study aimed to evaluate the antibacterial activity of Black Soldier Fly (Hermetia illucens) maggot extracts against Escherichia coli and Salmonella typhi. Extraction was performed using successive maceration with acetone, methanol, and water as solvents. Antibacterial activity was assessed using the disc diffusion method. The most active extract was further fractionated using n-hexane, dichloromethane, ethyl acetate, and water. The results showed that the acetone extract of maggots exhibited the highest antibacterial activity against S. typhi, with an inhibition zone of 8.13 ± 0.15 mm at a concentration of 10,000 ppm. Among the fractions, the n-hexane fraction demonstrated the strongest antibacterial activity, with inhibition zones of 8.37 ± 0.32 mm against E. coli and 9.00 ± 0.00 mm against S. typhi at a concentration of 2,500 ppm.*

Keywords: Antibacterial, *Escherichia coli,* Maggot BSF, *Salmonella typhi*

Abstrak: Penyakit tifus merupakan penyakit demam akut yang disebabkan oleh infeksi Salmonella typhi. Penelitian ini bertujuan untuk mengevaluasi aktivitas antibakteri ekstrak maggot Black Soldier Fly (Hermetia illucens) terhadap Escherichia coli dan Salmonella typhi. Ekstraksi dilakukan dengan metode maserasi bertingkat menggunakan pelarut aseton, metanol, dan air. Aktivitas antibakteri diuji menggunakan metode difusi cakram. Ekstrak terbaik kemudian difraksinasi menggunakan n-heksana, diklorometana, etil asetat, dan air. Hasil menunjukkan bahwa ekstrak aseton maggot memiliki aktivitas antibakteri tertinggi terhadap S. typhi dengan zona hambat sebesar 8,13 ± 0,15 mm pada konsentrasi 10.000 ppm. Fraksi n-heksana menunjukkan aktivitas tertinggi dibandingkan fraksi lainnya, dengan zona hambat 8,37 ± 0,32 mm terhadap E. coli dan 9,00 ± 0,00 mm terhadap S. typhi pada konsentrasi 2.500 ppm.

Kata kunci: Antibakteri, *Escherichia coli,* Maggot BSF, *Salmonella typhi*

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1. Introduction

Typhoid fever is an acute febrile illness caused by infection with the bacterium *Salmonella typhi* (Alba, 2016). Transmission occurs via the fecal–oral route, whereby the pathogen enters the human body through ingestion of contaminated food or water (Mogasale et al., 2016). Typhoid fever remains a significant public health concern in several countries. According to the World Health Organization (WHO), approximately 17 million deaths occur annually due to this disease. Asia reports the highest incidence, with an estimated 13 million cases each year. Since 2017, it has been estimated that between 800 and 100,000 individuals in Indonesia contract typhoid fever annually. Notably, 91% of these cases occur in children aged 3–19 years, with an annual mortality rate of around 20,000 deaths (Bahar et al., 2017).

The standard treatment for typhoid fever is antibiotic therapy. However, antibiotic resistance has become a serious global issue, affecting *S. typhi* among many other bacterial pathogens. Over the past decade, *S. typhi* strains carrying plasmid-encoded resistance to chloramphenicol—formerly the drug of choice for typhoid—have emerged. This resistance has been reported in the Indian subcontinent, Southeast Asia, and Africa (Cita, 2011). As a

result, alternative therapeutic approaches are being explored, one of which involves the use of Black Soldier Fly (BSF) maggots.

The Black Soldier Fly (*Hermetia illucens*), a member of the family Stratiomyidae (Diptera), is found in tropical and subtropical regions (46° N – 42° S). Its life cycle comprises five stages—egg, larva (maggot), prepupa, pupa, and adult—lasting approximately 24–38 days (Martínez-Sánchez et al., 2011). BSF maggots are efficient scavengers capable of thriving in extreme environmental conditions, such as compost and other organic waste, which are often inhabited by diverse bacteria and fungi. Consequently, BSF maggots possess a well-developed innate immune system. They are commonly used to degrade organic waste from households, restaurants, and hospitals. Notably, BSF maggots are rich in antimicrobial peptides (AMPs), which exhibit inhibitory activity against a variety of pathogenic microorganisms (Park et al., 2014).

In addition, BSF maggots are known to have a high lauric acid content, a fatty acid with natural antimicrobial properties (Kim & Rhee, 2016). They can also eliminate bacteria present in organic waste, including potential pathogens such as *S. typhi*, suggesting their potential as an alternative treatment for typhoid fever. Previous studies have shown that BSF maggot extracts are effective against Gram-negative bacteria such as *Klebsiella pneumoniae*, *Neisseria gonorrhoeae*, and *Shigella sonnei*. Among the extraction methods tested, polar solvents were found to be the most effective for isolating antibacterial compounds against Gram-negative bacteria (Choi et al., 2012).

To date, no studies have specifically investigated the bioactive compounds in BSF maggots and their antibacterial activity against *S. typhi*. This study aims to evaluate the antibacterial potential of BSF maggot extracts against *S. typhi*, with the goal of identifying a novel therapeutic option for typhoid fever. The working hypothesis is that BSF maggots contain AMPs and lauric acid capable of disrupting bacterial cell membranes, thereby exerting antibacterial effects against *S. typhi*. The findings of this research are expected to contribute valuable scientific information on the antibacterial properties of *H. illucens* maggots and their potential role as an alternative treatment for typhoid fever.

2. Metodhology

The equipment used in this study included glassware, a rotary vacuum evaporator, oven, micropipettes, mortar, magnetic stirrer, water bath, autoclave, shaker, desiccator, water bath, laminar air flow cabinet, sonicator, spreader, vernier caliper, Petri dishes, and inoculating loops. The materials used were Black Soldier Fly (BSF) maggots obtained from PT Biomagg Indonesia, dimethyl sulfoxide (DMSO), tryptic soy agar (TSA) medium, methanol, acetone, *n*-hexane, ethanol, ethyl acetate, dichloromethane, paper discs, filter paper, cotrimoxazole, distilled water, ethanol spirit, *Salmonella typhi* (obtained from the Microbiology Laboratory, Department of Biology, IPB University), and *Escherichia coli* (obtained from the Microbiology Laboratory, Department of Biology, IPB University).

2.1. Sample Preparation

Black Soldier Fly (BSF) maggots used in this study were obtained from PT Biomagg Indonesia. The samples were prepared in two forms: wet samples and powdered samples. The distinction was based on the method used to terminate the maggots. For the wet samples, BSF maggots were euthanized by adding acetone and left for 30 minutes, after which they were ground using a mortar. For the powdered samples, BSF maggots were euthanized by freezing at −20 °C for 24 hours, thawed by immersion in water, then dried in an oven at 60 °C for 24 hours. The dried maggots were then finely ground using a blender and weighed (Yantina 2016).

2.2. Moisture Content Determination of the Simplicia

Three empty covered porcelain crucibles were dried in an oven at 105 °C for 3 hours and cooled in a desiccator for 30 minutes before weighing. Approximately 2 g of BSF maggot sample was placed into each crucible, and the total weight was recorded. The crucibles containing the samples were then returned to the oven at 105 °C for 3 hours. Weighing was repeated three times until a constant weight was obtained (AOAC, 2005). The test was conducted in triplicate, and the moisture content was calculated using the following formula:

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

2.3. Successive Extraction of Maggots

Extraction was performed using the maceration method with three different solvents: acetone, methanol, and distilled water. Two sample types—wet maggots and powdered maggots—were used, with each sample weighing 20 g. The samples were placed into Erlenmeyer flasks, and 200 mL of 100% acetone was added. The mixtures were soaked and agitated in a shaker for 24 hours, followed by filtration through filter paper. The residue was re-macerated once more with 100% acetone. After the second acetone extraction, the residue was subjected to methanol extraction under the same conditions: soaking and shaking for 24 hours, followed by filtration. The methanol extract was further subjected to a second maceration step with 100% methanol.

Aqueous extraction was carried out by mixing 20 g of BSF maggots with distilled water at a 1:10 ratio, followed by heating in a water bath at 90 °C for 30 minutes. The macerates obtained from each extraction step were concentrated using a rotary vacuum evaporator at 50 °C (Susanti & Bachmid, 2016). The extraction yield was calculated using the following equation:

$$\text{Yield (\%)} = \frac{\text{Weight of Extract}}{\text{Weight of Maggots}} \times 100$$

2.4. Antibacterial Assay of Maggot Extracts

The antibacterial activity was assessed using the disc diffusion method. Initial screening was conducted against *Escherichia coli* to identify extracts with potential antibacterial activity. For this assay, 6 g of TSA powder was dissolved in sterile distilled water, heated, and stirred until boiling. The medium was sterilized in an autoclave at 121 °C for 15 minutes and cooled at room temperature for 30 minutes under sterile conditions. The medium was then poured into three sterile Petri dishes. Each dish was marked into seven equal sections: 0 (positive control), 1 (wet methanol extract), 2 (wet acetone extract), 3 (wet aqueous extract), 4 (powdered methanol extract), 5 (powdered acetone extract), 6 (powdered aqueous extract), and 7 (negative control).

Once solidified, the surface of the TSA medium was inoculated with *E. coli* suspension (10^6 CFU/mL) and evenly spread using a sterile glass spreader. Sterile paper discs were impregnated with 20 µL of extract at a concentration of 10,000 ppm (dissolved in 20% DMSO). Cotrimoxazole (250 ppm) served as the positive control, and 20% DMSO served as the negative control. The discs were placed on the surface of the inoculated agar and incubated at 37 °C for 24 hours. Antibacterial activity was determined by measuring the diameter of the inhibition zones around the discs (in mm) using a vernier caliper. The extract showing the highest inhibitory activity against *E. coli* was subsequently tested against *Salmonella typhi*.

For the *S. typhi* assay, the procedure was identical, except that each Petri dish was divided into three equal sections. TSA was prepared and sterilized as described above, inoculated with *S. typhi* suspension (10^6 CFU/mL), and spread evenly. Sterile discs containing 20 µL of extract at 10,000 ppm (in 20% DMSO) were placed on the agar surface, with cotrimoxazole (250 ppm) as the positive control and 20% DMSO as the negative control. Plates were incubated at 37 °C for 24 hours. The extract with the best antibacterial activity was selected for further fractionation (Febriyani et al., 2018, with modifications).

2.5. Fractionation of Maggot Extract

The maggot extract was fractionated using *n*-hexane (non-polar), dichloromethane, ethyl acetate (semi-polar), and water (polar) as solvents, based on polarity, via liquid–liquid partitioning in a separatory funnel. A total of 5 g of maggot extract was dissolved in 50 mL of *n*-hexane and 50 mL of water (1:1, v/v). The mixture was homogenized by shaking and allowed to stand for 30 minutes until two distinct layers formed: *n*-hexane (upper layer) and water (lower layer). The *n*-hexane layer was collected, while the aqueous layer was further

fractionated with 50 mL of dichloromethane to yield a dichloromethane fraction and an aqueous fraction. The aqueous layer was subsequently fractionated with 50 mL of ethyl acetate, producing ethyl acetate and aqueous fractions.

The final aqueous fraction was rinsed with an additional 50 mL of water to ensure complete recovery from the separatory funnel. Fractionation with each solvent was repeated three times. Four distinct fractions were obtained: *n*-hexane, dichloromethane, ethyl acetate, and water. Saturated NaCl solution was added to each fraction (except the aqueous fraction) using the same partitioning technique, followed by the addition of anhydrous Na_2SO_4 to remove residual moisture. All fractions were concentrated using a rotary vacuum evaporator at 50 °C (Hasim et al., 2018). The percentage yield of each maggot fraction was calculated using the following formula:

$$\text{Fraction Yield (\%)} = \frac{\text{Weight of Fraction Extract x Weight of Acetone Extract}}{\text{Sample Weight Before Extraction}} \times 100$$

2.6. Fractionation of Maggot Extract

Tryptic Soy Agar (TSA) medium was prepared by dissolving 6 g of TSA powder in sterile distilled water, followed by heating in a water bath and stirring until boiling. The medium was sterilized in an autoclave at 121 °C for 15 minutes, then cooled to room temperature for 30 minutes under sterile conditions. The sterile medium was poured into four sterile Petri dishes, each marked with lines dividing the surface into seven equal sections.

Once solidified, the surface of each TSA plate was inoculated with *Salmonella typhi* and *Escherichia coli* suspensions (10^6 CFU/mL) and evenly spread using a sterile glass spreader. Sterile paper discs were then impregnated with 20 µL of extract fraction at concentrations of 1,000 and 2,500 ppm (dissolved in 20% DMSO). Cotrimoxazole (250 ppm) served as the positive control, while 20% DMSO served as the negative control.

The discs were placed on the agar surface and incubated at 37 °C for 24 hours. Antibacterial activity was evaluated by measuring the diameter of the clear inhibition zones around each disc (in mm) using a vernier caliper (Febriyani et al., 2018, with modifications).

3. Result

3.1. Moisture Content and Yield of BSF Maggot Extracts

Two types of BSF maggot samples were prepared, namely wet maggots and powdered maggots, with the aim of screening their antibacterial activity against *Escherichia coli* and *Salmonella typhi*. From 200 g of wet maggots, 50.56 g of powdered maggots was obtained, corresponding to a yield of 25%.

Moisture content testing was carried out to assess the storage stability of the samples. As shown in Table 1, the moisture content of wet BSF maggots was 75%, while powdered BSF maggots contained only 6.67% moisture. Extraction by maceration yielded the highest percentage of wet maggot extract in acetone (12.76%), followed by aqueous extract (8.38%), and the lowest in methanol extract (2.49%). In powdered maggots, the acetone extract showed the highest yield (14.13%), followed by the aqueous extract (13.22%) and methanol extract (6.20%).

(**a**) (**b**)

Figure 1. Maggot BSF: (**a**) Wet sample (**b**) Powder sample

Table 1. Moisture Content and Yield of BSF Maggot Extracts

Sample	Moisture content	Yield
Methanol extract wet maggot		2.49%
Acetone extract wet maggot	75%	12.76%
Aquades extract wet maggot		8.38%
Methanol extract powder maggot		6.20%
Acetone extract powder maggot	6.67%	14.13%
Aquades extract powder maggot		13.22%

3.2. Antibacterial Activity of BSF Maggot Extracts

Antibacterial activity assays were performed to identify the most active extract capable of inhibiting Gram-negative bacteria, prior to further testing against *S. typhi*. As shown in Table 2, the methanol extract of powdered maggots at a concentration of 10,000 ppm exhibited the highest inhibitory activity against *E. coli*, producing an inhibition zone of 9.10 ± 0.01 mm. The lowest activity was observed in the acetone extract of wet maggots, which showed no inhibition (0.00 ± 0.00 mm), indicating no antibacterial activity.

Based on these findings, the powdered maggot extracts obtained with acetone and methanol were selected for further antibacterial testing against *S. typhi*. As shown in Table 3, the acetone extract at 10,000 ppm demonstrated antibacterial activity with an inhibition zone of 8.13 ± 0.001 mm. The positive control, cotrimoxazole at 250 ppm, produced inhibition zones of 16.73 ± 4.059 mm (*E. coli*) and 23.03 ± 0.25 mm (*S. typhi*), while the negative control (20% DMSO) showed no inhibition (0.00 ± 0.00 mm). Among the tested samples, the acetone extract demonstrated the strongest antibacterial activity and was therefore selected for fractionation.

Table 2. Moisture Content and Yield of BSF Maggot Extracts

Sample	Zone of inhibition (mm) Mean ± deviation standard
DMSO 20%	0.00±0.00
Cotrimoxazole (250 ppm)	16.73±4.059
Methanol extract wet maggot	0.00±0.00
Acetone extract wet maggot	2.33±4.041
Aquades extract wet maggot	4.53±3.93
Methanol extract powder maggot	8.63±1.95
Acetone extract powder maggot	9.10±0.794
Aquades extract powder maggot	4.70±4.07

Table 3. Antibacterial activity of BSF maggot powder extract against *S.typhi*

Sample	Zone of inhibition (mm) Mean ± deviation standard
DMSO 20%	0.00±0.00
Cotrimoxazole (250 ppm)	23.03±0.25
Methanol extract powder maggot	2.10±3.637
Acetone extract powder maggot	8.13 ±0.153

3.3. Yield and Antibacterial Activity of BSF Maggot Extract Fractions

The acetone extract of powdered maggots, which showed the highest antibacterial activity against *S. typhi*, was fractionated using successive liquid–liquid partitioning with n-hexane, dichloromethane, ethyl acetate, and water. The yields of the respective fractions were 5.35%, 1.11%, 0.66%, and 5.47%. Among them, the aqueous fraction had the highest yield, whereas the ethyl acetate fraction had the lowest.

The antibacterial activity of the fractions was tested against *E. coli* and *S. typhi* at concentrations of 1,000 ppm and 2,500 ppm. The n-hexane fraction exhibited the strongest activity against *E. coli*, with inhibition zones of 7.67 mm and 8.37 mm at 1,000 ppm and 2,500 ppm, respectively. Similarly, the n-hexane fraction also showed the highest activity against *S. typhi*, producing inhibition zones of 8 mm and 9 mm at 1,000 ppm and 2,500 ppm, respectively (Table 4, Table 5).

An increase in extract concentration consistently enhanced the inhibition zone diameter across all fractions. Cotrimoxazole (250 ppm) was used as the positive control, producing inhibition zones of 15 mm against *E. coli* and 17 mm against *S. typhi*. The negative control (20% DMSO) showed no inhibitory effect. DMSO was also used as the solvent for preparing all sample fractions.

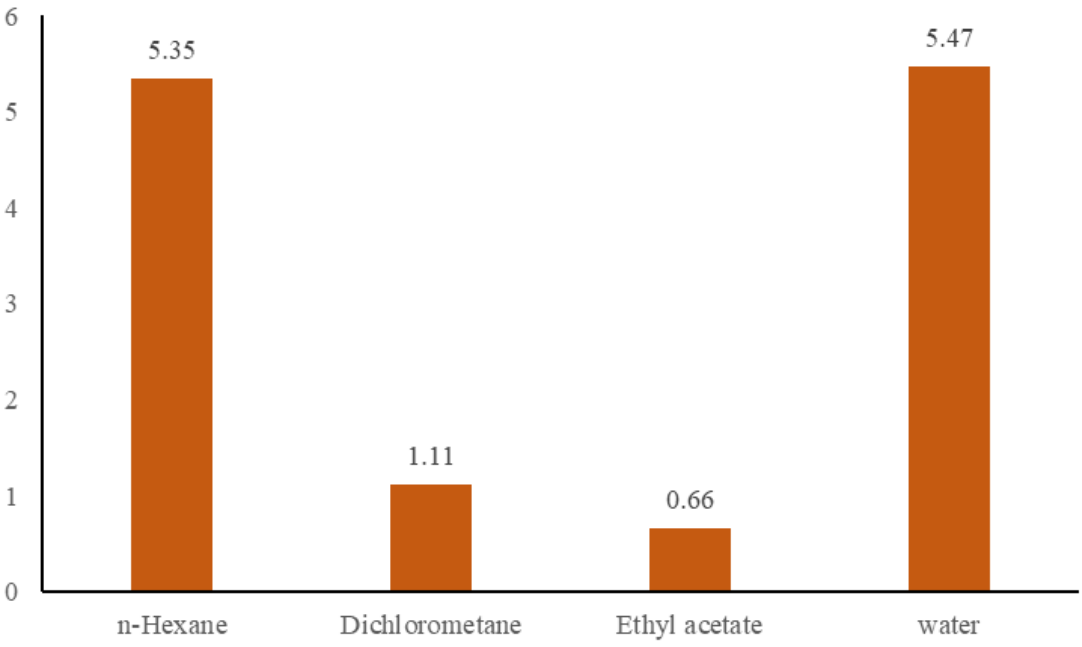

Figure 1. Yield BSF fraction

Table 4. Antibacterial activity of BSF fraction against *E. coli*

Sample	Zone of inhibition (mm) Mean ± deviation standard	
	1000 ppm	**2500 ppm**
DMSO 20%	0.00±0.000	0.00±0.000
Cotrimoxazole (250 ppm)	15.00±0.000	15.00±0.000
n-Hexane fraction	7.67±0.153	8.37±0.321
Dichlorometane fraction	7.17±0.289	7.73±0.153
Ethyl acetate fraction	7.10±0.100	7.53±0.058
Water fraction	7.60±0.436	7.90±0.173

Table 5. Antibacterial activity of BSF fraction against *S. typhi*

Sample	Zone of inhibition (mm) Mean ± deviation standard	
	1000 ppm	**2500 ppm**
DMSO 20%	0.00±0.000	0.00±0.000
Cotrimoxazole (250 ppm)	17.00±0.000	17.00±0.000
n-Hexane fraction	8.00±0.000	9.00±0.000
Dichlorometane fraction	7.27±0.252	7.70±0.300
Ethyl acetate fraction	7.27±0.252	7.67±0.153
Water fraction	7.20±0.100	8.00±0.000

4. Discussion

3.1. Moisture Content and Yield of Maggot Extract

The regulation of the Indonesian National Agency of Drug and Food Control (BPOM) number 12 of 2014 concerning the quality requirements for traditional medicines states that the permissible moisture content of a simplicia is less than 10%. Therefore, maggot powder with a moisture content of 6.67% is still considered suitable for use as a herbal material and meets the requirements for further testing. Herbal materials with a moisture content of more than 10% will affect the extract yield obtained. The extract will contain more water than the desired bioactive content (BPOM 2014).

BSF maggot samples were extracted using three solvents: acetone, methanol, and water. The yield values of the extracts obtained were then calculated. Extraction of maggot samples produced different yields depending on the type of sample and solvent used. Extracts of wet maggot samples were lower than those of maggot powder. This is because smaller particle sizes increase contact surface area and enhance interaction with solvents. Powder-sized samples make it easier for solvents to penetrate and bind to compounds within the sample (Taiz and Zeiger 2015).

Extraction is the separation of bioactive compounds from plants using selective solvents through specific procedures. During extraction, the solvent diffuses into the plant cell wall and dissolves bioactive compounds with a polarity level similar to that of the solvent used. The choice of the maceration (soaking) method was due to its simplicity and effectiveness compared to other methods, as maceration is suitable for extracting heat-sensitive compounds. Soaking maggot powder in certain solvents allows its bioactive compounds to be released (Falah et al. 2015).

The highest yield was obtained from extracts with acetone as solvent, followed by water and methanol. The results indicate that the bioactive components contained in the Black Soldier Fly maggot sample are more semi-polar in nature, so the acetone yield was higher than

other solvents, with 14.13% for maggot powder and 12.76% for wet maggots. According to Choi et al. (2012), maggot extracts using methanol and aquadest solvents produced yields of 2.0% and 0.52%, respectively, while acetone extracts had not been studied. These differences are due to the agrobio-physical conditions where the maggots grow. Weather, humidity, feed, physical and chemical conditions of the medium, and different extraction methods will affect the number of secondary metabolites produced (April 2016).

Extracts that had undergone antibacterial testing against *E. coli* and *S. typhi* were then fractionated using a liquid-liquid fractionation method. Liquid-liquid fractionation is a separation method based on the distribution of one or more compounds between two immiscible or nearly immiscible solvents. This fractionation is a bioactivity-guided isolation step for identifying active compounds from natural products (Laila 2016). The extract was fractionated using n-hexane (non-polar), dichloromethane (semi-polar), ethyl acetate (semi-polar), and water (polar) to separate chemical compounds based on their polarity, thus simplifying their components and facilitating subsequent processes. The order of highest fraction yields was water 5.47%, n-hexane 5.35%, dichloromethane 1.11%, and ethyl acetate 0.66%. Fraction yield values indicate the level of secondary metabolites from BSF maggots dissolved in the solvent according to their polarity (Septiani 2017).

3.2. Antibacterial Activity of BSF Maggot Extracts

The preliminary antibacterial activity test was carried out using the disc diffusion method. The disc diffusion method was chosen because it can determine the sensitivity of a sample and estimate the minimum inhibitory concentration that can visually inhibit bacteria, has high flexibility, and is easy to apply (Agnes 2015). Antibacterial activity is indicated by a clear zone showing inhibition of microorganism growth by antimicrobial agents on the agar medium surface (Eko 2013). The preliminary test was carried out on two types of maggot extracts to assess antibacterial activity against *E. coli*. The results of *E. coli* antibacterial testing aimed to optimize extracts with antibacterial activity, which were then tested for antibacterial activity against the typhoid-causing bacterium *S. typhi*.

Data in Table 2 shows that methanol extract of maggot powder and acetone extract of maggot powder exhibited inhibition zones greater than 5 mm. According to Eko (2013), inhibition zones greater than 5 mm up to 10 mm indicate antibacterial activity, albeit at a low level. Acetone, being semi-polar, is able to extract semi-polar to non-polar compounds (Nur 2013). Acetone extract can extract lauric acid, which is non-polar, from the maggots. According to Choi et al. (2012), maggot extracts are effective against Gram-negative bacteria because methanol solvent extracts can denature and precipitate large proteins and polypeptides, thereby allowing antimicrobial peptides (AMPs) contained in BSF maggots to be absorbed and act as antibacterials.

Antibacterial activity testing against *S. typhi* using methanol extract of maggot powder and acetone extract of maggot powder showed that acetone extract of maggot powder had antibacterial activity against *S. typhi* (Table 3). This is because acetone extract is able to extract two active compounds in maggots, including lauric acid, so the antibacterial spectrum of acetone extract is broader than that of methanol extract. Koolman (1997) mentioned that antibacterial selectivity and inhibition against test bacteria are divided into narrow-spectrum and broad-spectrum antibacterials. Narrow-spectrum antibacterials inhibit the growth of certain Gram bacteria, whereas broad-spectrum antibacterials inhibit the growth of both Gram-positive and Gram-negative bacteria.

The antibacterial mechanism of maggot extracts involves lauric acid, which can damage bacterial cell membranes, thereby increasing hydrogen ion uptake that acts as a strong bactericidal agent in the cytoplasm. This hydrogen ion uptake then penetrates into bacterial cells (Kim and Rhee 2016). The acetone extract in the antibacterial activity test against *E. coli* showed a lower inhibition zone diameter than methanol extract. This is because, according to Yanti and Mitika (2017), differences in antibacterial diffusion rates in agar media result in different inhibition zone diameters, affecting activity. However, in antibacterial testing against *S. typhi*, acetone extract showed higher activity than methanol extract because acetone extract contained more lauric acid compared to methanol extract.

In this study, cotrimoxazole was used as the positive control. Cotrimoxazole has replaced chloramphenicol and is now widely used as the drug of choice for typhoid because *S. typhi* has developed resistance to chloramphenicol (Anggun et al. 2015). This resistance occurs

because *S. typhi* produces the enzyme chloramphenicol acetyltransferase, which prevents chloramphenicol from inhibiting protein synthesis associated with the 50S ribosomal subunit (Utami and Eka 2012). The mechanism of cotrimoxazole is that it inhibits enzymes involved in folic acid synthesis in bacteria, thereby preventing the formation of nucleic acids, DNA, and RNA (Porth et al. 2012).

The inhibition zone diameter of cotrimoxazole in antibacterial testing against *E. coli* was smaller than in antibacterial testing against *S. typhi*. According to Utami and Eka (2012), this is because cotrimoxazole is more effective at inhibiting folic acid synthesis enzymes in *S. typhi* than in *E. coli*. DMSO was used as the negative control because it does not inhibit bacterial growth, thus not affecting antibacterial activity testing, and also served as the solvent for extract samples (Wildan et al. 2015).

The acetone maggot extract fractions were then tested for antibacterial activity against *E. coli* and *S. typhi*. The determination of extract concentrations in this study was based on previous studies (Park et al. 2012), namely at concentrations of 1000, 2500, up to 100,000 ppm. This study used concentrations of 1000 and 2500 ppm to determine the lower concentrations capable of inhibition. According to Park et al. (2014), maggot extracts exhibit bacteriostatic (inhibitory) properties, as the minimum inhibitory concentration in the aqueous fraction was 12,500 ppm and in the ethyl acetate fraction more than 100,000 ppm.

Based on Tables 4 and 5, the fraction with the highest antibacterial activity against *E. coli* and *S. typhi* at concentrations of 1000 ppm and 2500 ppm was the n-hexane fraction. The n-hexane fraction produced higher results than other fractions because BSF maggots contain lauric acid with antibacterial activity. The mechanism of lauric acid as an antibacterial is that it damages bacterial cell membranes, thereby increasing hydrogen ion uptake that acts as a strong bactericidal agent in the cytoplasm, which then penetrates bacterial cells (Kim and Rhee 2016).

Based on Tables 4 and 5, increasing the concentration from 1000 ppm to 2500 ppm in all fractions resulted in increased inhibition zones. The higher the concentration of antibacterial active compounds used, the faster bacteria are killed (Widiana 2012). However, the increase in antibacterial activity from 1000 ppm to 2500 ppm was not significant. According to Yanti and Mitika (2017), this is because inhibition zone diameters are not always directly proportional to concentration increases, as differences in diffusion rates of antibacterial compounds in agar media, types, and concentrations of antibacterial compounds also result in different inhibition zone diameters and affect activity. Moreover, the concentration variations tested in antibacterial activity of fractions were too small, so greater variations in concentration are needed to achieve significantly different results. Based on their mechanism of action, antibacterial agents are classified into bactericidal and bacteriostatic. Bactericidal antibacterials are substances that kill bacteria, whereas bacteriostatic antibacterials inhibit their growth. Some antibacterial substances act as bacteriostatic at low concentrations and bactericidal at high concentrations (Koolman 1997).

5. Conclusion

The Black Soldier Fly (BSF) maggot exhibits antibacterial properties against *E. coli* and *S. typhi*. The BSF maggot extract with the highest antibacterial activity was the acetone extract, whereas the fractionation result with the highest antibacterial activity was the n-hexane fraction. Both the extract and the fractionation products of BSF maggots are categorized as having low antibacterial activity.

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