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Phytochemical Potential of Maggot Extract Antimicrobial for *Escherichia Coli sp*. in Diabetic Ulcers of Diabetes Mellitus II

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ABSTRACT

Diabetes Mellitus (DM) is a metabolic disorder related to the relative deficiency of insulin secretion, affecting the metabolism of carbohydrates, fats, and proteins, leading to chronic hyperglycemia caused by genetic and environmental factors. This condition significantly impacts the quality of life, primarily due to complications such as diabetic ulcers. Diabetic foot ulcers are non-traumatic lesions on the skin of the foot in individuals with diabetes mellitus, resulting from repeated pressure and associated diabetes complications related to peripheral neuropathy. The healing of wounds can be facilitated through Maggot Debridement Therapy, a treatment involving the application of maggots or larvae of the Black Soldier Fly (BSF). These maggots secrete enzymes that dissolve necrotic tissue, disinfect the wound, and stimulate wound healing. This study aims to analyze the effectiveness of methanol extract from Maggot (*H. illucens*) in inhibiting the migration of bacterial cultures and its potential as an antimicrobial agent against bacteria found in diabetic foot ulcers (DM II). The maggot was extracted using the maceration method with methanol, and its content was determined through phytochemical and GC-MS tests. The antibacterial test was conducted using E. coli. Phytochemical testing revealed that the methanol extract of maggot contains saponin, as evidenced by the formation of foam after the addition of distilled water and agitation. The results of the antibacterial test indicated that the methanol extract of Maggot (*Hermetia illucens*) can inhibit the growth of Escherichia coli bacteria concentration of 12,5%.

Keywords: Antimicrobial, diabetic ulcers, maggot, methanol extract.

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1. Introduction

Diabetes Mellitus (DM) is a disorder of carbohydrate, fat, and protein metabolism associated with a relative deficiency in insulin secretion. It is characterized by chronic hyperglycemia caused by both genetic and environmental factors [1]. Diabetes mellitus is one of the Non-Communicable Diseases (NCDs) with a rising prevalence, reaching 8.5% in 2018 from 6.9% in 2013 [2]. In 2017, Indonesia ranked 6th globally with a population of 10.7 million, and if left untreated, it is projected to reach 21.3 million by 2030 [3]. This disease is the third leading cause of death in Indonesia, following stroke and coronary heart disease [4]. Most diabetes-related deaths in the age group of 45–54 occur in urban populations compared to rural areas [5]. Currently, Type II diabetes is not only prevalent in adults but is also increasing among children and teenagers [6].

This disease significantly impacts the quality of life, influenced by complications, one of which is diabetic ulcers. Diabetic foot ulcers are non-traumatic lesions on the skin of the feet of diabetes patients caused by repetitive pressure, compounded by complications related to diabetes-related peripheral neuropathy. Peripheral neuropathy results in the loss of sensation in the distal areas of the feet, and healing is often hindered by the development of infections. Infections, including those caused by bacteria such as *Staphylococcus aureus* sp., *Staphylococcus epidermidis* sp., *Citrobacter freundii* sp., *Escherichia coli* sp., *and Proteus mirabilis* sp., are common [7].

One alternative therapy that can be considered is methanol extract. This extract is found in animals, including relatives of flies (Diptera family), commonly bred, especially in the Special Region of Yogyakarta (DIY) known as Maggot (*Hermetia illuciens sp*). Maggots are generally used as animal feed, and there has been limited research on their antibacterial properties against various bacteria. Medical treatment for diabetic ulcer patients typically involves antibiotics, but this complicates healing due to antibiotic resistance [8]. Maggots contain protein and active compounds such as alkaloids, flavonoids, saponins, terpenoids, and triterpenoids [9]. Certain flavonoid groups, such as flavonols like quercetin, have the potential as antibacterial agents for diabetic ulcers by increasing the permeability of bacterial cell membranes, causing denaturation [10].

Maggots (*Hermetia illuciens* sp.) from the Special Region of Yogyakarta (DIY) contain antioxidant compounds in the form of flavonoids, making them a potential alternative therapy for diabetic ulcer infections. This may reduce side effects, lower the cost of conventional treatment, and improve the quality of life for patients. Maggot has become one of the sought-after cultivation methods in DIY as a means of biodegradation and livestock feed. Therefore, the potential of maggots in DIY needs to be highlighted, as they can also enhance household economics. This study will assess the potential of Maggot from DIY as an antibacterial agent through tests for toxicity and inhibition of bacterial chemotaxis migration in diabetic ulcer bacteria.

2. Materials and Methods

The materials used in this research consisted of maggot extraction results obtained from people's farms in Yogyakarta with methanol prepared to be continued in the test. Next, namely the phytochemical test to obtain target compounds in the form of terpenoids and is used for bacterial preparations with two tests, including the test cytotoxicity and cell migration tests to determine bacterial growth as well cell migration that occurs after adding maggot methanol extract.

2.1. Preparation of Maggot (*Hermetia illuciens* sp.) samples and methanol extraction

Maggots used in this research were purchased from maggot breeders located around the Special Region of Yogyakarta. The preparation involved taking a sample of 2 kg of maggots, sorting, and washing them. Subsequently, they were dried using an oven at a temperature of 50°C for 7 hours. Once dried, the samples were blended into a powder. Prepared sample, weighing 500 grams, underwent maceration using 3 liters of methanol for 3 intervals of 24 hours each. The mixture was stirred every 24 hours for 30 minutes. Following this, the maggot sample extract was filtered using a membrane filter and concentrated using an evaporator at a temperature of 55°C until a thick extract was obtained.

2.2. Phytochemical test

Extract 1 mL maggot methanol was taken, then magnesium powder was added to taste and 10 drops of concentrated hydrochloric acid. Phytochemical tests, including: Alkaloid Test, A total of 2 mL of

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sample (±0.05% w/v) was dissolved in 2 mL of 2% HCl (v/v), heated for 5 minutes and filtered. The filtrate obtained is dripped with the reagent Dragendorff as much as 2-3 drops. The presence of alkaloid compounds is indicated by formation of an orange precipitate. Flavonoid test of 2 mL sample (0.05% w/v) dissolved in 2 mL of methanol, then added Mg powder and concentrated HCl as many as 5 drops. The presence of flavonoid compounds is indicated by their formation red or orange. A total of 2 mL of sample (±0.05% w/v) was dissolved in distilled water in a test tube, then added 10 drops of KOH and heat in a 50°C water bath for 5 minutes, shaking for 15 minutes. If foam forms the maximum height of 1 cm and remaining stable for 15 minutes indicates the presence of saponin compounds. Terpenoid test as much as 2 mL of sample (±0.05% w/v) was added with 1 mL Liebermann Burchard reagent. The presence of terpenoid compounds is addressed with the formation of a dark blue or blackish green color. Polyphenol TestA total of 2 mL of sample (±0.05% w/v) was dissolved in 10 mL distilled water, heated for 5 minutes and filtered. The filtrate formed was added with 4 to 5 drops of $FeCl_3$ 5% (w/v). The presence of phenol is indicated by the formation of a dark blue or green color black.

2.3. Bacterial culture and inhibition test

Bacterial culture was performed by inoculating endophytic bacterial isolates, *Escherichia coli* sp. on sterile NB media. The cultures were then incubated at 37°C for 24 hours inside an incubator with agitation using a shaker. Agitation aims to ensure aeration during incubation, allowing bacteria to release their metabolites into the media. The obtained maggot extract was tested against *Escherichia coli* sp. and *Staphylococcus aureus* sp. (cultures from diabetic ulcer bacteria) using the disc diffusion method. The clear zone diameters were measured to determine the antibacterial activity. Stated that the concentration of nanoparticles with the largest clear zone underwent minimum inhibitory concentration (MIC) measurement using a calliper and repetition was carried out four times [11].

2.4. Data analysis

The obtained data are quantitative and were analyzed using the statistical method of One-Way Analysis of Variance (ANOVA) to determine the differences in outcomes among the various test and control groups.

3. Results

3.1. Maggot methanol extraction

The extraction of secondary metabolite compounds from Maggot (*Hermetia illuciens* sp.) is carried out through the maceration method using 300 grams of maggot powder with 1 liter of methanol solvent. The yield results from the maggot methanol extraction are 11.73%.

3.2. Gas chromatography-mass spectrometry (GC-MS) analysis

Gas chromatography is a highly versatile analytical technique used for the separation and analysis of mixtures of several components. The results of gas chromatography are presented in the form of a chromatogram, which is a visual representation of the methanol extract from the maggot, as seen in **Figure 1** and structure as shown in **Figure 2**. Additionally, the identification of each peak in the chromatogram is carried out by matching the Mass Spectrometry (MS) spectrum of each peak with the Wiley database. Based on the results of GC-MS in **Figure 1**, it is revealed that there are three main compounds in the methanol extract from maggots, as observed from its qualitative analysis in sequence, namely Tetradecanoic acid, 9-Hexadecenoic acid, and 9,12-Octadecadienoic acid. Based on the results of the above GC-MS, there are antioxidant, anticancer, and antibacterial compounds, as found in the mentioned compounds. The results of the color identification test conducted on each sample extract indicate that the positive methanol extract contains saponin triterpene compounds, as evidenced by the formation of a brown color ring (**Figure 3**).

3.3. Phytochemical test

Phytochemical testing was conducted to identify the active compounds present in insects. In this study, the testing was performed by taking a small sample from the maceration extract, then adding reagents according to the compounds to be identified. The results of phytochemical testing on the methanol extract of Maggot (*Hermetia illuciens sp*) showed the presence of a bioactive compound, namely saponin (**Table 1**). Based on the phytochemical test conducted, it is known that only one test was successful, which is the saponin test, where a foam of 0.5 cm height persisted for more than 15 seconds.

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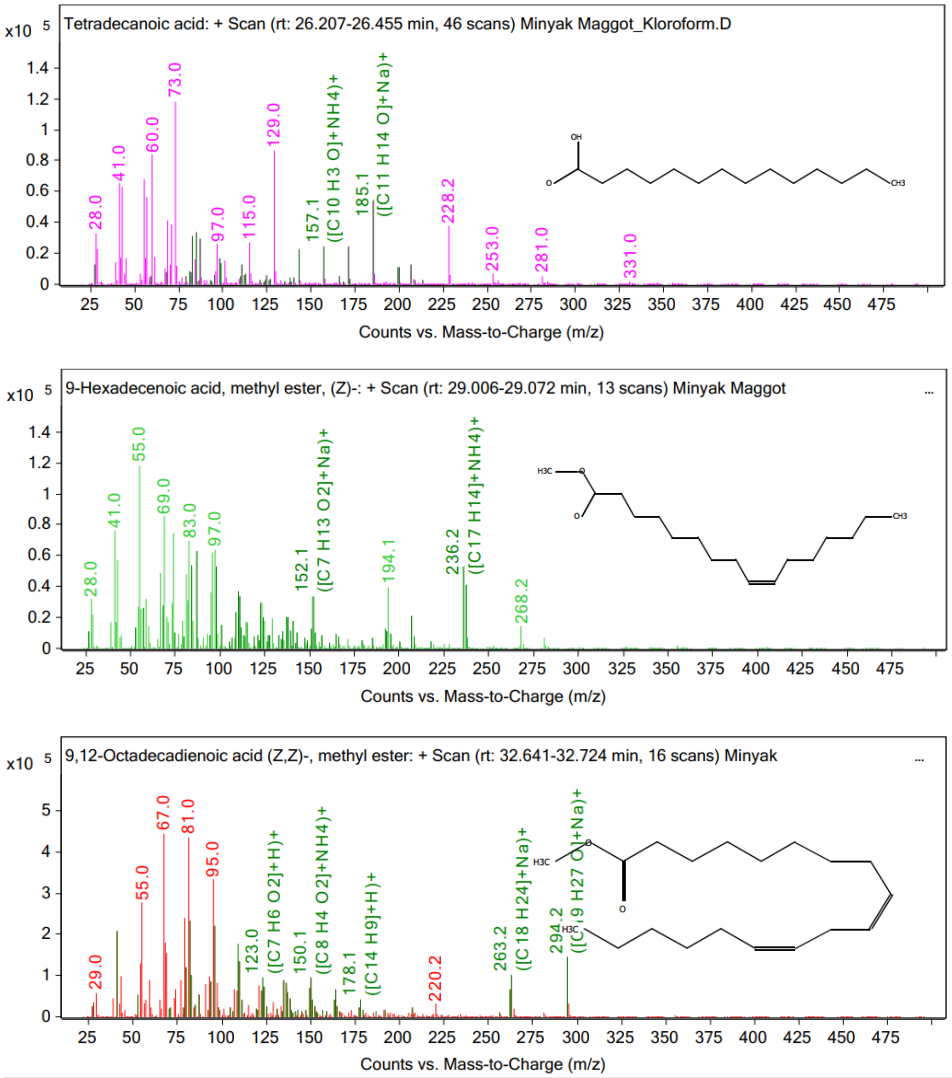
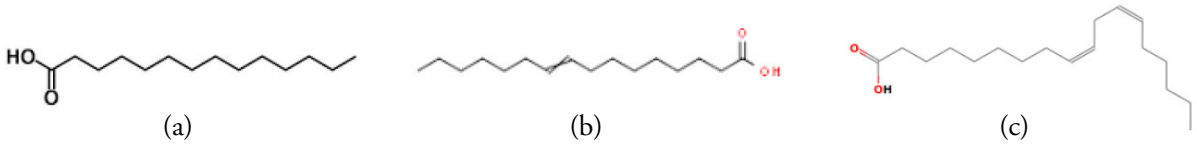


Figure 1. a: Tetradecanoic acid; b: 9-Hexadecenoic acid; and c: 9,12-Octadecadienoic acid



(a) (b) (c)

Figure 2. The structure of the compounds contained in the methanol extract of Maggot (*Hermetia illuciens* sp.) in Yogyakarta, Indonesia. a: Tetradeconic acid; b: 9-Hexadecenoic acid, c: 9,12-Octadecadienoic acid

(a) (b) (c) (d) (e)

Figure 3. Maggot (*Hermetia illuciens* sp.) phytochemical test results. a: Flavonoid; b: Alkaloid; c: Terpenoid; d: Polyphenols; e. Saponins

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Table 1. Phytochemical test of methanol extract of Maggot (*Hermetia illuciens* L)

Phytochemical test	Methanol extract	indicator
Alkaloid	-	Not formed red precipitate
Flavonoid	-	No color change occurs turn black reddish
Terpenoid	-	No red or purple color formation
Polyphenol	-	No formation of blackish brown and blackish green color
Saponin	+	Foam is formed which is stable

3.4. In vitro antibacterial assay

The activity test in this research utilized the well diffusion method. This method was chosen because it allows for easier observation of the diameter of the clear zones, not only on the surface but also reaching into the medium. Based on the above research results, a table of inhibition zone data generated by the methanol extract of Maggot (*Hermetia illuciens* sp.) against *Escherichia coli* sp. can be created (**Table 2**). The inhibition zones formed in all treatment groups of methanol extract of bajakah tampala stem indicate the presence of inhibitory activity against *Escherichia coli* sp. There are differences in the inhibition zones formed at each concentration.

Table 2. Results of bacterial inhibition

Treatment	Inhibition zone diameter				Average diameter
	I	II	III	IV	(mm) ± SD
Concentration 12,5%	10,1	9,95	9,25	9,9	9,8 ± 0,376
Concentration 25%	11,45	11,5	10,45	13,45	11,71 ± 0,501
Concentration 50%	15,9	15,35	16,7	15,4	15,83 ± 0,626
Concentration 75%	19,8	20,6	20,4	20,5	20,32 ± 0,359
Control Positif	30,05	30,15	31,15	31,85	30,8 ± 0,858
Control Negatif	-	-	-	-	0 ± 0

4. Discussion

The high yield of 11.73% from the methanol extraction of maggot is attributed to the abundance of bioactive components it contains. States that the higher the extraction yield, the greater the content of substances of interest in a raw material [9]. Based on the results of GC-MS in **Figure 1**, it is revealed that there are three main compounds in the methanol extract from maggots, as observed from its qualitative analysis in sequence, namely tetradecanoic acid, 9-hexadecenoic acid, and 9,12-Octadecadienoic acid. Based on the results of the above GC-MS, there are antioxidant, anticancer, and antibacterial compounds, as found in the mentioned compounds. The presence of hexadecanoic acid and its esters likely contributes to antioxidant and antimicrobial activities [11]. Those certain compounds such as carpesterol, ethyl phenyl benzene, tetradecanoic acid ethyl ester, and 1,3-Diphenyl-1-(2-hydroxyphenyl) butane are major contributors to antioxidants. Octadecanoic acid, found at peak 4 with an area percentage of 14.45%, has a molecular formula of $C_{18}H_{36}O_2$ and belongs to the group of fatty acids used for antibacterial and antioxidant purposes. In addition to Maggot, compounds like hexadecanoic acid are also present in the methanol extract of *Justicia adhatoda* (Linn) leaves, which can be utilized as an insecticide [12].

Saponin is one of the secondary metabolite compounds that exhibits antibacterial effects. The mechanism of saponin as an antibacterial agent involves reacting with porins (transmembrane proteins) on the outer membrane of bacterial cells, forming strong polymer bonds that result in the damage of porins. The damage to porins, which serve as the entry and exit points for compounds, reduces the permeability of the bacterial cell membrane, leading to a deficiency in nutrients. This, in turn, inhibits bacterial growth or causes cell death. The potential reasons for the unsuccessful phytochemical tests, other than the saponin test in this study, could be attributed to inappropriate methods, the use of expired samples, and/or the improper selection of reducing agents. The higher the concentration of the

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extract used, the larger the inhibition zone formed [13]. By examining the average diameter values of the inhibition zones formed at each concentration, the methanol extract of bajakah tampala stem is classified based on its activity as weak to very strong. The larger the inhibition zone produced, the greater the diameter of the inhibition zone inside it, indicating a higher content of compounds acting as antibacterial agents in the methanol extract of Maggot (*Hermetia illuciens* sp.)[14].

This extract plays a role in inhibiting the growth of Gram-positive bacteria such as Escherichia coli due to its saponin content [15]. Saponin disrupts the surface tension of the cell wall, allowing antibacterial substances to easily enter the cell and interfere with metabolism, ultimately leading to bacterial death [16][17]. The negative control used was distilled water (aquadest) because it has no effect on bacteria. In the positive control group with 10% povidone iodine, it showed the largest average diameter of the inhibition zone against the growth of Escherichia coli bacteria. The minimum inhibitory concentration (MIC) value of the methanol extract from the stem of bajakah tampala is 12.5% concentration, as it is the smallest concentration that exhibits an inhibition zone.

5. Conclusion

The methanol extract of Maggot (*Hermetia illuciens* sp.) is positive for containing saponin, as indicated by the formation of foam after the addition of distilled water and agitation. Additionally, the methanol extract of Maggot (*Hermetia illuciens* sp.) demonstrates the ability to inhibit the growth of Escherichia coli bacteria in concentration 12,5%.

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