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Antibacterial Activity Test of Maggot (*Hermetia illucens*) Extracts Against Respiratory Pathogenic Bacteria

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ABSTRACT

The bacteria that cause pneumonia are Streptococcus pneumoniae, Haemophilus influenzae, and Staphylococcus aureus. Maggot extract (Hermetia illucens) contains fatty acids and has antimicrobial potency. Studies regarding its antibacterial activity against respiratory pathogenic bacteria have not been reported. This research aimed to predict the biological activity of maggot extract through PASS online and in vitro study against S. pneumoniae, H. influenzae, and S. aureus. Maggot extract was obtained from maceration using methanol and ethyl acetate. Upper and lower phases were formed after the ethyl acetate extract was centrifuged. The result showed ethyl acetate extract has antibacterial activity against three tested bacteria. PASS analysis predicted its antibacterial mechanism as a peptidoglycan glycosyltransferase inhibitor. Antibacterial activity of the lower phase extract against S. pneumoniae is not significantly different from chloramphenicol, and its activity against H. influenzae is higher than chloramphenicol. Minimum inhibitory concentration and minimum bactericidal concentration of the lower phase extract against three tested bacteria was 0.625% (v/v), and the upper phase was 1.25–2.5% (v/v). Overall results indicate the potential of maggot extract to be further developed as a respiratory pathogenic antibacterial candidate.

Keywords: *ethyl acetate, H. influenzae, methanol, S. aureus, S. pneumoniae*

ABSTRAK

Bakteri yang dapat menyebabkan pneumonia adalah bakteri Streptococcus pneumoniae, Haemophilus influenzae, dan Staphylococcus aureus. Maggot (Hermetia illucens) mengandung asam lemak melimpah yang berpotensi sebagai antimikroba dan belum pernah diteliti aktivitas antibakterinya terhadap bakteri patogen pernapasan. Penelitian ini bertujuan melakukan prediksi aktivitas biologis senyawa menggunakan PASS online dan analisis in vitro ekstrak maggot terhadap S. pneumoniae, H. influenzae, dan S. aureus. Ekstrak maggot diperoleh dari proses maserasi dengan pelarut metanol dan etil asetat. Fase atas dan fase bawah terbentuk setelah ekstrak etil asetat disentrifugasi. Hasil uji menunjukkan bahwa ekstrak etil asetat maggot memiliki aktivitas antibakteri terhadap ketiga bakteri uji. Mekanisme antibakteri berdasarkan analisis PASS adalah sebagai inhibitor peptidoglikan glikosiltransferase. Secara statistika, aktivitas antibakteri ekstrak etil asetat fase bawah terhadap S. pneumoniae adalah tidak berbeda nyata dengan antibiotik kloramfenikol, dan aktivitasnya terhadap H. influenzae lebih tinggi dibandingkan antibiotik kloramfenikol. Konsentrasi hambat minimum dan konsentrasi bunuh minimum oleh ekstrak etil asetat fase bawah terhadap ketiga bakteri uji

sebesar 0,625% (v/v), serta fase atas sebesar 1,25-2,5% (v/v). Keseluruhan hasil pengujian menunjukkan potensi ekstrak maggot untuk dikembangkan sebagai kandidat antibakteri patogen pernapasan.

***Kata kunci**: etil asetat, H. influenzae, metanol, S. aureus, S. pneumoniae*

1. INTRODUCTION

Acute Respiratory Infection (ARI) remains a critical global health threat, encompassing infections of the respiratory tract ranging from the nasal passages to the alveoli (Prasekti *et al.* 2018). ARI is currently the leading cause of infectious mortality among children in Indonesia (Kemenkes RI 2018). This high fatality rate poses a significant challenge to the Sustainable Development Goals (SDGs), specifically third goal regarding "good health and well-being," which aims to reduce neonatal and under-five mortality rates (Rakernas 2015). ARI persist as a global public health priority due to their significant contribution to worldwide morbidity and mortality (Chen *et al.* 2024). Furthermore, WHO in 2018 identified Indonesia as having the highest ARI-related mortality rate in Southeast Asia, with approximately 20,084 deaths reported among infants and toddlers. These figures are exacerbated by the synergy between respiratory viruses and bacterial pathogens, which can severely impair the immune system and increase antibiotic intolerance (Zhu *et al.* 2020).

The highest mortality rates in ARI cases are attributed to *Streptococcus pneumoniae* infection, followed by *Haemophilus influenzae* and *Staphylococcus aureus* (Fusvita & Umar 2016). While antibiotics are traditionally employed to inhibit these pathogens (Fu *et al*. 2023), the imprudent use of such therapeutics, particularly in pediatric populations, can lead to multi-drug resistance and compromise immature immune systems (Elshobary *et al.* 2025). Consequently, biomedical innovation is required to develop optimal antibacterial agents. This study explores the larvae of the Black Soldier Fly (*Hermetia illucens*), commonly known as maggot, as a novel therapeutic candidate. *Hermetia illucens* is a member of the Stratiomyidae family, prevalent in tropical climates and frequently utilized for organic waste biodegradation (Camperio *et al.* 2025).

According to Franco et al. (2024), maggot larvae contain diverse fatty acids with significant antimicrobial potential. These fatty acids address bacterial resistance by targeting specific sites, such as the inhibition of peptidoglycan cell wall biosynthesis. Research by Marusich *et al.* (2020) indicated that the abundant fatty acid content in maggots consists primarily of lauric acid (34.43%) and palmitic acid (19.28%), both of which exhibit antibacterial activity against Gram-positive and Gram-negative bacteria. This concentration is typically highest between 15 and 30 days of maggot. In addition to antimicrobial properties, the diverse amino acid profile in maggots is expected to enhance immune cell formation, making it a suitable candidate for pediatric patients with developing immune systems (Wardhana 2016).

The use of maggot extracts specifically as a treatment candidate for ARI caused by *S. pneumoniae, H. influenzae*, and *S. aureus* has not been reported. Therefore, this study aims to analyze the antibacterial potential of *Hermetia illucens* extracts obtained via methanol and ethyl acetate extraction against these specific ARI pathogens. The study utilizes the WAY2DRUG PASS web-based tool to predict active compounds and biological activities, followed by in vitro assays to determine the inhibition zone, Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC).

2. MATERIALS AND METHODS

The study utilized *Hermetia illucens* larvae (15 and 30 days old) and bacterial isolates of *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247, and *S. aureus* ATCC 25293. Key extraction solvents included ethyl acetate and 96% methanol. Specialized media for antibacterial testing consisted of Tryptic Soy Agar (TSA), Blood Agar, Chocolate Agar, Haemophilus Test Medium (HTM) Broth, and Mueller Hinton Broth (MHB). In silico analysis was performed using the WAY2DRUG PASS platform with molecular data retrieved from the PubChem database. Primary analytical equipment included a rotary vacuum evaporator for extract concentration and a 37 °C anaerobic incubator for pathogen cultivation.

Biological Activity Prediction

The biological potential of *Hermetia illucens larvae* (maggot) compounds was analyzed using the WAY2DRUG PASS (Prediction of Activity Spectra for Substances) server. Structural data (SMILES) for lipid-group compounds and other larval constituents were retrieved from the PubChem database. Antibacterial potential was predicted based on the structure-activity relationship algorithms of the PASS software (Filimonov et al. 2014).

Sample Preparation and Extraction

Following the modified method of Choi et al. (2012), 600 g of larvae were rinsed with distilled water, homogenized for 2 minutes, and mixed with two different solvent methanol (96%) or ethyl acetate at a 1:10 (w/v) ratio. The two mixtures were macerated for 24 hours at 150 rpm. The resulting filtrates were concentrated using a rotary evaporator at 56 °C to a semi-solid state and stored at 4 °C.

Bacterial Strains and Culture Conditions

The antibacterial activity was evaluated against *Streptococcus pneumoniae* ATCC 49619, *Haemophilus influenzae* ATCC 49247, and *Staphylococcus aureus* ATCC 25923. According to CLSI (2016) guidelines, strains were subcultured on Blood Agar (*S. pneumoniae*), Chocolate Agar (*H. influenzae*), and Tryptic Soy Agar (*S. aureus*). *S. pneumoniae* and *H. influenzae* were incubated at 37°C under 5% CO2 for 20 hours, while S. aureus was incubated aerobically at 37 °C for 20 hours.

Antibacterial Assay

Disk Diffusion Method. Extracts were prepared at 100% (v/v) concentration; ethyl acetate extracts were centrifuged at 11,200 rcf for 4 minutes prior to use (CLSI 2016). Bacterial suspensions were standardized to 0.5 McFarland (3 × 108 CFU/mL) using a densitometer (Puidokas et al. 2019). Suspensions were inoculated onto Mueller-Hinton Agar (MHA) supplemented with 5% sheep blood (S. *pneumoniae*), Haemophilus Test Media (HTM) (*H. influenzae*), and MHA (*S. aureus*) (CLSI 2016). Disks containing 20 µL of extract were applied. Chloramphenicol (30 µg/disk) and distilled water served as positive and negative controls, respectively. Inhibition zones were measured after 20 hours of incubation under species-specific conditions.

Determination of MIC and MBC. Minimum Inhibitory Concentration (MIC) was determined via the microdilution method in 96-well plates (CLSI 2016). Extracts were serially diluted to final concentrations ranging from 0.625% to 10% (v/v) in specific growth broths (LHB-supplemented MHB for *S. pneumoniae*, HTM broth for *H. influenzae*, and MHB for S. aureus). Vancomycin (20 µg/mL) was used as the positive control. The MIC was defined as the lowest concentration with no visible turbidity. To determine the Minimum Bactericidal Concentration (MBC), 10 µL from non-turbid wells were cultured onto agar media; the MBC

was defined as the lowest concentration resulting in no bacterial growth.

Statistical Analysis

Data are presented as mean ± standard deviation. Statistical significance was analyzed using one-way ANOVA followed by Duncan's Multiple Range Test (α = 0.05) via SPSS 22.0 to determine significant differences between extract treatments and controls.

3. RESULTS

Biological Activity Prediction of Maggot Compounds

The biological activity spectra of 16 compounds identified in *Hermetia illucens*, predominantly lipids, were predicted using PASS (Prediction of Activity Spectra for Active Substances). The analysis revealed potential antibacterial and anti-inflammatory properties (Figure 1 and Table 1). The compounds demonstrated antibacterial potential with a mean Pa (Probability to be active) of 0.389, specifically through the inhibition of peptidoglycan glycosyltransferase (Pa = 0.568). Campesterol exhibited the highest inhibitory potential against peptidoglycan glycosyltransferase (Pa = 0.871). For anti-inflammatory activity, the collective Pa was 0.639, with alpha-tocotrienol showing the highest individual score (Pa = 0.866). Beta-sitosterol and chitin recorded the highest antibacterial scores (Pa = 0.585).

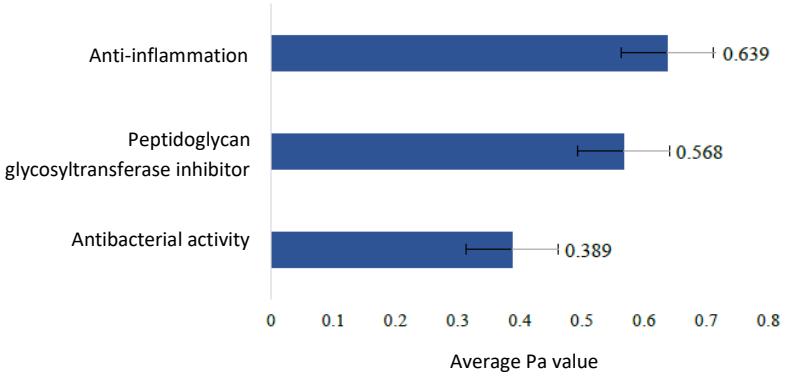


Figure 1. Relative score of PASS server test results

Table 1. Results of PASS analysis of active compounds in maggots

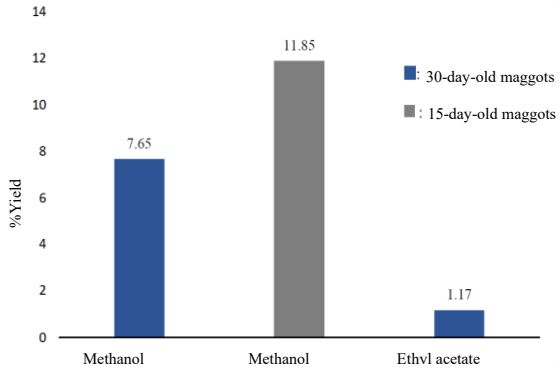
Ligand	Pubchem ID	Antibacterial activity	Peptidoglycan glycosyltransferase inhibitor	Anti-inflammation
Campesterol	173183	0,328	0,871	0,348
Alpha-Tocopherol	14985	0,314	0,315	0,814
Alpha-Tocotrienol	5282347	0,445	0,356	0,866
Beta-Sitosterol	222284	0,585	0,780	0,467
Chitin	6857375	0,585	0,322	0,383
Adipic acid	196	0,543	0,843	0,510
Tannin	250395	0,386	0,345	0,735
Beta-Tocopherol	6857447	0,393	0,369	0,756
Gamma-Tocopherol	92729	0,339	0,321	0,775
Beta-Tocotrienol	5282348	0,404	0,358	0,835
Gamma-Tocotrienol	5282349	0,336	0,310	0,846
Lauric acid	3893	0,300	0,814	0,515

Ligand	Pubchem ID	Antibacterial activity	Peptidoglycan glycosyltransferase inhibitor	Anti-inflammation
Myristic acid	11005	0,300	0,814	0,515
Linoleic acid	5280450	0,335	0,720	0,730
Oleic acid	445639	0,332	0,743	0,614
Palmitic acid	985	0,300	0,814	0,515

Yield of *Hermetia illucens* Extracts

Extraction of *H. illucens* at 15 and 30 days of age using maceration with methanol and ethyl acetate yielded varying amounts of bioactive compounds (Figure 2). From an initial sample weight of 650 g, the highest yield was obtained from 15-day-old maggots extracted with methanol (11.85%). The methanol extract of 30-day-old maggots yielded 7.65%, while the ethyl acetate extract of 15-day-old maggots produced the lowest yield at 1.17%.

Figure 2. Maggot extracts yield



Antibacterial Activity of Maggot Extracts

The disc diffusion assay indicated that antibacterial activity was restricted to ethyl acetate extracts, while methanol extracts showed no inhibition against *Streptococcus pneumoniae*, *Haemophilus influenzae*, or *Staphylococcus aureus* (Figure 3). Upon centrifugation, the ethyl acetate extract separated into upper and lower phases, both of which were tested.

The lower phase of the ethyl acetate extract consistently demonstrated significantly higher antibacterial activity compared to the upper phase across all tested pathogens (Figures 3 and 4). Against *S. aureus*, the lower and upper phases produced inhibition zones of 11.95 mm and 7.05 mm, respectively. For *S. pneumoniae*, the lower phase achieved a zone of 26.95 mm, which was statistically comparable to the chloramphenicol positive control, while the upper phase reached 22.00 mm and induced hemolysis in the media. In the case of *H. influenzae*, the lower phase (36.50 mm) significantly outperformed the positive control, whereas the upper phase (26.50 mm) showed lower activity.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values for the ethyl acetate phases are detailed in Table 2. The lower phase exhibited a uniform MIC and MBC of 0.625% (v/v) against all three bacteria. The upper phase showed an MIC and MBC of 1.25% (v/v) for *S. pneumoniae* and *H. influenzae*, but required a higher MBC of 2.5% (v/v) for *S. aureus*.

Table 2. MIC and MBC of maggot extract to S. *aureus*, S. *pneumoniae*, and H. *influenzae*

	Extract	*S. aureus*	*S. pneumoniae*	*H. influenzae*
MIC (%) (v/v)	Ethyl acetate (A)	1.25	1.25	1.25
	Ethyl acetate (B)	0.625	0.625	0.625
MBC (%) (v/v)	Ethyl acetate (A)	2.5	1.25	1.25
	Ethyl acetate (B)	0.625	0.625	0.625

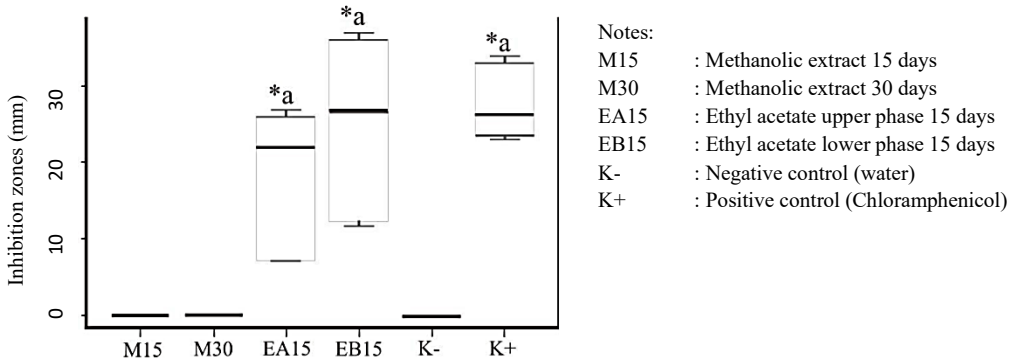


Figure 3. Boxplot of inhibition zone diameters for four maggot extracts and chloramphenicol against three test bacteria. (*) Identical letters above the data plots indicate no significant difference at P > 0.

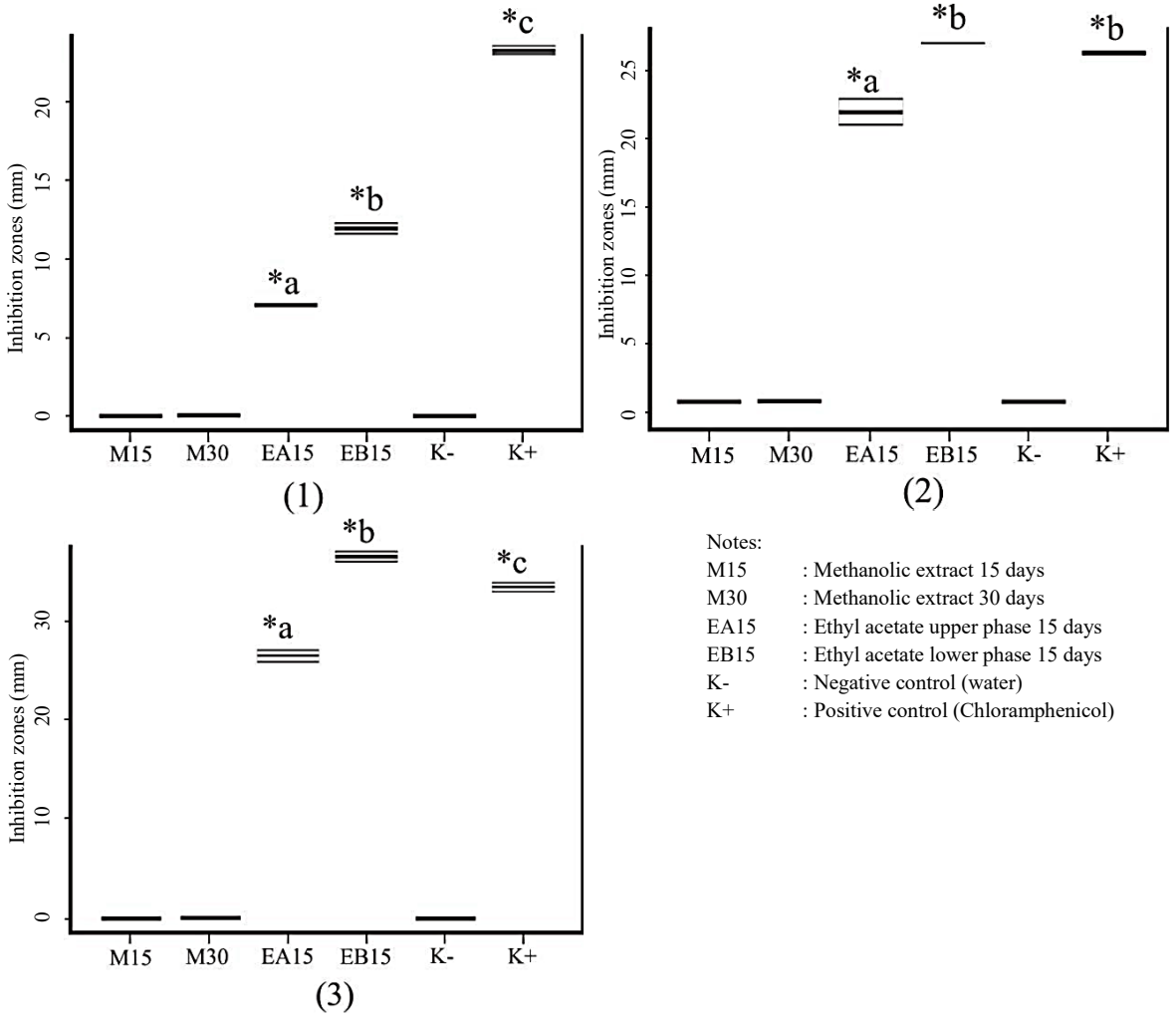


Figure 4. Boxplot of inhibition zone diameters for four maggot extracts and chloramphenicol against *S. aureus* (1), *S. pneumoniae* (2), and *H. influenzae* (3). (*) Different letters above data plots indicate statistically significant differences at P < 0.05.

4. DISCUSSION

Bioactive Compound Profiling via PASS Analysis

The in silico prediction using PASS Online successfully identified the biological potential of 16 predominant compounds in Hermetia illucens, primarily lipid-based. The results indicated significant anti-inflammatory activity (Pa > 0.7) for eight compounds, including α-tocopherol, α-tocotrienol, and

linoleic acid. These findings align with Mulyati (2021), who reported that Vitamin E derivatives in maggot extracts function as potent antioxidants and cell regenerators, mitigating mitochondrial dysfunction caused by oxidative stress during respiratory infections. Furthermore, the antibacterial potential was linked to the inhibition of peptidoglycan glycosyltransferase (Pa > 0.7) by compounds such as lauric, myristic, and palmitic acids. This mechanism is critical as peptidoglycan and essential for bacterial cell wall integrity; its inhibition leads to osmotic lysis and cell death, consistent with the structural biological roles described by Karlina (2013).

Extraction Yield and Solvent Efficacy

Maceration with methanol, yielded the highest extract recovery (11.85%) compared to ethyl acetate (1.17%). This disparity is attributed to the amphiphilic nature of methanol, which possesses both polar hydroxyl and non-polar methyl groups, allowing for a broader extraction range of bioactive metabolites (Romadanu *et al*. 2014). Conversely, the lower yield of ethyl acetate is due to the weaker hydrogen bonding capacity of its methoxy group (CH_3O-) compared to methanol, resulting in a more selective extraction of semi-polar compounds (Wardhani & Sulistyani 2012). Despite the higher yield in methanol, the bioactive concentration appears solvent-dependent, as evidenced by the subsequent biological assays.

Antibacterial Activity Against Respiratory Pathogens

Antibacterial screening revealed that only ethyl acetate extracts exhibited potent inhibitory activity across all tested pathogens. Specifically, the extract showed "very strong" activity against S. pneumoniae and H. influenzae (inhibition zones > 20 mm) and "moderate to strong" activity against S. aureus. The efficacy of the ethyl acetate extract, despite its lower yield, suggests a higher concentration of lipophilic antibacterial agents such as lauric acid. Kim & Rhee (2016) noted that H. illucens contains high concentrations of lauric acid (~49.18%), which acts as a natural antibiotic by targeting microbial cell membranes.

The lower activity in methanol extracts contradicts with Choi *et al.* (2012), potentially due to variations in substrate quality and environmental conditions in Indonesia, which significantly influence the nutrient and antimicrobial peptide (AMP) profiles of the larvae (Park 2016). Furthermore, the ethyl acetate bottom phase demonstrated superior efficacy compared to the top phase, likely due to a higher concentration of semi-polar tannins and fatty acids. Tannins exert antibacterial effects by precipitating proteins and inactivating essential enzymes like DNA topoisomerase (Ngajow *et al.* 2013).

Hemolytic Activity and Minimum Inhibitory/Bactericidal Concentrations (MIC/MBC)

The observation of hemolysis in S. pneumoniae assays suggests the extract's ability to disrupt erythrocyte membranes. However, previous in vivo studies on poultry indicated no significant changes in Mean Corpuscular Hemoglobin Concentration (MCHC), suggesting that maggot-derived products may not induce intravascular hemolysis at certain dosages (Irawan 2020).

The MIC and MBC values further quantify this potency. The ethyl acetate bottom phase achieved a simultaneous MIC/MBC of 0.625% (v/v) for all pathogens, indicating high efficiency. In contrast, the top phase required higher concentrations (1.25–2.5%) to achieve bactericidal effects. These low values suggest a synergistic interaction between the maggot's lipid components and secondary metabolites, enhancing the overall antimicrobial flux

compared to isolated compounds (Anwar et al. 2021).

5. CONCLUSION

The ethyl acetate extract of Hermetia illucens larvae demonstrates significant antibacterial activity against Streptococcus pneumoniae, Haemophilus influenzae, and Staphylococcus aureus, likely through the inhibition of peptidoglycan glycosyltransferase as predicted by PASS analysis. Statistical evaluation reveals that the lower-phase extract matches the efficacy of chloramphenicol against S. pneumoniae and surpasses it against H. influenzae, with minimum inhibitory and bactericidal concentrations (MIC/MBC) as low as 0.625% (v/v). These findings underscore the potential of maggot-derived extracts as viable therapeutic candidates for addressing respiratory pathogens.

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