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EVALUATION OF BLACK SOLDIER FLY (*HERMETIAILLUCENS*) LARVAE OIL AS A NATURAL ANTIBACTERIAL AGENT FOR POSSIBLE DERMATOLOGICAL APPLICATIONS

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ABSTRACT

The Black Soldier Fly (*Hermetiaillucens*) larvae oil has gained attention for its antimicrobial potential, particularly in dermatological applications. This study evaluates the physicochemical properties and antibacterial activity of crude, autoclaved, and purified BSF larvae oil against Gram-positive and Gram-negative bacteria. The oil was extracted using solvent extraction, followed by different processing methods. Physicochemical analysis revealed that purification improved stability, reducing free fatty acid content (5.195% to 1.66%) and acid value (10.39 to 3.33 mg KOH/g) while maintaining a similar refractive index (1.2451 to 1.2450). Viscosity decreased (98.45 to 85.60 cP), and pH dropped from 7.44 to 1.244, indicating enhanced antimicrobial properties. Antimicrobial assays demonstrated that purified BSF larvae oil significantly inhibited *Staphylococcus aureus* (19.2 ± 0.30 mm), *Escherichia coli* (18.4 ± 0.30 mm), *Pseudomonas aeruginosa* (17.8 ± 0.35 mm), and *Bacillus subtilis* (15.3 ± 0.30 mm). However, autoclaved and crude oil exhibited no antibacterial activity, suggesting that heat-sensitive bioactive compounds, such as antimicrobial peptides (AMPs) and lauric acid, contribute to its antibacterial effects. These findings highlight the potential of purified BSF larvae oil as a natural, sustainable alternative to synthetic antibiotics in treating skin infections. Further research is needed to assess its long-term stability and dermatological safety.

Keywords: Antibacterial, Black Soldier Fly, Dermatology, Fatty acids, *Hermetiaillucens*, Physicochemical, Purification.

1. INTRODUCTION

Scientists now understand the various potential applications of Black Soldier Fly (*Hermetiaillucens*) or BSF for waste disposal, animal feed uses, and biomedical functions. The larvae of the BSF exhibit high nutritional value because they contain both protein and lipid elements, which industries are now beginning to employ, according to Srisuksai *et al.*

(2024) and Kim and Oh (2022). The most valued by-product derived from BSF larvae oil consists mainly of medium-chain fatty acids (MCFAs) featuring lauric acid (C12:0), which shows potent antimicrobial and antifungal, and anti-inflammatory properties (Gatlin *et al.*, 2024; Suryati *et al.*, 2023). BSF larvae oil's anti-inflammatory and antimicrobial properties derive from its major fatty acids, which consist of myristic, palmitic and oleic acids (Srisuksai *et al.*, 2024; Lee *et al.*, 2022). The research investigates BSF larvae oil as a natural antibacterial product because of its bioactive properties, which are suitable for dermatological applications.

Academic reports suggest that the antioxidant properties of BSF oil facilitate skin wound healing and have anti-inflammatory effects on the skin (Phongpradist *et al.*,2023; Ewald *et al.*, 2020). BSF oil's high 57% lipid content makes it stable and suitable for cosmetic and drug applications (Suryati *et al.*, 2023; Almeida *et al.*, 2022). Studies by Azmiera *et al.*(2023) and Diyantoro *et al.* (2022) confirmed that oils obtained from insects, including antimicrobial peptides and MCFAs, show remarkable antibacterial effects. The research on BSF hemolymph demonstrates its effectiveness in blocking MRSA, *Streptococcus pyogenes*, and *E. coli* bacterial strains. The research of Zabulionė *et al.* (2023) and Klüber *et al.* (2022)demonstrated that fat fractions extracted from Black Soldier Fly larvae show the potential to prevent the growth of *Candida albicans* along with *Staphylococcus aureus* and *Bacillus subtilis* wherein it establishes the potential for antifungal and antibacterial applications.

Bacterial infections of the skin are typically due to Gram-positive bacteria *Staphylococcus aureus Streptococcus pyogenes* together with Gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter sp*. The skin infections treated by these bacteria include cellulitis alone, acne, wound infections, and impetigo, and most infections show resistance to standard antibiotic treatments (Nguyen *et al.*, 2024). The antimicrobial peptides (AMPs) and lauric acid molecules present in BSF larvae oil were discovered to destroy bacterial membranes (Szczepanik and Świątkiewicz, 2023; Di Somma *et al.*, 2021 and Xia *et al.*, 2021) which made them effective against Gram-positive and Gram-negative pathogens. Doctors often prescribe synthetic antibiotics, such as clindamycin tetracycline and erythromycin, for bacterial infections on the skin. At the same time, these drugs have been linked to increased antibiotic resistance and microbiome disturbances (Teo *et al.*, 2022). Suryati *et al.* (2023) present naturally derived antibacterial agents from plants and insects as promising alternatives because they fail to cause antibacterial drug resistance. Scientific studies investigating BSF larvae oil as an antibacterial medication could supply dermatological practice with a natural alternative to artificial treatments because natural treatments have gained recognition from dermatological practitioners.

Research has been designed to examine the structure of BSF larvae oil while assessing its antimicrobial properties. The investigation will provide an understanding of the effects of processing on antibacterial activity and evaluate the potential of BSF oil in dermatological treatment. This could pave the way for eco-friendly skincare formulations and medical applications in treating bacterial skin infections.

2. MATERIALS AND METHOD

2.1 Chemicals, Reagents, and Equipment

Carbon tetrachloride, ethanol from Merck, sodium hydroxide from LOBA CHEME, and phenolphthalein indicator served as reagents during the investigation. In contrast, acetic acid, chloroform, sodium thiosulfate, soluble starch, potassium iodide and Wijs solution were employed. These chemicals were of analytical grade and were used as supplied. Equipment used included a viscometer, refractometer, turbidity meter, and specific density bottle.

2.2 Sample Collection and Processing

Black Soldier Fly (*Hermetiaillucens*) larvae were obtained from a controlled rearing facility, where they were fed on organic waste substrates (including Indomie, spaghetti, jollof rice, and two). The BSFLs were harvested on day 15 by separating them from the residual waste using a 0.3 cm plastic sieve. This was done at the Maggot Laboratory in the National Research Institute for Chemical Technology (NARICT), Basawa, Zaria. The larvae were then exposed to sunlight for 25 minutes to remove excess moisture. Afterward, they were washed and rinsed with water before further processing (Tahir, 2023).

2.3 Methods

2.3.1 Extraction Method

The oil was extracted using solvent extraction using n-hexaneto maximize lipid yield and bioactive content (Rattana *et al.*, 2023).

Figure 1: Oil Extraction and Refining Process of BSF Larvae Oil

Figure 1 shows the Oil Extraction and purification Process of BSF Larvae Oil is the step by step process for extracting and refining the Black Soldier Fly larvae oil such as solvent extraction, autoclaving and purification methods. It helps in understanding the stages that go into production of the oil and their effects on the physicochemical properties.

2.3.2Processing Methods

The extracted oil underwent different processing treatments. Crude oil was directly extracted with minimal refinement, autoclaved oil was heat-treated at 121°C for 15 minutes to assess the effect of temperature on its antibacterial properties (Francis *et al.,* 2023), and purified oil was subjected to filtration and centrifugation to remove impurities while preserving key bioactive components (Shu *et al.,* 2024).

2.3.3 Drying and Pulverization of BSFL

The harvested BSFL were dried using a biobase freeze dryer (Model BK-FD12P) at a temperature of -68.4°C cold trap with a running time of 4 hours 32 minutes per day for four days. One hundred ten grams of the freeze-dried BSFL sample was ground using a ceramic pestle and mortar (Housia *et al.,* 2014 modified).

2.3.4 Physicochemical Analysis of BSF Larvae Oil

The physicochemical parameters of BSFL oil were analyzed following standard analytical methods. Acid Value (AV), Free Fatty Acid (FFA), and Peroxide Value (PV) were determined using standard methods. The fatty acid composition was determined. Three significant tests were performed, including acid value, peroxide value, and iodine value. Researchers measured skin compatibility using a digital pH meter. At the same time, viscosity evaluation occurred with a Brookfield viscometer for oil consistency assessment, and peroxide and anisidine value assays helped estimate shelf-life (Achuoth *et al.,* 2024). The refractive index instrument, the refractometer, analyzed the substance, while the specific density bottle revealed specific gravity information.

2.3.5 Refining of Crude BSFL Oil

A specialized sequence that included degumming and refinement allowed the testing of BSFL oil (CBSFLO). The procedure involved placing 20 mL CBSFLO into a test tube before centrifuging this mixture at 4000 rpm for thirty minutes. The oil received 1.0 mL phosphoric acid and sulfuric acid (H_2SO_4) addition at 70°C while stirring its content for thirty minutes on a hot plate magnetic stirrer. RBSFLO was obtained from the mixture after subjecting it to 30 minutes of centrifugation at 4000 rpm (Cang Mai *et al.,* 2019)

2.3.6 Antibacterial Analysis

BSF larvae oil underwent antibacterial testing against Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* together with Gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae* by using clinical bacterial isolates as per Zabulionė *et al.* (2023). Antibacterial BSF oil samples were tested through the agar well diffusion procedure. Sterile wells were made in Mueller-Hinton agar plates, which received filled oil samples before being incubated at 37°C for 24 hours. A measurement of the inhibition zone (mm) appeared on the data record (Nguyen *et al.,* 2024).

2.3.7Application on Skin Models

Krittika *et al.* (2024) assessed bacterial colonization and wound healing by applying BSF oil to ex vivo human skin samples.

Observation of Inflammation, Bacterial Reduction, and Healing Effects

- Histological analysis is a method that helps investigators view the state of skin epidermal structures and identify inflammatory reactions.

- Bacterial reduction assays: Quantified microbial load before and after treatment.
- According to Weththasinghe *et al*. (2022), assessments of wound healing took place within 7 to 14 days to measure tissue regeneration and collagen deposition.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Analysis

Table 1.Physicochemical Evaluation of Black Soldier Fly (*Hermetiaillucens*) Larvae Oil

Parameter	Crude Oil	Purified Oil
Refractive Index	1.2451	1.245
Viscosity (cp)	98.45	85.60
pH	7.44	1.244
Free Fatty Acid (FFA)	5.195	1.66
Acid Value	10.39	3.33
Peroxide Value	0.287	0.297
Specific Gravity	0.940	0.920

Lab tests show that black soldier fly larvae oil (BSFLO) undergoes positive changes during the crude oil's (CBSFLO) transition to refined oil (RBSFLO). The refractive index remains nearly constant (1.46553 to 1.46524), indicating minimal alteration in chemical structure. However, viscosity decreases notably (98.45 to 85.60), enhancing the oil's spreadability, which is advantageous for topical applications. A drastic reduction in pH from 7.44 to 1.44 makes RBSFLO more acidic, a feature often linked to enhanced antimicrobial properties, though it may raise concerns regarding skin tolerance without further formulation adjustments.

Purification also reduces free fatty acid (FFA) content (5.19% to 1.66%) and acid value (10.39 to 3.33), indicating improved stability and lower susceptibility to rancidity, essential for shelf life extension—the peroxide value slightly declines (0.297 to 0.287), suggesting limited lipid oxidation post-purification. The specific gravity of the water showed a minor decrease from 0.940 to 0.920, which could be attributed to the removal of heavier impurities during the refining process. The findings support previous research examining insect oil refinement because it improved stability and utility. As identified in studies, the reduced FFA mean,sures, and peroxide figures improve the oxidative stability of refined oils for medical and cosmetic purposes (Surendra *et al.*, 2016). More research needs to be conducted about the solution's pH level because it exhibits similar antimicrobial properties as tested formulations, yet its benefits for skin tissue need evaluation (Kim *et al.*, 2020).

Scientific research on insect oils demonstrates the pharmaceutical and skin health benefits that refining operations can provide. The reduction of FFA and acid value in refined oils matches previous research about how refined oils maintained their stability to function correctly when used topically (Surendra *et al.*, 2016). Scientific evidence shows that processed oils experience improved oxidative stability when the peroxide value decreases (Maszewska *et al.*, 2018). The pH level of RBSFLO reaches values similar to those found in skin treatment solutions because acids help prevent microbial growth (Kim *et al.*, 2020).

Evidence shows that RBSFLO presents improved characteristics for possible applications in antimicrobial medical treatments alongside skin-related products. Further examination is needed to verify the solution's safe application to the skin, even though its low pH shows potential for microbial inhibition. Prioritizing the delivery of acceptable formulation results requires evaluating both purification results and practical product needs.

3.2 Antimicrobial Analysis

Table 2. Antimicrobial Activity of BSF Larvae Oil against Selected Bacterial Isolates

Isolates	**Purified BSF Larvae Oil (mm)**	**Autoclaved BSF Larvae Oil (mm)**	**Crude BSF Larvae Oil (mm)**
Escherichia coli	18.4 ± 0.30	0.0 ± 0.00	0.0 ± 0.00
Pseudomonas aeruginosa	17.8 ± 0.35	0.0 ± 0.00	0.0 ± 0.00
Bacillus subtilis	15.3 ± 0.30	0.0 ± 0.00	0.0 ± 0.00
Staphylococcus aureus	19.2 ± 0.30	0.0 ± 0.00	0.0 ± 0.00

Autoclaved and crude BSF larvae oil displayed no antibacterial effect against tested bacterial isolates according to the determination (0.0 ± 0.00 mm), whereas purified BSF larvae oil proved effective for all bacterial isolates. *Staphylococcus aureus* achieved the largest inhibition zone of 19.2 ± 0.30 mm compared to *Escherichia coli* (18.4 ± 0.30 mm), *Pseudomonas aeruginosa* reached (17.8 ± 0.35 mm) and *Bacillus subtilis* had (15.3 ± 0.30 mm). Research by Azmiera *et al.* (2023) and Zabulionė *et al.* (2023) has shown that antimicrobial peptides (AMPs) and medium-chain fatty acids (MCFAs) with special prominence of lauric acid make BSF larvae effective as a bacterial fighter. Heat-sensitive bioactive compounds in autoclaved oil are responsible for antimicrobial effects because heat treatment destroys AMPs and fatty acids found in BSF-derived extracts (Xia *et al.,* 2021).

The antibacterial zone measurements from this study exceeded previous findings of Zabulionė *et al.* (2023) regarding BSF fat fractions when measuring *E. coli* and *S. aureus* growth inhibition. The purification process increased the amount of antimicrobial compounds available in BSF larvae oil, thus showing potential as a natural antibacterial agent for dermatological applications. The antibacterial properties in both autoclaved and crude oils became absent because low-temperature extraction procedures combined with purification methods are necessary to maintain bioactive compounds. The research demonstrates how bioactive compounds extracted from BSF have the potential to serve as a renewable solution for antibiotic alternatives designed explicitly for skin use since they fight bacterial skin infections through heat-resistant antimicrobial peptides and fatty acids. Additional research is needed to investigate BSF oil stability in skincare products and its long-term antibacterial properties.

4. CONCLUSION

This research shows that purified BSF larvae oil exhibits strong antimicrobial properties by blocking Gram-positive and Gram-negative bacteria. Improving physical attributes and decreasing free fatty acids through purification processes made BSF larvae oil appropriate for dermatological product development. Additional research must assess how long the purified BSF larvae oil remains stable while investigating which ingredients work best with it and its results when used in skincare products.

Author Contributions

The conceptualization, project management, writing of original draft, reviewing and editing of the manuscript have all been done by all authors. They were also engaged in reviewing, editing, validation, and overseeing the MP. This research work has been read and approved by all authors.

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The data will be available upon request.

Conflicts of Interest

The authors have no conflicts of interest to disclose.

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