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Antibacterial Activities of Blowfly (*Lucilla sericata*) and Housefly (*Musca domestica*) Maggots Extract on Some Selected Isolates

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Abstract: Antimicrobial substances have played a pivotal role in global health since the discovery of penicillin in 1941. However, the rise of antimicrobial resistance poses a significant threat to public health. This study focuses on the potential antibacterial properties of maggot extracts from *Lucilia sericata* and *Musca domestica* on selected bacterial isolates. The study was conducted in the Federal Capital Territory, Abuja, Nigeria, with maggot collection, extraction, and antibacterial assays carried out through agar-well diffusion methods. The results indicate dose-dependent antibacterial activity, with the aqueous extract showing the highest efficacy. The minimum inhibitory concentrations were determined, highlighting varying sensitivities among tested bacteria. The findings suggest the potential of maggot extracts as alternative antimicrobial agents, emphasizing their efficacy against *Staphylococcus aureus*. This research provides a scientific basis for considering maggot extracts in the development of new therapies, particularly for infections caused by antibiotic-resistant bacteria.

Key Words: *Antimicrobial resistance, Maggot extracts, Antibacterial activity, Minimum inhibitory concentration (MIC), Staphylococcus aureus*

1 Introduction

Antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoan, as well as killing viruses. The discovery of antimicrobials like penicillin and tetracycline paved the way for better health for millions around the world. Before 1941, the year where penicillin was discovered, there were no true cure existed for some diseases such as gonorrhoea (a venereal disease involving inflammatory discharge from the urethra or vagina), strep throat, or pneumonia (a lung infection in which the air sacs fill with pus). Often, the infected wound patients have a wounded limb removed, or face death from the infection. Research into antibacterial activity is crucial for safeguarding the health of millions worldwide. The quest for new cures against diseases caused by microorganisms underscores the significance of this endeavor, as it directly impacts human health and well-being.

Some of the microorganisms, especially bacteria are becoming resistant to more and more antibacterial agents. By making a new research, antibacterial agents that can kill or inhibit the growth of other bacteria can be found (Cosgrove *et al*., 2003). Now, most of these infections can be cured easily with a short course of antibacterial.

Microorganisms, especially bacteria are becoming resistant to more and more antimicrobial agent. They are becoming resistant more quickly than new drugs that are being made available. Therefore, future research in antimicrobial therapy may focus on finding the new antimicrobial that can overcome this problem, or treat infections with alternative means.

The increase of life-threatening infections that are resistant to commonly used antibiotics has become a worldwide problem (Enerijiofi and Isola, 2019). It is becoming an important cause of morbidity in immune-compromised patients particularly in developing countries (Al-Bari *et al*., 2006). The increasing prevalence of multi-drug resistant strain of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics has raised the spectre of untreatable bacterial infection and adds urgency to search for new infection - fighting strategies (Akortha et al., 2010; Zy *et al*., 2005, Rojas *et al*., 2006).

Presently, there are global problems of emergence of multiple antibiotics resistance as well as emergence of new and resurrection of previously eradicated disease. There is need to search for new and more

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potent antimicrobial compounds of natural origin to complement the existing synthetic antimicrobial drugs that are gradually becoming less potent against pathogenic microorganisms (Adesina *et al*., 2013).

The continuous spread of multi-drugs resistant pathogen has become a serious threat to public health and major concern for infection control practitioners' worldwide (Ovuru *et al.*, 2023). In addition to the increasing cost of drug regimens, this scenario has paved way for the re-emergence of previously controlled disease and has contributed substantially to the high frequency of opportunistic and chronic infection cases in developing countries (Fernandez *et al*., 2003 and Ako-Nai *et al*., 2003.)

The extract of fly maggot has been shown to completely lyse the cell wall of many bacteria including those that infect chronic wounds. As the population ages, the number of patients suffering from bacteria infected chronic wounds attributable to diseases such as diabetes mellitus and peripheral vascular disease is on the rise. This poses a significant impact on the health care system, because of the chronicity of care required and the associated costs.

i. The aim of this study was to determine the antibacterial effects of *Lucillia sericata* and *Musca domestica* maggot extracts against some selected bacterial isolates.

2 Materials and Methods

2.1 Study Area

The study area for this research is Federal Capital Territory (FCT) Abuja. The territory is located just north of the confluence of the River Niger and River Benue. It is bordered by the states of Niger to the West and North, Kaduna to the Northeast and South, and Kogi to the Southwest. The Federal Capital Territory lies between latitude 8.25 and 9.20 North of the equator and longitude 6.45 and 7.23 East of the Greenwich Meridian. Abuja is geographically located in the centre of the country.

2.2 Collection and Preparation of Live Maggot

The maggots of *Lucilia sericata* and *Musca domestica* were jointly collected by allowing flies to feed on cattle liver in different transparent plastic containers covered with a rubber net to enable the flies to feed and lay their eggs effectively. The flies were allowed to escape by opening the rubber net after 2 hours. After an incubation period of 24 hours, the eggs hatched and developed into larvae known as maggots. The first generation of larvae was allowed to turn into pupae to get a new breed of flies, which were reintroduced into another fresh transparent plastic container containing fresh cattle liver for feeding and laying of eggs. This process was repeated thrice to aseptically obtain clean flies' maggots since they were not allowed to roam outside the study environment and feed on dirt. It took around 7 days for a fly life cycle to be completed depending on the temperature, and maggots grow well in the dark.

2.3 Harvesting of Maggots

The third instar maggots were harvested from the transparent plastic containers containing cattle liver by removing them with a sterile teaspoon and transferring them into a sterile universal bottle and taken to the laboratory.

2.4 Decontamination of Maggots

Whole live maggots were decontaminated by soaking in 70% alcohol for 5 minutes. Thereafter, they were transferred into another sterile container covered with a rubber net to prevent maggots from escaping while allowing alcohol to evaporate from the body of the maggots. Maggots were kept in this decontaminated container for 30 minutes to allow the recovery of normal metabolism by maggots to produce efficient extracts which are located in the guts.

2.5 Maggot Extraction Procedures

2.5.1 Aqueous Extraction

This was carried out according to the method of Suchi et al. (2010). Maggots numbering 200, 400, 600, and 800 were respectively pulverized in a plastic laboratory mortar with a plastic laboratory pestle. The paste was collected and soaked in 2 ml of sterile water and allowed to incubate for 1 hour at room temperature. The mixture of pulverized maggot and sterile water was transferred into a centrifuge test tube to remove the particulate by centrifugation at 5,000g for 15 minutes at room temperature (25±2ºC). The supernatant was collected for antibacterial screening.

2.5.2 Saline Water Extraction

This was carried out according to the method of Suchi et al. (2010). Maggots numbering 200, 400, 600, and 800 were respectively pulverized in a plastic

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laboratory mortar with a plastic laboratory pestle. The paste was collected and soaked in 2 ml of saline water and allowed to incubate for 1 hour at 28ºC. The mixture of pulverized maggot and saline water was transferred into a centrifuge test tube to remove the particulate by centrifuging at 5,000g for 15 minutes at room temperature (25±2ºC). The supernatant was collected for antibacterial screening.

2.5.3 Phosphate Buffer Saline Extraction

This was carried out according to the method of Suchi et al. (2010). Maggots numbering 200, 400, 600, and 800 were respectively pulverized to paste in a plastic laboratory mortar with a plastic laboratory pestle. The paste was collected and soaked in 2 ml of PBS and allowed to incubate for 1 hour at room temperature. The mixture of pulverized maggot and PBS was transferred into a centrifuge test tube to remove the particulate by centrifuging it at 5,000g for 15 minutes at room temperature (25±2ºC). The supernatant was collected for antibacterial screening.

2.6 Preparation of Extract Concentrations

Ahmed and Nancy (2012) state that the total protein measured from native excretory/secretory product of 100 larvae per 1 ml distilled water was 0.9 mg. Therefore, 200 maggots per 2 ml of distilled water will be 0.9 mg.

2.7 Identification of Bacterial Isolates Used

The bacterial isolates were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. They were clinical isolates obtained from University of Abuja Teaching Hospital, Abuja, Nigeria. The cultures were gram-stained for proper confirmation using standard gram staining procedures. The cultures were maintained on nutrient agar slants at 36ºC after which they were stored in the refrigerator and later re-identified and sub-cultured by biochemical test (Cheesbrough, 2005; Oyeleke and Manga, 2008).

2.8 Preparation of Inoculum

Inoculums for each organism were prepared in a test tube of 5 ml of saline water by touching the fresh colonies with a sterile wire loop to obtain a very small portion of the organism and adding it to the 5 ml of saline water. This was stirred for proper mixing and to obtain a low bacterial load.

2.9 Preparation of the Control Antibiotic (Ampiclox) for the Antibacterial Activity

For the control, a known standard antibiotic ampiclox was used just like that of the stock solution. The standard antibiotic was weighed into different concentrations (50, 100, 200, 250, and 500 mg/ml) using a weighing balance. It was then dissolved in 1 ml of sterile water for injection in sterile universal containers that have been properly labelled to obtain stock cultures of it.

2.10 Antibacterial Assay

The antibacterial assay was performed using the agar-well diffusion method as described by Okoli and Iroegbu (2004) to determine the growth inhibition of the test organisms by the maggot extracts. 25 ml each of the prepared Muller Hinton Agar was poured into sterile Petri dishes that were already properly labelled for easy identification and left to solidify. Using sterile pipettes, the agar was aseptically inoculated uniformly by flooding with 1 ml suspension of the three test organisms which had been prepared using a loop full of the inoculum in saline water. The plates were then rocked carefully for the inoculum to spread around the agar and allowed to dry.

Different agar wells were then made on the plates with the aid of a sterile cork borer (6 mm in diameter). Four wells were made on the agar, sufficiently spaced away from the edge of the plate and 25 mm from well to well to prevent overlapping of zones and labelled properly according to the three different crude extract concentrations at the bottom of the Petri dish. The same was done for the standard control plates for easy identification. The stock solutions of the crude extracts of the aqueous, saline water, and phosphate buffer saline were introduced (2-3 drops) into each agar well using a sterile Pasteur pipette, according to the labels including that of the standard antibiotics' plates.

The plates were then incubated at 37ºC for 24 hours after which the sensitivity of the test organisms to aqueous, saline water, and PBS extracts of maggots were determined by measuring the zone of inhibition. The measurement of the zone of inhibition was carried out using a transparent meter rule and read to the nearest millimetres. The diameter of the clear zone was taken as an index of the degree of sensitivity.

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2.11 Determination of Minimum Inhibitory Concentration (MIC)

The MIC values of the extracts were determined using the dilution method of the extracts. Various concentrations (0.9, 1.8, 2.7, and 3.6 mg/ml) of the maggot extracts were prepared. 36 test tubes were set up; 2 ml of nutrient broth was pipetted into each sterile test tube that was already labelled properly. Control tubes were also set up: Tube A (test tubes containing extracts and broth), Tube B (test tube containing broth and each inoculum), and Tube C (test tube containing broth only). Using sterile Pasteur pipettes, 0.2 ml suspension of the test organisms was introduced into the test tubes according to their labels. Also, 2-3 drops of the different stock solutions were introduced using sterile Pasteur pipettes into the test tubes containing both broth and inoculum. The preparation was incubated at 37°C for 24 hours after which the test tubes were observed for turbidity or clearness. The least concentration where no turbidity was observed was noted as the minimal inhibitory concentration (MIC) value.

2.12 Determination of Minimum Bactericidal Concentration (MBC)

The least concentration of maggot extracts that has an antibacterial effect on the organism is considered as the minimum bactericidal concentration (MBC). This was determined from the broth dilution resulting from MIC tubes by subculturing to the surface of freshly prepared nutrient agar plates by using a sterile inoculating loop to streak the plate as described by Vollekova et al. [12pt]article graphicx array amsmath

RESULTS

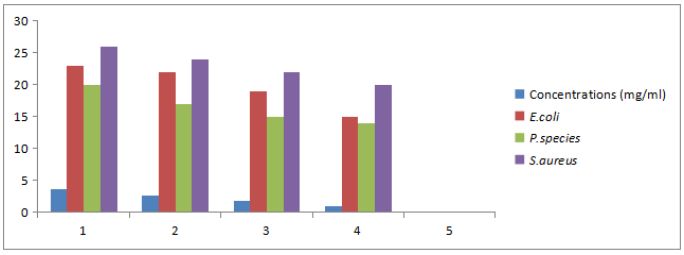

Figure 1: Bar chart representing the efficacy of different concentrations in mg/ml (aqueous) against tested organisms compared to each other.

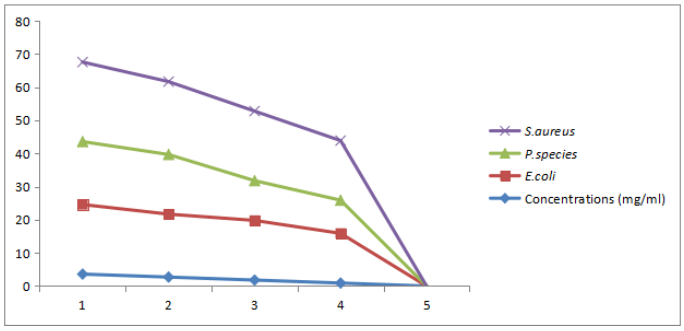

Figure 2: Stark line with markers chart representing the rate of efficacy of extract (aqueous) against the tested organisms.

3 Discussion

A total yield of 30% soluble excretion/secretion was obtained for the aqueous, saline water, and phosphate buffer saline (PBS) extracts, with an original weight of 0.4 g/g of the whole maggot of *Lucilia sericata* and *Musca domestica*. The physical characteristics are indicated in Table 1. Tables **??** to **??** show the results of the antibacterial pattern of the extracts against the test organisms.

The results obtained from this study demonstrated a dose-dependent activity of *L. sericata* and *M. domestica* maggot or larva extracts (aqueous, saline water, and PBS) at tested doses (0.9, 1.8, 2.7, and 3.6 mg/ml) on the test organisms (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*). Maggot excretion/secretions located in their mid-gut contained protein or antibacterial peptide, as noted by Ai et al. (2008, 2012), Hon et al. (2007), and Cao et al. (2011), who extracted chitosan, HF-1 (a novel antibacterial peptide), and Lectin, respectively.

The bar chart, stacked line with markers chart, and pie chart illustrate the efficacy of the different concentrations in mg/ml (0.9, 1.8, 2.7, and 3.6 mg/ml) compared to each other, the efficacy of extracts (aqueous, saline water, and PBS) against the tested organisms, and the growth level of tested organisms (*E. coli*, *P. aeruginosa*, and *S. aureus*) compared to each other, respectively.

The minimum inhibitory concentration (MIC) of the aqueous maggot extract against *E. coli*, *P. aeruginosa*, and *S. aureus* was 1.8 mg/ml, as shown in Table **??**. The MIC of the saline water maggot extract against *E. coli* and *P. aeruginosa* was 3.6 mg/ml, while for *S. aureus* it was 1.8 mg/ml, as shown in Table **??**. The

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Table 1: Physical characteristics of the Maggot Extract

Maggot parts	Solvent	Weight of maggot (g/g)	% Yield	Colour	Odour	Texture
Whole maggot	Aqueous	0.4	30	Yellow	Unpleasant	Oily
	Saline water	0.4	30	Yellow	Unpleasant	Oily
	PBS	0.4	10	Yellow	Unpleasant	Oily

Table 2: Zone Diameter of inhibition (mm) of the aqueous extract on test organisms

Microorganisms	Concentration (mg/ml)			
	0.9	1.8	2.7	3.6
Escherichia coli	15 ± 0.0	19 ± 0.0	22 ± 0.0	10 ± 0.0
Pseudomonas aeruginosa	14 ± 0.0	15 ± 0.0	17 ± 0.0	11 ± 0.0
Staphylococcus aureus	20 ± 0.0	22 ± 0.0	24 ± 0.0	26 ± 0.0

The table shows the zone diameter of inhibition for test organisms at different concentrations of the aqueous extract.

MIC of the PBS maggot extract against *P. aeruginosa* and *S. aureus* was 3.6 mg/ml, with no MIC against *E. coli*, as shown in Table **??**.

The minimum bactericidal concentration (MBC) of the aqueous maggot extract against *E. coli* and *P. aeruginosa* was 3.6 mg/ml, while for *S. aureus* it was 2.7 mg/ml, as shown in Table **??**. The MBC of the saline water maggot extract against *S. aureus* was 3.6 mg/ml, with no MBC for *E. coli* and *P. aeruginosa*, as shown in Table **??**. There was no MBC for the PBS maggot extract against *E. coli*, *P. aeruginosa*, and *S. aureus*, as shown in Table **??**.

The largest zone of inhibition was obtained with the aqueous extract against *S. aureus*, and the lowest was obtained with the PBS extract against *E. coli*. The highest MIC was observed with the aqueous extract on *E. coli*, *P. aeruginosa*, and *S. aureus* (1.8 mg/ml), while the lowest MIC was found with the PBS extract on *P. aeruginosa* and *S. aureus*, with no MIC on *E. coli* using PBS. The highest MBC was observed with the aqueous extract on *S. aureus* (2.7 mg/ml), and the lowest MBC was obtained with the saline water extract on *S. aureus* (3.6 mg/ml), but no MBC was obtained from PBS. It is evident from the results of this study that *S. aureus* was more susceptible to the maggot extract compared to *E. coli*, which showed the highest resistance, followed by *P. aeruginosa*. These findings agree with several literature reports (Meylaers et al., 2004; Liang et al., 2006; Wang et al., 2006; Jin et al., 2006; Cancado et al., 2007; Xu et al., 2007; Hou et al., 2007; Ai et al., 2008, 2012; Ren et al., 2009; Cao et al., 2011; Yoon et al., 2008; Jang et al., 2007; Suchi et al., 2010), where maggot extract was found to possess significant antimicrobial activities against several pathogens, especially *S. aureus*. In general, the low activity of the PBS extract on tested organisms might be due to the actions of different components that might have denatured the protein constituents of the maggot extract. This can be observed in the aqueous extract, which has the highest activity on tested organisms since it contains only two molecules of hydrogen and one molecule of oxygen.

4 Recommendation

It is recommended that while extracting excretions/secretions from maggots, a neutral solvent or sterile water should be used to obtain the extract in its natural form. The use of maggot extract is not yet prevalent in Nigeria; this can be improved or encouraged by health workers or the Ministry of Health as it serves as a good and cost-effective alternative to antibiotics.

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Table 3: Zone diameter of inhibition (mm) of the control Antibiotic on test organisms

Microorganisms	Concentration (mg/ml)				
	50	100	200	250	500
E. coli	26	31	32	36	38
P. aeruginosa	26	27	34	35	36
S. aureus	29	30	31	33	34

Table 4: Minimum inhibitory concentration (MIC) of aqueous extracts on test organisms

Microorganisms	Concentration (mg/ml)			
	0.9	1.8	2.7	3.6
E. coli	-	+MIC	+	+
P. aeruginosa	-	+MIC	+	+
S. aureus	-	+MIC	+	+

This is the lowest concentration of the extract required to inhibit the growth of the organism.
Key: += clear (no growth), - = turbid (growth)

5 Conclusion

From the study, it can be concluded that the aqueous extract is more efficient than extracts obtained from other solvents and should be preferred for obtaining maggot extract. These studies provide a scientific basis for the clinical or traditional use of maggot extract or whole maggot for therapy, especially for infections caused by *S. aureus*, which is sensitive to maggot extract.

6 Conflict of Interest

All authors declare that there is no conflict of interest. The study was conducted impartially and without bias.

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Table 5: Minimum inhibitory concentration (MIC) of saline water extracts on test organisms

Microorganisms	Concentration (mg/ml)			
	0.9	1.8	2.7	3.6
E. coli	-	-	-	+MIC
P. aeruginosa	-	-	+	+MIC
S. aureus	-	+MIC	+	+

This is the lowest concentration of the extract required to inhibit the growth of the organism.
Key: += clear (no growth), - = turbid (growth)

Table 6: Minimum inhibitory concentration (MIC) of PBS extracts on test organisms

Microorganisms	Concentration (mg/ml)			
	0.9	1.8	2.7	3.6
E. coli	-	-	-	-
P. aeruginosa	-	-	-	+MIC
S. aureus	-	-	-	+MIC

This is the lowest concentration of the extract required to inhibit the growth of the organism.
Key: += clear (no growth), - = turbid (growth)

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Table 7: Minimum Bactericidal Concentration (MBC) of aqueous extracts on test organisms

Microorganisms	Concentration (mg/ml)			
	0.9	1.8	2.7	3.6
E. coli	+	+	+	-MBC
P. aeruginosa	+	+	+	-MBC
S. aureus	+	+	-MBC	-

This is the lowest concentration of the extract required to kill the organism completely.
Key: += bacterial growth, - = no bacterial growth

Table 8: Minimum Bactericidal Concentration (MBC) of saline water extracts on test organisms

Microorganisms	Concentration (mg/ml)			
	0.9	1.8	2.7	3.6
E. coli	+	+	+	+
P. aeruginosa	+	+	+	+
S. aureus	+	+	+	-MBC

This is the lowest concentration of the extract required to kill the organism completely.
Key: += bacterial growth, - = no bacterial growth

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Table 9: Minimum Bactericidal Concentration (MBC) of PBS extracts on test organisms

Microorganisms	Concentration (mg/ml)			
	0.9	1.8	2.7	3.6
E. coli	+	+	+	+
P. aeruginosa	+	+	+	+
S. aureus	+	+	+	+

This is the lowest concentration of the extract required to kill the organism completely.
Key: += bacterial growth, - = no bacterial growth

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