Indonesian Journal on Health Science and Medicine

Vol. 3 No. 2 (2026): Oktober
DOI: 10.21070/ijhsm.v3i2.497

IJHSM

Indonesian Journal on Health Science and Medicine

UNIVERSITAS MUHAMMADIYAH SIDOARJO

ISSN 3063-8186 (online), https://ijhsm.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

Indonesian Journal on Health Science and Medicine
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ISSN 3063-8186 (online), https://ijhsm.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

Indonesian Journal on Health Science and Medicine

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Indonesian Journal on Health Science and Medicine
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Indonesian Journal on Health Science and Medicine

Vol. 3 No. 2 (2026): Oktober
DOI: 10.21070/ijhsm.v3i2.497

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ISSN 3063-8186 (online), https://ijhsm.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

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Biological activity of cinnamon and cardamom oils on some virulence factors of Pseudomonas aeruginosa

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Abstract

General Background: Drug-resistant *Pseudomonas aeruginosa* poses a severe global health hazard, demanding alternative therapeutic strategies. **Specific Background:** Plant-derived essential oils such as cinnamon and cardamom oils are investigated for their potential to target bacterial virulence factors without triggering antimicrobial resistance. **Knowledge Gap:** However, the comparative efficacy of cinnamon oil versus cardamom oil in suppressing biofilm development, pigment production, and bacterial motility remains insufficiently characterized. **Aims:** This study evaluated the comparative biological activity of cinnamon and cardamom oils against biofilm formation, pyocyanin production, and swarming motility in a clinical isolate of *P. aeruginosa*. **Results:** Microtiter plate assays revealed that cinnamon oil successfully reduced strong biofilm formation to weak biofilm formation at 0.8 µl/ml and suppressed pyocyanin production at 0.4–0.8 µl/ml, while significantly restricting swarming motility; conversely, cardamom oil showed no effect on biofilm formation at any tested concentration and exhibited lower efficacy in inhibiting pigment production and movement. **Novelty:** This study demonstrates the superior, concentration-dependent anti-virulence potency of cinnamon oil over cardamom oil against *P. aeruginosa*. **Implications:** These findings support the potential application of cinnamon oil as a natural therapeutic agent to attenuate key virulence factors in multidrug-resistant

Keywords : Cinnamon Oil, Cardamom Oil, Pseudomonas Aeruginosa, Biofilm Formation, Pyocyanin Production

Key Findings Highlights

- Cinnamon oil effectively converted strong biofilm production to weak biofilm production at 0.8 µl/ml, whereas cardamom oil exhibited no anti-biofilm activity at any tested concentration.
- Pyocyanin pigment synthesis was fully suppressed by cinnamon oil at concentrations between 0.4 and 0.8 µl/ml, demonstrating significantly higher inhibitory activity than cardamom oil.
- Swarming motility on agar was markedly restricted by cinnamon oil compared to cardamom oil and untreated controls.

Published date: 2026-08-09

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Introduction:

Pseudomonas aeruginosa is a Gram-negative bacterium, which is rod shaped, motile, non-fermenting, and a member of the Pseudomonas genus. These bacteria are widespread in the environment; they are normally found in soil, water, plants, and humans. They are microorganisms, which have the ability to live in harsh environments at different temperatures ranging from 4 – 42 degrees centigrade. Adaptability of *Pseudomonas aeruginosa* is what enables it to survive in hospital surfaces for as long as six months. *Pseudomonas aeruginosa* can adapt to hosts by making harmful substances. These substances help it infect and cause disease. Examples include biofilms, pyocyanin pigment and proteases. These factors make it harder to treat infections and create a problem in hospitals. *Pseudomonas aeruginosa* is a type of bacteria that causes infections in hospitals. These infections include pneumonia from ventilators, infections in care units and infections from central lines. It also causes infections in sites, urinary tract infections and burn wound infections. The formation of a layer called biofilms is key to *Pseudomonas aeruginosa* survival. Biofilms protect it from antibiotics. The bodys immune cells. Biofilms are made of proteins, DNA and sugars. Biofilms help *Pseudomonas aeruginosa* share genes that make it resistant to antibiotics. This makes it harder to treat infections. *Pseudomonas aeruginosa* uses biofilms to survive and dominate in the lungs of people with fibrosis. *Pseudomonas aeruginosa* colonizes surfaces, including medical equipment and food industry tools. Removing biofilms is important for treating infections and controlling their spread. Pyocyanin is another substance made by *Pseudomonas aeruginosa*. It can harm cells. Make it harder for the body to fight infections. It also causes the death of neutrophils which're important for fighting infections. These processes contribute to *Pseudomonas aeruginosa* causing disease. *Pseudomonas aeruginosa* uses biofilms and substances like pyocyanin to survive and cause infections. It is a concern, for hospitals and healthcare facilities, whereas pyocyanin-producing strains are more virulent and more resistant to many drugs than non-pyocyanin-producing strains (20, 21).

Swarming motility is another weapon for *Pseudomonas aeruginosa*that increases its effectiveness and pathogenicity. Swarming motility is the rapid and coordinated translocation for the multicellular activity of some bacterial species across semi- solid surfaces by flagella. It is an important virulence factor associated with its antibiotic resistance (22, 23).

When we talk about *Pseudomonas aeruginosa* we see that some special helpers, called antivirulence factors can stop the bacteria from doing things. We found this out by using kinds of essential oils that can prevent the bacteria from making biofilms and doing other harmful things.

Cinnamon is a kind of food that has antimicrobial properties, which means it can help fight off bad germs. People trust cinnamon. Use it every day so it is a good choice. We often use cinnamon to help with problems like nausea, vomiting and diarrhea. That is why cinnamon oil is used in some toothpastes to help keep our teeth clean.

Cardamom oil is also a product that can help with many health problems, like colds, bronchitis and asthma. It can even help people who have trouble eating. Some studies show that cardamom has powers that can help keep us healthy like stopping germs and even fighting off cancer.

In the few decades we have seen that bacteria are getting stronger and can fight off antibiotics. This is a problem so we need to find new ways to stop the bacteria from doing bad things. Of trying to kill the bacteria we want to stop them from being so harmful. So our study is about seeing how natural oils, like cinnamon and cardamom oils can affect *Pseudomonas aeruginosa*. This study aims to evaluate the efficacy of these oils in inhibiting biofilm formation, pyocyanin production, and swarming motility in Pseudomonas aeruginosa.

Materials and Methods:

The isolate of *Pseudomonas aeruginosa*was obtained from the Department of Bioligy Sciences /College of Science /University of Mosul .

1. Solution and Stain

A: The Normal Saline Solution

The solution was prepared by dissolving 0.9 g of sodium chloride in 100 cm^3 of distilled water (29).

B-Crystal Violet Stain with 1% concentration

To make the stain, we dissolved 1 g of dye powder into 100 cm^3 of distilled water. After filtering the mixture, we transferred it to a sterile glass bottle for storage at room temperature (30 °C).

2: Culture Media

1- **Trypic Soy Broth (TSB) :**

Prepared by dissolving 30 grams in one liter of distilled water (HIMEDIA).

2- Nutrient Broth Medium (N.B.) :

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Prepared by dissolving 25 grams in one liter of distilled water (HIMEDIA).

3- Swarm Agar Medium :

To get started you need to mix some things. First take one gram of glucose. Add it to one hundred milliliters of distilled water. Then do the same with half a gram of bactoagar. Next add six tenths of a gram of bactopeptone to the water. After that put in two tenths of a gram of yeast extract. All of these things need to be dissolved in the one hundred milliliters of distilled water.

1- Biofilms Formation

A: Evaluation of Biofilm Formation by *Pseudomonas aeruginosa* :

The researchers checked how *Pseudomonas aeruginosa* forms biofilms using plates.

They used 96-well plates from Fisher Scientific in Pittsburgh, PA.

1. They filled a medium called TSB with *Pseudomonas aeruginosa* that had been activated beforehand and keep at 37°C for one day.

The growth of the bacteria was compared to a McFarland tube.

2. They put 200 µL of the TSB medium into the first column of wells.

This was used as a control to compare with the samples.

They made three copies of this to make sure it was accurate.

3. They put 200 µL of the *Pseudomonas aeruginosa* into the three wells of the second column.

The plate was then. Kept at 37, °C for 18 to 24 hours.

1. Wash the wells with saline to get rid of any loose bacterial cells.
2. Add 200 µL of crystal violet solution, which was 1% concentration to each well and let it sit for 45 minutes. After a thorough rinse with distilled water, we set the plate aside on the bench to air-dry at room temperature for about 45 minutes.
3. Next add 200 µL of ethanol which was 99% pure, to each well and mix it up like it shows in figure 1.

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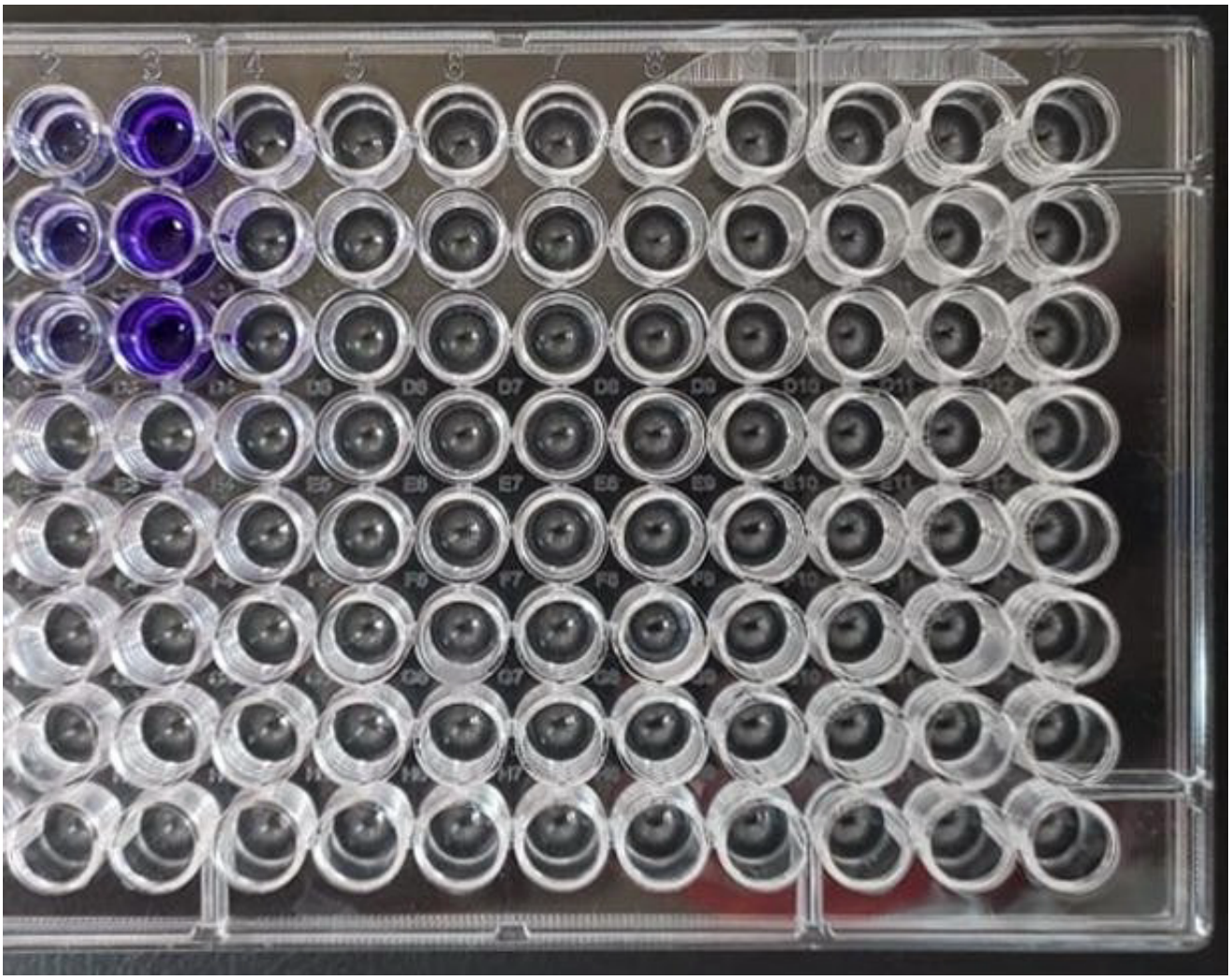

Figure 1. Figure (1): The microtiter plate After adding (99 %) ethanol.

4. The amount of light that the sample absorbed was measured at a wavelength of 630 nanometers using an ELIZA reader device that was made in America.

- The isolate is non-biofilms-forming :_ If the absorbance rate of the control factor is greater than, or equal to the absorbance rate of the bacterial isolate ($A^o \leq A^o$ C).
- The isolate is weakly biofilms-forming:_ If the bacterial isolate absorbance rate is greater than the control's absorbance ,equal to, or smaller than twice the absorbance rate for control (A^o C< $A^o \leq 2x$ A^o C).
- The isolate is moderately in biofilms formation: _If the absorbance rate of the isolate is greater than twice the absorbance of the control, smaller, or equal to four times the absorbance of control (2x $A^o < A^o \leq 4x A^o$ C) .
- The isolate is a strong biofilms-former : _If the absorbance rate of the isolate is greater than four times that of the absorbance of control ($A^o > 4x A^o$ C).

The absorbance rate of the isolate under test was compared with the control, and the result was recorded (31,32,33).

B: Disruption of Biofilm Formation using Cinnamon and Cardamom Oils:_

The people at AL_EMAD Company made cinnamon oil and cardamom oil that we used to see how these oils affect the formation of biofilms by the *Pseudomonas aeruginosa* isolate we were studying. We bought these oils from a shop that sells herbs.

We made amounts of cinnamon oil and cardamom oil by mixing them with TSB. The amounts we used were 0.1, 0.2 0.4 and 0.8 µl/ml.

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We used plates called 96-well flat-bottom polystyrene microtiter plates from Fisher Scientific in Pittsburgh, PA.

The quantity of cinnamon oil was added to the first column of the microtiter plates, with 300 µL volume being added thrice in case of control. In the second column, 290 µL quantity of cinnamon oil was mixed with 10 µL bacterial inoculum, and this process was also repeated thrice.

We did the thing for the other amounts of cinnamon oil one after the other in the next columns.

We also did the thing for the cardamom oil but we started from 4 row of the first column

We kept the plates at 37°C for 24 hours. After that we looked at how the different amounts of cinnamon oil and cardamom oil affected the formation of biofilms. We did this by following the steps we did before. We can see the results in figures 2, 3 and, in 31 32 and 33.

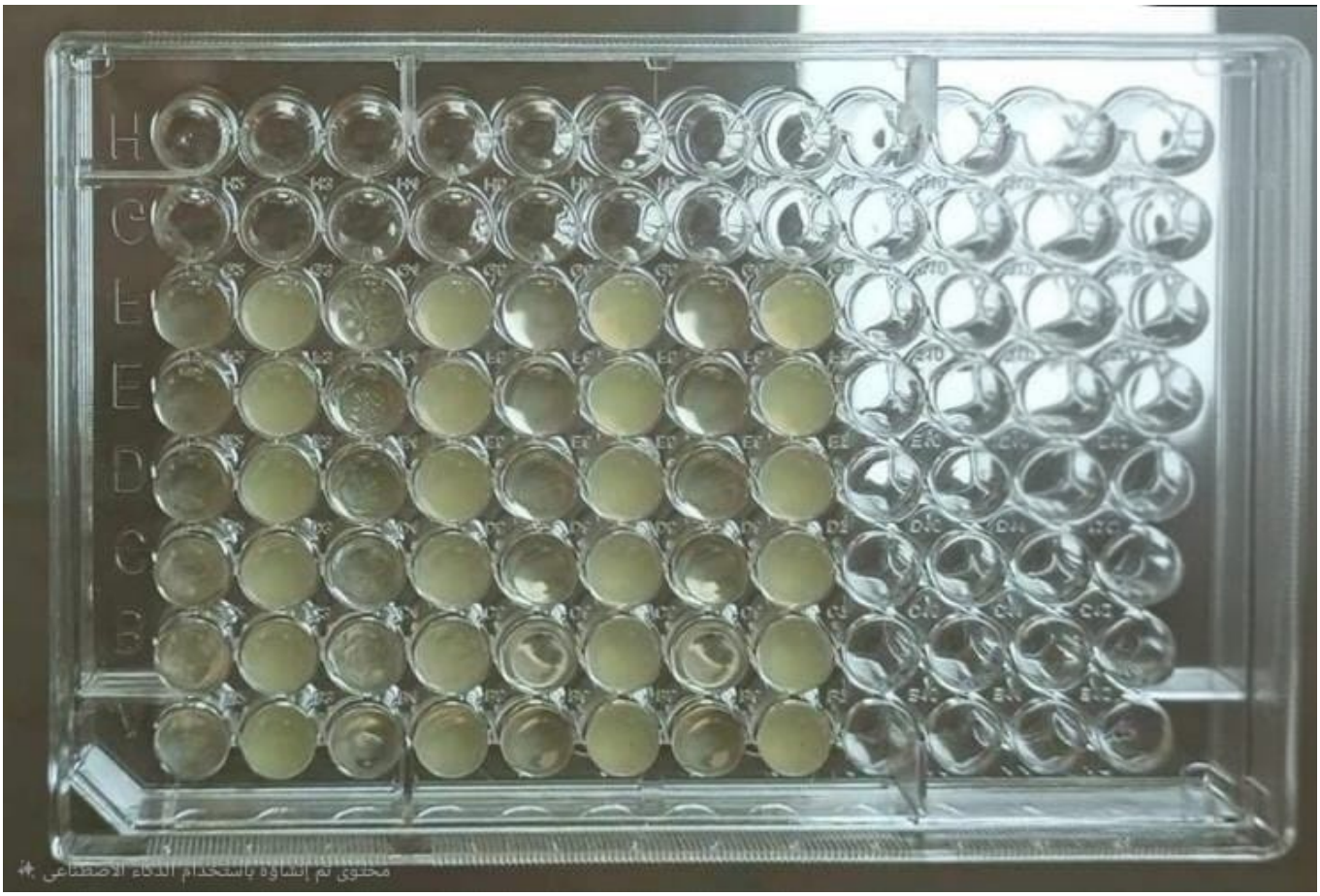

Figure 2. Figure(2):The microtiter plate After incubation for 24 h.

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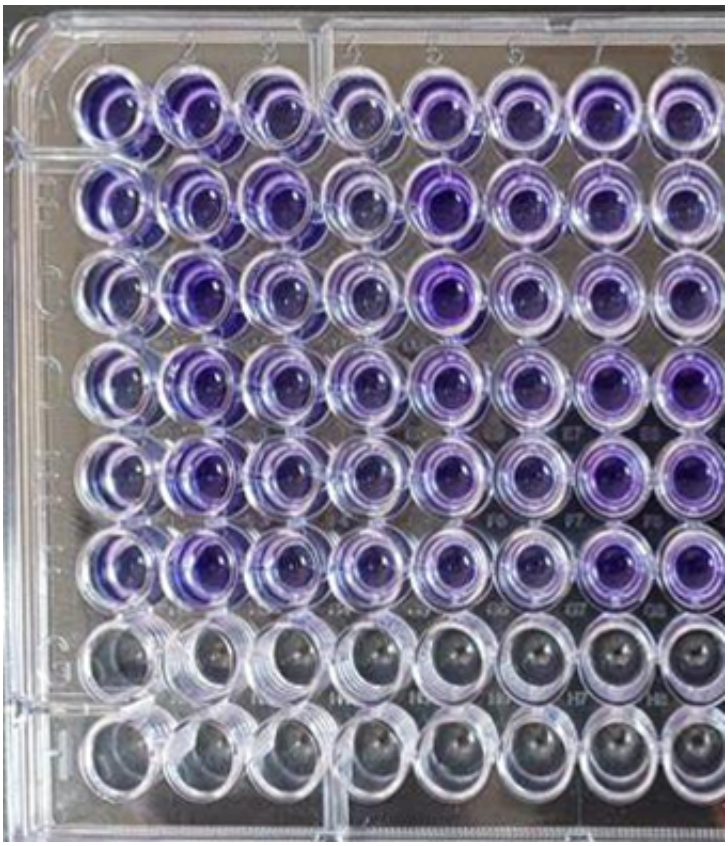

Figure 3. **Figure(3): The microtiter plate After adding crystal violet**

2- Pyocyanin Production

The effects of cinnamon and cardamom oils on pigment production in Pyocyanin were determined through an experiment carried out as follows: Nutrient Broth (N.B.) was prepared using the concentrations of (0.1, 0.2, 0.4, and 0.8 µl/ml) cinnamon and cardamom oils individually, and a control without any oil at all. All these preparations were inoculated with Pseudomonas aeruginosa -0.5 maccfarland- and incubated at 37oC for 24 hours (34).

3- Swarming Motility Assays

Swarming motility test was carried out following the previously described procedure (24). In brief, overnight grown cultures of Pseudomonas aeruginosa were spotted onto swarm plates of nutrient agar containing either cinnamon or cardamom oils (0.2 µl/ml) or no oils and then incubated upright at 37°C for 24 hours.

Results and Discussion

1-Biofilms formation

Complications related to bacterial biofilm infections result in significant complications for healthcare from both morbidity rates as well as heightened mortality rates. To assess whether the *Pseudomonas aeruginosa* isolate had the capacity to produce biofilm, we will assess through titration using microtiter plates, followed by ELIZA readings and calculations that yield results that correspond with the control will yield evidence demonstrating that this isolate indeed formed a greater level of biofilm than that of the control, as shown in figure 4.

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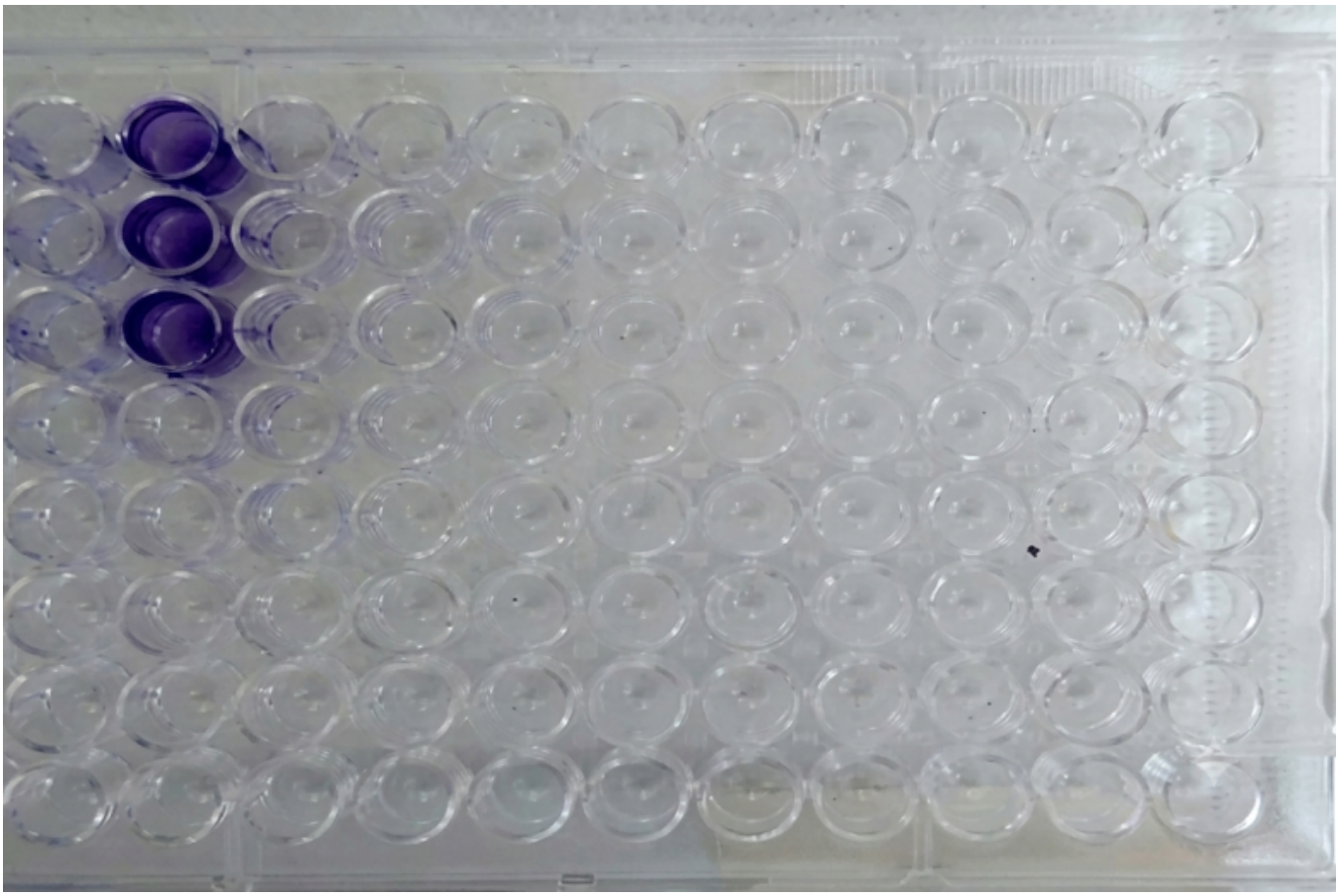

Figure 4. **Figure (4) : Biofilm formation by *Pseudomonas aeruginosa***

These findings align to the work of Kunwar et al. (35), who demonstrated that certain *Pseudomonas aeruginosa* strains recovered from burn wounds possess a strong capacity for biofilm production. Similarly, Abdelraheem et al. (36) observed that of their clinical isolates 14% were robust biofilm formers, while Tuon et al. (37) underscored the critical role this species plays in establishing these structured communities. The underlying process of *Pseudomonas aeruginosa* biofilm development follows a clear sequence: it begins when planktonic, free-floating bacteria loosely attach to a conditioned surface. This initial, reversible step quickly transitions into permanent anchoring as surface adhesins lock the cells in place. Next, the bacteria secrete a protective extracellular matrix, allowing the colony to mature fully. The cycle concludes when cells detach from the mature matrix and disperse to colonize new sites (38).

2. Efficacy of Cinnamon and Cardamom Oils against Biofilm Formation:_

In comparison with controls, all samples of cinnamon oil demonstrated an ability to change strong biofilm production of isolate to weak biofilm production at a concentration of only 0.8 µl/ml (figure 5). The cardamom oil did not provide any impact at any concentration with respect to the isolated strong biofilm-producing ability (figure 5).

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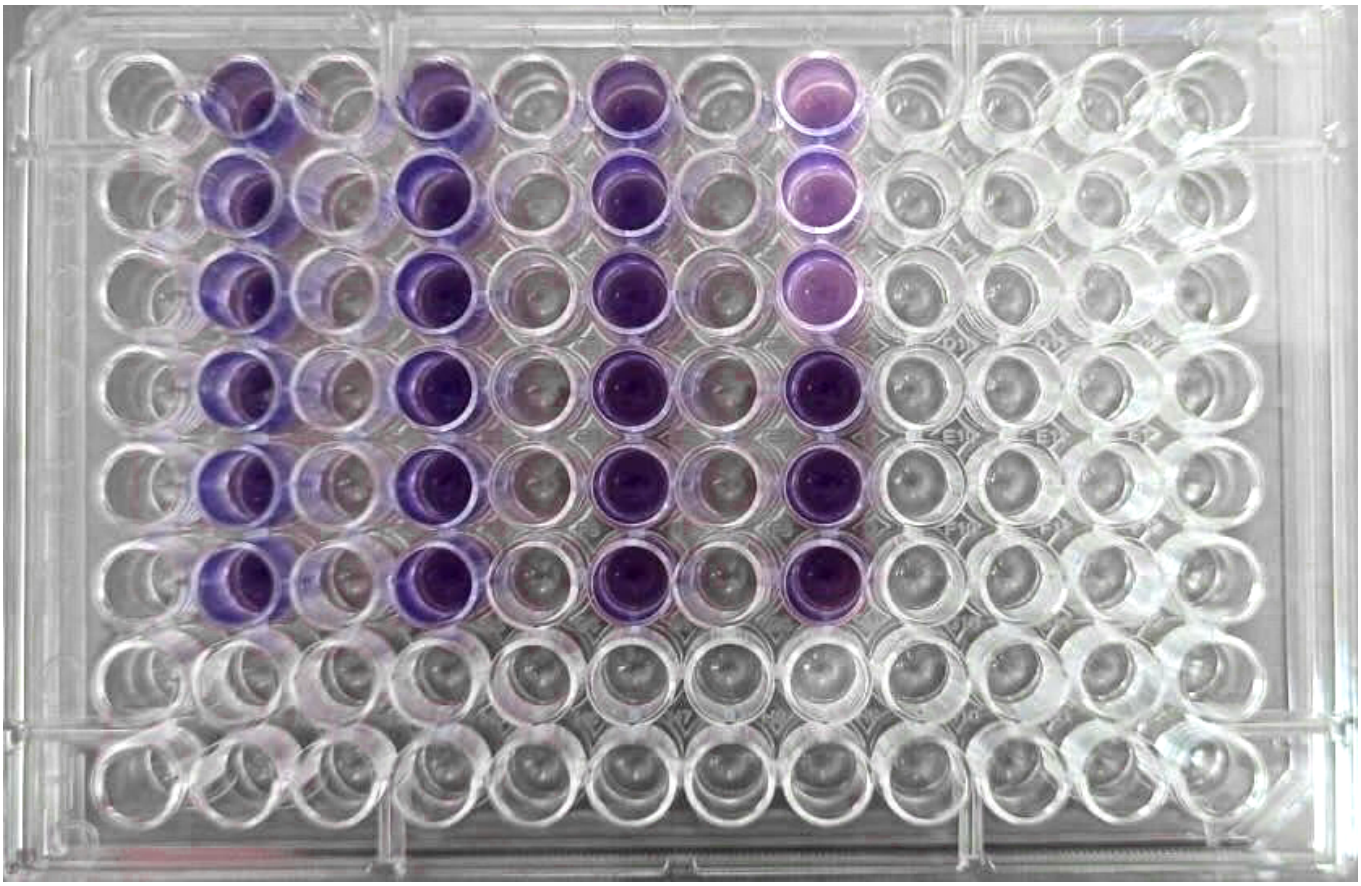

Figure 5. Figure(5): Cinnamon and Cardamom Oils: Impact on Biofilm Formation

As reported by Kalia et al. (24), the oil of cinnamon has a strong inhibition of Pseudomonas aeruginosa biofilms at the concentration of 0.8 µl/ml. The reason for the inhibition is due to the depletion of structural components of biofilms by cinnamon oil. These include bound extracellular DNA and extracellular polymeric substances (EPS). Furthermore, rather than targeting a single pathogen, Wijesinghe et al. (39) have shown the inhibitory effect of cinnamon oil on biofilms in the case of Pseudomonas aeruginosa, Staphylococcus aureus, and Klebsiella pneumoniae.

This anti-biofilm activity largely stems from cinnamaldehyde, the primary active constituent making up roughly 65% of cinnamon oil. Research indicates that cinnamaldehyde obstructs quorum sensing, impairs bacterial motility, and halts the production of alginate (24, 40, 41). Because alginate acts as a critical scaffolding component that maintains the structural integrity of the extracellular matrix, blocking its synthesis directly prevents the biofilm from reaching full maturation.

3- Pyocyanin Production

The results obtained for the effects of cinnamon oil and cardamom oil on pyocyanin production indicated that the effectiveness of cinnamon oil was higher than cardamom oil. The use of cinnamon oil suppressed the production of the color at an amount, ranging from 0.4 to 0.8 µl/ml. At the use of 0.2 µl/ml of cinnamon oil, there was suppression in the production of the color in comparison with the control group as illustrated in figure 6. Cardamom oil had production of the color at 0.8 µl/ml as shown in figure 7. Both cinnamon oil and cardamom oil had effects on pyocyanin production. However, cinnamon oil was more effective than cardamom oil. The control group had the production of color while cinnamon oil and cardamom oil inhibited the production. The production of pyocyanin was inhibited when cinnamon oil and cardamom oil were applied. Cinnamon oil and cardamom oil both inhibited pyocyanin production and cinnamon oil was the one.

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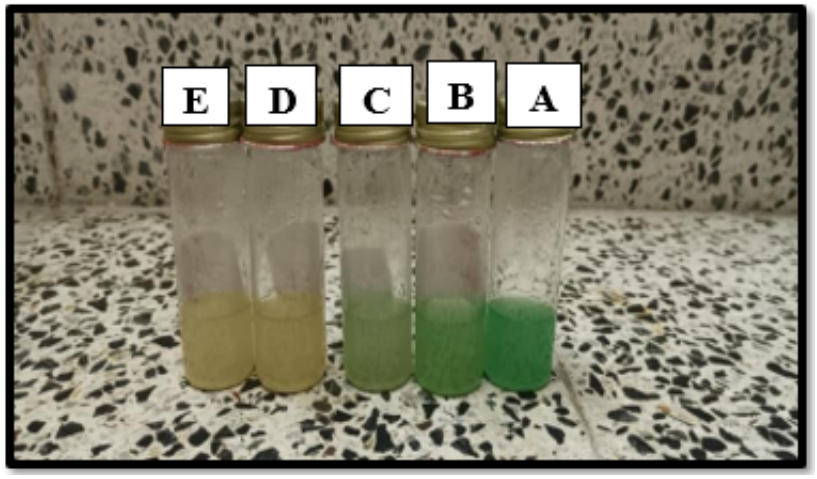

Figure 6. **Figure(6): Inhibitory Effect of Cinnamon Oil (µl /ml) on Pyocyanin Production A:control , B:0.1 , C:0.2 , D:0.4 ,E:0.8**

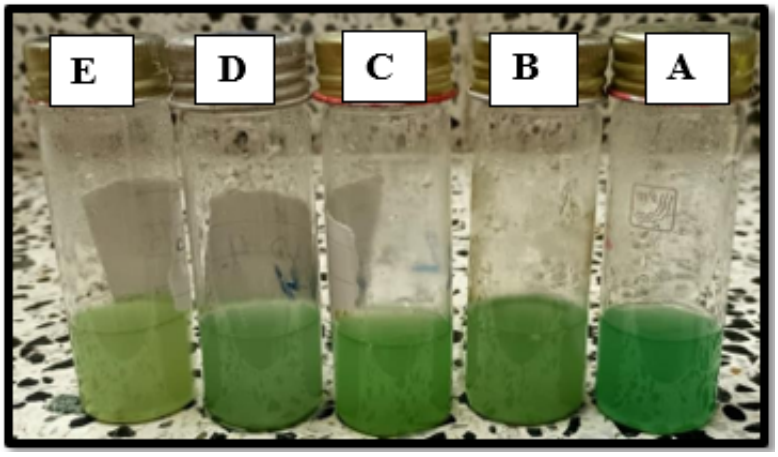

Figure 7. **Figure (7): Impact of Cardamom Oil (µl /ml) on Bacterial Pyocyanin Production**

A:control , B:0.1 , C:0.2 , D:0.4 , E:0.8

The outcome of cinnamon oil is proven by the research done by Kalia et al. (24), who discovered that the inhibitory effect of different concentrations of cinnamon oil on pigment production was 22% at a concentration of 0.2 µl/ml. In addition to that, Farisa Banu et al. (42) observed that cinnamon oil has an inhibitory effect on the production of pyocyanin by Pseudomonas aeruginosa. The authors believe that the inhibitory effect of pyocyanin could be due to the presence of either LasR or rhl inhibitor present in essential oils because of the presence of rhl in P.aeruginosa.

Regarding cardamom oil, cardamom oil has been demonstrated to possess biological activity by many researchers. Alam et al. (43), have shown cardamom oil's infectious potential for inhibiting *Pseudomonas aeruginosa* growth.

4- Swarming motility Assays

The application of cinnamon oil adheres to the findings in Kalia et al. (24). In their study, Kalia et al. (24) used different concentrations of cinnamon oil, where one of these concentrations, 0.2 µl/ml, resulted in the reduction of pigment synthesis by 22%. Further, the damning effects of cinnamon oil on the synthesis of pyocyanin in Pseudomonas aeruginosa were examined by Farisa Banu et al. (42). Farisa Banu et al. (42) concluded that the inhibition of pyocyanin is attributed to the presence of either LasR or rhl receptor antagonists in the essential oil. But the rhl receptors of Pseudomonas aeruginosa assist in gene expression for pigment synthesis.

In addition to cinnamon oil, there are many references for biological activity of cardamom oil. In the study by Alam et al., it was proven that cardamom oil can inhibit the growth of Pseudomonas aeruginosa. (9).

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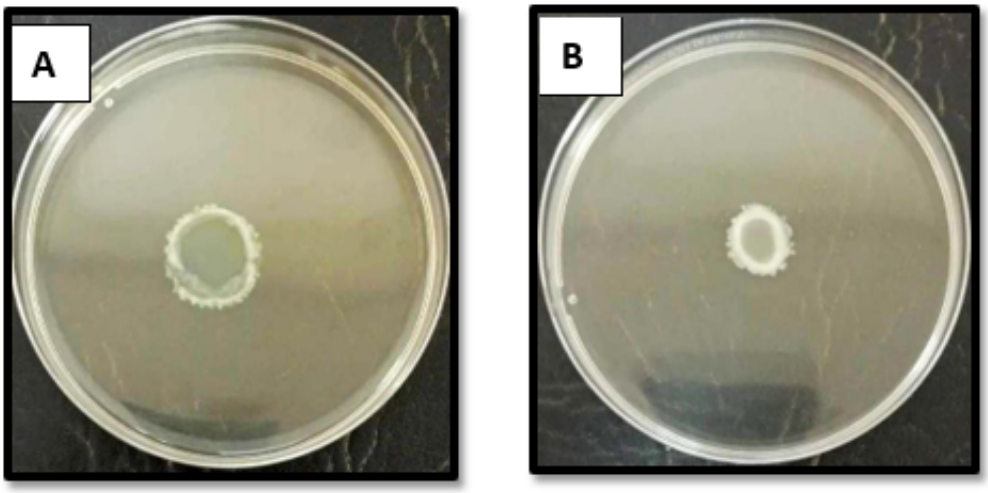

Figure 8. Figure(8): Impact of Cinnamon and Cardamom Oils (0.2 µl/ml) on Swarm Agar Growth and Motility.

A:Control B: Cardamom oil C: Cinnamon oil

Agha (44) observed that cinnamon oil has an inhibitory effect on the swarming motility of Pseudomonas, and that the oils from cinnamon and eucalyptus trees are better than any other oil in producing this effect. Kalia1 et al. also showed that swarming motility was inhibited by cinnamon oil. Cinnamon oil has been demonstrated to reduce the expression of fliC and rhlA which are involved in flagella synthesis and rhamnolipid production (45).

Also, Tuba et al. discovered that the swarming motility of Pseudomonas aeruginosa was inhibited by a compound known as Cinnamaldehyde (CAD), which is an extract of cinnamon oil, by modulating the intracellular signaling pathways that influence movement and biofilm formation (46). Noumi et al. (47) found that cardamom oil reduced swarming motility by reducing the size of the colony compared with the control group. According to Akrayi (48), there is a phenolic compound in the oil responsible for inhibiting the movement of microorganisms by attaching itself to the cell wall phospholipids and proteins

Conclusions:

According to the recent research, it was found that cinnamon oil is more efficient than cardamom oil in terms of inhibiting the biofilm formation of Pseudomonas aeruginosa at a concentration of 0.8 µl/ml. In addition, it was found that the cardamom oil does not affect the biofilm formation at any concentration. It was also observed that the cinnamon oil was capable of inhibiting the synthesis of pigment pyocyanin at concentrations of (0.4 and 0.8) µl/ml whereas the lowest amount of pigment was synthesized at a concentration of 0.8 µl/ml in case of cardamom oil.

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ISSN 3063-8186 (online), https://ijhsm.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo