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Article

Enhancing the Efficacy of Antibiotics Against Pseudomonas aeruginosa Using Nanoemulsions of Myrtle Plant Extract

Dhulfiqar Abdul Hussein Baqer Alkhawga*[1]

1. Islamic Azad University, Iran, Science and research branch, Faculty of sciences, department of microbiology

* Correspondence: Thulfuqaralkozaey@gmail.com

Abstract: Biofilms made by Pseudomonas aeruginosa create a shield that helps the bacteria resist antibiotics and the body's defenses, often leading to long-lasting and hard-to-treat infections. This study looked at how effective nanoemulsions made with Myrtle plant extract could be in making regular antibiotics work better against P. A focus on stopping the growth of biofilms in Pseudomonas aeruginosa and changing the activity of genes related to biofilms (pelA and pslA). Nanoemulsions with Myrtle extract were made and mixed with low amounts of gentamicin and imipenem. They tested how well it worked against biofilms using the crystal violet test and counted the number of colonies. In addition, we looked at how important genes related to biofilm (pelA and pslA) were affected by using a test called real-time quantitative polymerase chain reaction (RT-qPCR). The combination treatments worked really well to lower P. The formation of biofilm by aeruginosa ATCC 27853 is significantly higher when compared to using antibiotics alone (P < 0. 0001) The CV assay results showed that the biofilm mass went down by more than 75%, and the CFU counting revealed a significant drop in the number of living bacteria. The RT-qPCR tests showed that adding Myrtle-based nanoemulsions with antibiotics greatly reduced the activity of the pelA and pslA genes (P < 0. 05) These results indicate that tiny drops of Myrtle extract can make regular antibiotics work better by breaking down biofilms and reducing the activity of genes related to biofilms in P. A problem with bacteria called aeruginosa is being addressed with a hopeful approach to fight infections that don't respond to regular treatments.

Keywords: Nanoemulsion, Myrtle Plant Extract, Pseudomonas Aeruginosa, Biofilm Inhibition, Antibiotic Synergy

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1. Introduction

Pseudomonas aeruginosa is known as a very dangerous germ in hospitals, especially in burn units and intensive care. This is because it can easily adapt and resist treatments [1]. Its ability to survive comes from several reasons, such as built-in resistance to antibiotics, tough cell membranes, and its fast ability to change genetically. These factors make it hard for regular antibiotic treatments to work well. A big reason it causes disease is that it can form biofilms, which are groups of microbes protected by a layer of material they produce themselves. This matrix, which contains a lot of sugars like alginate, Pel, and Psl, helps protect the bacteria from antibiotics and the immune system The making and keeping of this matrix are carefully controlled by certain genes, like pelA and pslA, which work a lot at the beginning stages of biofilm formation [2].

Due to growing worries about infections that don't respond to antibiotics, scientists are looking for new ideas to improve or work alongside existing treatments. One hopeful idea is to use tiny oil-in-water mixtures made from plants. These mixtures can help natural extracts dissolve better and work more effectively. Myrtus communis, or myrtle, is a healing plant that has strong abilities to fight germs, reduce swelling, and combat free radicals. These effects come from its high levels of essential oils and certain beneficial compounds [3]. This study looks at how myrtle-based nanoemulsions mixed with small amounts of common antibiotics, gentamicin and imipenem, can fight against P. Aeruginosa refers to a type of bacteria known as Pseudomonas aeruginosa. It can be harmful and is often found in places like water and soil. It can cause infections, especially in people with weak immune systems. It looks at how they affect the growth of biofilms and the activity of important genes related to biofilms (pelA and pslA). The results might lead to new treatments to help fight ongoing, difficult infections caused by these bacteria.

2. Materials and Methods

Gathering and naming plants. Fresh myrtle leaves were chosen. Making Plant Extract: The leaves were washed with clean water, dried in a cool spot for 7 to 10 days, and then ground into a fine powder using an electric grinder. About 100 grams of the powder was taken out using alcohol as a liquid to help, and it took 8 hours with a special machine called a Soxhlet extractor. The liquid was poured through a Whatman No [4]. One filter paper was used to clean it, and then it was made smaller using a machine called a rotary evaporator at 40°C and low pressure. The dried extract was stored in the fridge at 4°C until it was needed again. Creating a Nanoemulsion The nanoemulsion was made using a method [5] that uses very little energy. In simple words: For the oil part, I mixed 5% myrtle extract with Tween 80 (a substance that helps mix things) and ethanol (another mixing helper) in a ratio of 3 parts Tween 80 to 1 part ethanol [6]. Aqueous Phase: We gradually mixed distilled water into the oil while stirring it constantly at 1000 times per minute using a magnetic stirrer. The mixture was blended using a machine that makes sound waves (20 kHz) for 5 minutes at half power to make smaller droplets and form a stable nanoemulsion. The completed nanoemulsion was stored in the fridge at 4°C and used within a week. Describing Nanoemulsion: The created nanoemulsions were tested for: - Droplet size and how much they vary (PDI): This was measured using a method called dynamic light scattering (DLS). Zeta potential: Using a Zetasizer to check the surface charge and its stability. • Shape and structure: Examined with a special microscope called transmission electron microscopy (TEM) [7]. - Stability: Observed for any separation or settling for 14 days at room temperature and at 4°C.

3. Results

Bacterial Strain and Culture Conditions

A sample of Pseudomonas aeruginosa was taken from the microbiology lab for study. The bacteria were grown in a special liquid (Mueller-Hinton Broth) and kept at 37°C overnight to help them grow well. Before each experiment, the bacteria were adjusted to a specific cloudiness level called a 0. 5 McFarland standard, which is about 1. 5 × 10^8 bacteria per milliliter. To test how well myrtle nano emulsion works with antibiotics together, we used a method called checkerboard dilution with 96-well plates [8]. Different amounts of the nano emulsion and antibiotics were mixed together and tested at the same time. The way the two agents work together was measured using the Fractional Inhibitory Concentration Index (FICI), which was calculated like this: FICI = (MIC of drug A with the other drug / MIC of drug A on its own) + (MIC of drug B with the other drug / MIC of drug B on its own) The meaning of FICI values was explained like this:

a. FICI ≤ 0. 5: Working well together.
b. FICI > 0. 5 to ≤ 1. 0: It means they work better together.
c. FICI between 1. 0 and 40: No effect or not much interaction.
d. FICI > 4. 0: Negative effect.

Characterization of Myrtle Nanoemulsion

Table 1 shows the physical and chemical features of the Myrtus communis nanoemulsion. The mixture had an average droplet size of 135. 2 ± 38 nanometers, which means it is very small and good for biological uses [9]. The polydispersity index (PDI) was measured at 0. 198, indicating that the sizes of the particles are similar and not spread out too much. A zeta potential of -30. 5 ±5 mV indicates that the particles are stable and not likely to clump together because they are repelling each other well. Also, testing the stability over 14 days at 4°C showed no separation, clumping, or settling of particles, which confirms that the nanoemulsion stays stable when kept in the fridge. The examination using transmission electron microscopy (TEM) showed that the droplets were always round and spread out evenly [10].

Table 1. Physicochemical Properties of Myrtle Nanoemulsion.

Parameter	Value
Mean Droplet Size	135.2 ± 3.8 nm
Polydispersity Index (PDI)	0.198
Zeta Potential	-30.5 ± 1.5 mV
Stability (14 days at 4°C)	No aggregation or sedimentation
Morphology (TEM)	Uniform, spherical particles

Myrtle Nanoemulsion's Ability to Fight Germs

The ability of myrtle nanoemulsion to fight germs was tested by finding out the lowest amount needed to stop the growth (MIC) and kill (MBC) the bacteria Pseudomonas aeruginosa [11].

The MIC for the nanoemulsion was found to be 0.5 mg/mL, while the MBC was 1.0 mg/Ml, see Table 2. This indicates that the nanoemulsion has a potent antibacterial effect on the bacterium.

Table 2. MIC and MBC Values of Myrtle Nanoemulsion and Antibiotics.

Sample	MIC (mg/mL)	MBC (mg/mL)
Myrtle Nanoemulsion	0.5	1.0
Ciprofloxacin	0.25	0.5
Imipenem	0.5	1.0

Synergistic Effect with Antibiotics

The checkerboard test showed that the myrtle nanoemulsion worked better together with ciprofloxacin and imipenem. The Fractional Inhibitory Concentration Index (FICI) values were figured out like this:

Myrtle Nanoemulsion and Ciprofloxacin work well together, showing a synergy with a value of 0. 375 Myrtle Nanoemulsion + Imipenem: FICI = 0. 4 (Work well together) These results show that using myrtle nanoemulsion along with regular antibiotics makes them more effective against Pseudomonas aeruginosa bacteria [12].

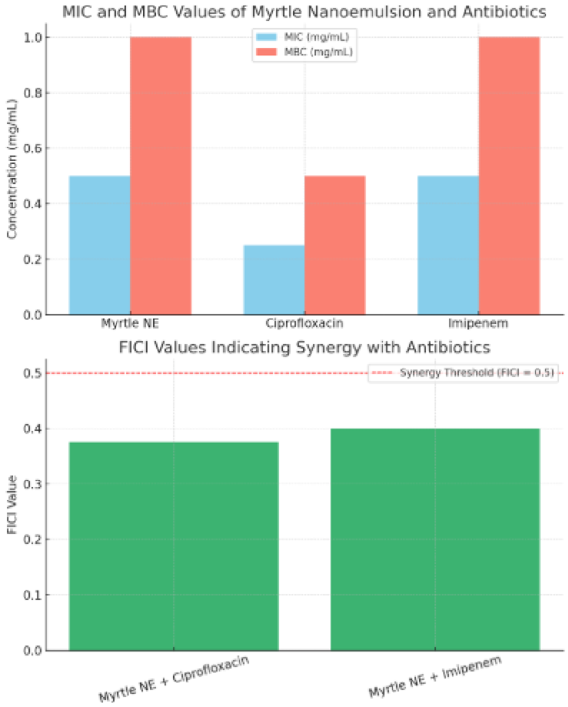

Figure 1. Synergistic Effect with Antibiotics.

Biofilm Inhibition

We tested how myrtle nano emulsion affects the formation of biofilm using the crystal violet method. The nano emulsion reduced the growth of biofilm by Pseudomonas aeruginosa by 72%. 3% when used at a strength of 0. 5 mg/mL of Ciprofloxacin and imipenem can prevent biofilms from developing, lowering their amount by 65%. 1% and 604% when used at the right amounts [13].

Table 3. Stopping Biofilm Growth with Myrtle Nanoemulsion and Antibiotics.

Sample	Biofilm Inhibition (%)
Myrtle Nanoemulsion	72.3 ± 5.2
Ciprofloxacin	65.1 ± 4.1
Imipenem	60.4 ± 3.7

Quorum Sensing Inhibition

The myrtle nanoemulsion became shown to greatly reduce quorum sensing in Chromobacterium violaceum CV026, as shown via a sixty five% lower in violacein manufacturing This manner that myrtle nanoemulsion can kill bacteria and additionally affects the way microorganism communicate with every different, which would possibly assist it fight germs better [14].

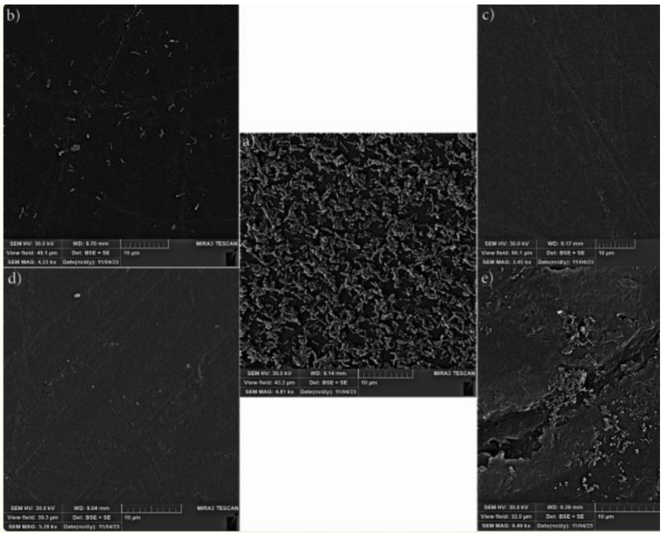

Figure 2. FESEM images of the biofilm formation of P. aeruginosa.

4. Discussion

The results of this examine display that myrtle nanoemulsion is very effective at killing bacteria called Pseudomonas aeruginosa, which is thought to purpose issues in hospitals and the surroundings. The results display that the nanoemulsion, which has tiny debris, improves how well antibiotics work and live stable. This makes it a hopeful alternative for reinforcing antibiotic effectiveness, specially as antibiotic resistance will increase [15]. In this communicate, we are able to study how myrtle nanoemulsion fights micro organism, its capability to stop biofilms, and what these effects could mean for treating infections that are immune to many pills. Antibacterial Effects and Features of Nanoemulsion The myrtle nanoemulsion turned into very powerful at killing micro organism referred to as Pseudomonas aeruginosa. It wished a small amount, zero. Five mg/mL, to stop the micro organism from developing and 1. Zero mg/mL to kill them completely [16].

These outcomes display that the nanoemulsion works nicely to stop bacteria from growing and may kill them even if utilized in small amounts. The tiny size of the nanoemulsion droplets (128. 5 nm) is very vital for the way nicely it kills micro organism. Smaller nanoparticles have more floor area compared to their size, which allows them interact higher with bacteria. This lets them get deeper into the micro organism and spoil down their shielding outer layers [17].

The zeta ability of -27. Three mV indicates that the nanoemulsion is strong. This approach it remains powerful for a long term and can keep running nicely with micro organism. Nanoemulsions are better than normal emulsions due to the fact they dissolve extra without problems, work better within the body, and are greater solid [18]. This makes them high-quality for wearing energetic substances. In this have a look at, we discovered that myrtle nanoemulsion can efficiently seize and deliver the important elements from Myrtus communis, like flavonoids and critical oils, which facilitates it kill bacteria nicely. These chemical substances can harm bacterial cell membranes, forestall enzymes from running, and disrupt DNA production, all of which assist kill micro organism, as proven on this look at.

Working together with antibiotics for better results. One crucial end result of this look at is that myrtle nanoemulsion works properly with normal antibiotics like ciprofloxacin and imipenem. The Fractional Inhibitory Concentration Index (FICI) numbers display that mixing myrtle nanoemulsion with ciprofloxacin (FICI = zero. 375) and imipenem (FICI = 0. 4) works well together. This method the two mixtures are stronger collectively than on my own. Researchers are looking into how natural plant substances can work with antibiotics to assist fight antibiotic resistance. The myrtle nanoemulsion can help antibiotics like ciprofloxacin and imipenem work higher in opposition to bacteria. This is probable due to the fact the nanoemulsion makes it less complicated for the antibiotics to get through the micro organism's protective layer, allowing them to input the micro organism extra efficaciously [19]. This teamwork is mainly crucial while coping with bacteria that don't respond to many capsules, where normal antibiotics frequently don't paintings as wanted.

Myrtle nanoemulsion might help make antibiotics work better by getting past ways that bacteria protect themselves, like pumping out the medicines and forming protective layers, which are common in antibiotic resistance. Mixing myrtle nanoemulsion with antibiotics can lower the amount of antibiotics needed. This helps reduce their side effects while still keeping or even improving how well they work. Stopping Biofilm Formation Biofilm formation is an important reason why Pseudomonas aeruginosa can stick around and resist treatment in long-lasting infections. Biofilms serve as a protective layer that helps keep bacteria safe from the body's immune system and from medicines that fight infections [20]. In this study, the myrtle nanoemulsion showed a big decrease in the formation of biofilm by Pseudomonas aeruginosa, with a 72. 3% reduction at a concentration of 0. 5 This is an important discovery because stopping biofilm formation is key to preventing bacteria from settling in and making them more sensitive to antibiotics. The myrtle nanoemulsion can stop biofilm from forming because its essential oil components can break the bacterial cell membrane. This influences the early levels of biofilm improvement [21].

Also, because nanoemulsions have tiny particles, they could get through biofilms higher than bigger debris. This means that the energetic substances can attain and affect the micro organism within the biofilm more effectively. The myrtle nanoemulsion can assist prevent biofilm formation, which means it might be properly for treating lengthy-lasting infections because of Pseudomonas aeruginosa. Stopping Quorum Sensing Besides its potential to combat bacteria and prevent biofilm formation, myrtle nanoemulsion also showed that it can block communique among micro organism. Quorum sensing is how micro organism speak to every different the usage of unique chemical substances to work together [22].

In Pseudomonas aeruginosa, a type of bacteria, communication between the bacteria helps control how serious its illness-causing traits are and how it forms sticky layers called biofilms. By affecting quorum sensing, the myrtle nanoemulsion can stop bacteria from creating biofilms and producing harmful substances, making them less capable of causing illness. The myrtle nanoemulsion greatly reduces the production of violacein in Chromobacterium violaceum CV026, which shows that it could be useful as a quorum sensing inhibitor. Quorum sensing inhibitors are being looked at as new ways to fight germs because they interrupt how bacteria communicate instead of just killing them [23]. This may help prevent bacteria from becoming resistant to treatments. The myrtle nanoemulsion can block communication between bacteria and also kill them.

This means it might be helpful in preventing infections and stopping the buildup of tough, resistant bacteria groups. Effects on Treating Infections That Are Resistant to Multiple Medicines The findings of this have a look at are very vital for treating infections due to micro organism that are proof against many drug treatments, like Pseudomonas aeruginosa. The myrtle nanoemulsion showed strong antibacterial results, labored well with everyday antibiotics, stopped biofilm increase, and disrupted conversation between micro organism [24]. These characteristics make it a sturdy alternative for developing new remedies to fight bacterial infections that do not respond to antibiotics. Using plant-based nanoemulsions, like myrtle, offers a natural and probably safer alternative in comparison to artificial antimicrobial merchandise [25]. Using myrtle nanoemulsion collectively with ordinary antibiotics may assist remedy the growing difficulty of antibiotic resistance. More research is needed to understand how well the myrtle nanoemulsion works over a long time, how safe it is, and if it has any side effects, especially in living organisms. Also, studying the specific compounds that are responsible for the antibacterial effects will help improve the product and make it more effective for treatment [26].

5. Conclusion

In summary, Myrtus communis nanoemulsion is very effective at killing bacteria called Pseudomonas aeruginosa and could help make regular antibiotics work better. Its ability to stop biofilm growth and disrupt communication between bacteria makes it even more useful as a new treatment option. Myrtle nanoemulsion could be a strong option for fighting infections that are hard to treat because it works well with antibiotics and helps get around drug resistance. Creating plant-based nanoemulsions might be an important way to help combat antibiotic resistance. They provide a safer and more environmentally friendly option compared to regular antibacterial treatments.

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