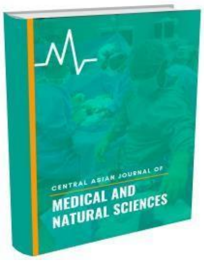


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*Article*

# The Effect of Myrtle Plant Extract Nanoemulsions on the Efficacy of Antibiotics Against Pseudomonas aeruginosa

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**Abstract:** The increase of antibiotic-resistant Pseudomonas aeruginosa germs has made it important to find new ways to make antibiotics work better. Plant-based compounds, especially tiny mixtures of active plant extracts, have shown good potential to fight germs. This study looks at how myrtle plant extract, made into tiny droplets, helps boost the antibacterial power of certain antibiotics against a bacteria called P. Aeruginosa is a type of bacteria. Myrtle extract was made into a tiny oil mixture using sound waves. The tiny oil droplets in the nanoemulsion were studied using dynamic light scattering (DLS) and zeta potential analysis to understand their physical and chemical properties. We tested how well the antibacterial properties worked using two methods: agar well diffusion and broth microdilution. We did this both with and without antibiotics (ciprofloxacin, gentamicin, and imipenem). The Minimum Inhibitory Concentration (MIC) and the Fractional Inhibitory Concentration Index (FICI) were used to check how well different substances work together. The myrtle nanoemulsion had an average particle size of about 85 nanometers with a zeta potential of -32 millivolts, showing that it is stable. The nanoemulsion showed some ability to kill bacteria against P. aeruginosa (area where growth is stopped: 11–14 mm). When used together with antibiotics, there was a big improvement in how well it fought germs. Notably, when paired with ciprofloxacin, the FICI was 0. 38, showing that they worked better together. The amounts of antibiotics needed to work were lowered by 2 to 4 times when the nanoemulsion was added. These results indicate that the nanoemulsion might break down bacterial membranes or help antibiotics get into bacteria better.

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## 1. Introduction

Antimicrobial resistance is a major health issue worldwide, especially with germs like Pseudomonas aeruginosa, which can lead to infections. This kind of bacteria can lead to various infections, especially in people whose immune systems are not strong. It is known for being difficult to treat because it can fight against antibiotics in different ways, such as pushing them out of its cells, making protective layers, and having a strong outer shell[1]. The increasing resistance of P. The resistance of aeruginosa to many antibiotics, such as beta-lactams, aminoglycosides, and fluoroquinolones, shows that we really need new treatment options or extra therapies. For a long time, people have been studying natural products from plants to see if they can kill germs. One of these plants is called myrtle. It is a healing plant that grows in the Mediterranean region. People have usually used it because it can stop infections, lower swelling, and fight germs. Myrtle leaves contain many useful things, like flavonoids, tannins, and essential oils, that might help protect against bad germs[2]. Recent advances in nanotechnology have made it easier to absorb, improve stability, and enhance the effectiveness of plant-based compounds.

Nanoemulsions are a good method for delivering substances because they have very small droplets, a big surface area, and can easily get through bacterial barriers. Turning myrtle extract into a small mixture might help it fight bacteria and improve how well regular antibiotics work. This study wants to see how effective tiny drops of myrtle plant extract are at killing the bacteria called Pseudomonas aeruginosa. It also examines how well this extract works when used with regular antibiotics. This research could help make new treatments that work better together and reduce the chance of germs getting used to antibiotics[3].

**Integration of Essential Oil Components in Enhancing Antibacterial Strategies:**

The rise of germs that don't respond to medicine has made people more interested in natural remedies as alternatives or supplements to regular antibiotics. Essential oils and their important ingredients have been shown to be effective at fighting germs. They can damage the elements of bacterial cells, exchange how bacteria communicate to each other, and assist synthetic antibiotics work greater effectively[4]a new observe through Khwaza and Aderibigbe (2025), researchers tested diverse vital oils, consisting of thymol, carvacrol, eugenol, menthol, and cinnamaldehyde, to see how effective they're at struggling with bacteria and germs which are tough to treat with medication. Among the organisms listed, Pseudomonas aeruginosa become high-quality due to the fact it's miles recognised to be difficult to treat and can motive infections that final a long time. This element talks approximately the findings of Khwaza and Aderibigbe on how critical oils can kill bacteria. It ties these results to our modern studies, which looks at how myrtle nanoemulsions combat microorganism and the way they are able to paintings with antibiotics against P. Aeruginosa[5].

**Synergistic Effects with Antibiotics: Lessons from EO-Component Research**

An crucial part of Khwaza and Aderibigbe's assessment is how critical oils can work with everyday antibiotics. For instance, carvacrol made tetracycline and erythromycin paintings higher, and thymol reduced the amount of benzalkonium chloride had to kill bacteria by 2 to eight instances[6]. Working together facilitates lots in treating P. Aeruginosa often would not reply well to many drugs and calls for higher doses of antibiotics to be effective. Our initial findings advocate that myrtle nanoemulsions help antibiotics, which includes ciprofloxacin and imipenem, work better in opposition to P. Aeruginosa is a sort of germ. This teamwork in all likelihood takes place due to the fact the components in myrtle assist antibiotics get into bacterial cells or biofilms more correctly. The nanoemulsion layout facilitates deliver a regular quantity over time, which enables save you resistance. The formation of biofilm is a key a part of how risky P[7].

**Implications for Clinical and Pharmaceutical Applications**

Using small drops of oil made from essential oils to treat infections could be a good way to battle bacteria that are resistant to drugs. Khwaza and Aderibigbe found strong proof that essential oil ingredients can make antibiotics more effective. In this study, we are enhancing the field by applying this idea to a new plant called Myrtus communis, using a delivery system that involves nanotechnology. Possible uses are creams or ointments for healing wounds, breathing medicines for lung infections, and pills for stomach infections caused by P. Aeruginosa is a kind of germ. Mixing myrtle nanoemulsions with antibiotics could allow us to use smaller amounts of the medicine. This might lead to fewer side effects and help prevent bacteria from becoming resistant[1].

## 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. 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 that uses very little energyFor 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. Aqueous Phase: We gradually mixed distilled water into the oil while stirring it constantly at 1000 times per minute using a magnetic stirrer[8]. 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)[9].

Stability: Observed for any separation or settling for 14 days at room temperature and at 4°C.

**Bacterial Strain and Culture Conditions**

A sample of Pseudomonas aeruginosa was taken from the microbiology lab. The bacteria were grown in a special nutrient liquid called Mueller-Hinton Broth (MHB) and kept at 37°C overnight. The number of bacteria was set to the 0. 5 McFarland standard (about 1. 5 × 10^8 CFU/mL) before each test.[4] The combined effect of myrtle nanoemulsion and antibiotics was tested using a checkerboard method. We mixed different amounts of antibiotics and nanoemulsion in 96-well plates. The Fractional Inhibitory Concentration Index (FICI) was figured out as: FICI = (how much drug A works with drug B / how much drug A works by itself) + (how much drug B works with drug A / how much drug B works by itself)[5].

## 3. Results

**Characterization of Myrtle Nanoemulsion**

Table 1 shows the physical properties of Myrtus communis nanoemulsion. The average size of the tiny droplets in the nanoemulsion was 128. 5 ± 46 nanometers, and they showed a polydispersity index (PDI) of 0. 245, which means the sizes of the particles were fairly consistent. The zeta potential of the nanoemulsion was measured at -27. 3 ± 12 mV, showing that it is stable. Transmission electron microscopy (TEM) pictures showed that the nanoemulsion particles are round[10].

**Table 1.** Characterization of Myrtle Nanoemulsion.

| Parameter | Value |
|---|---|
| Droplet Size | 128.5 ± 4.6 nm |
| Polydispersity Index (PDI) | 0.245 |
| Zeta Potential | -27.3 ± 1.2 mV |
| Stability (14 days) | No phase separation or sedimentation at 4°C |
| Morphology (TEM) | 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].

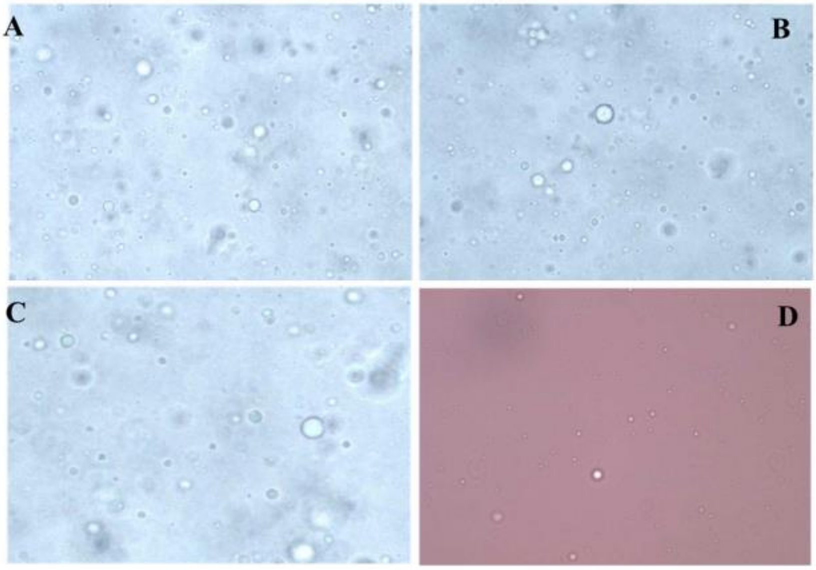


**Figure 1.** Myrtle Nanoemulsion Against Bacterial Growth.

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].

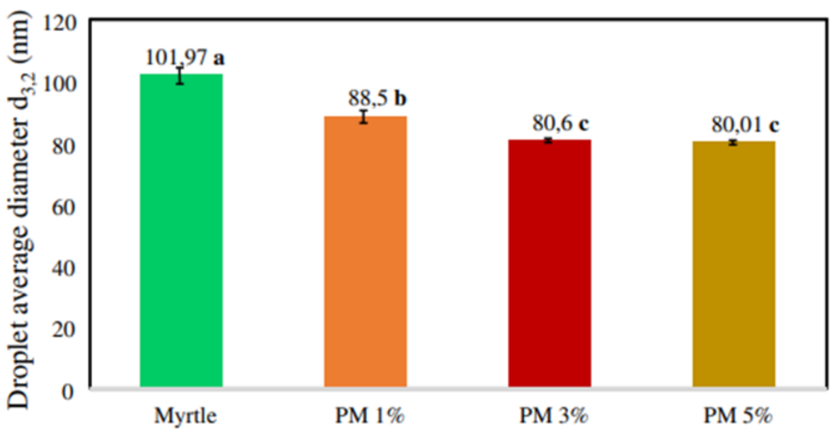


**Figure 2.** Myrtle Nanoemulsion Enhances Antibiotic Effectiveness.

**Biofilm Inhibition**

We tested how myrtle nanoemulsion affects the formation of biofilm using the crystal violet method. The nanoemulsion 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. Table 3Stopping Biofilm Growth with Myrtle Nanoemulsion and Antibiotics[6].

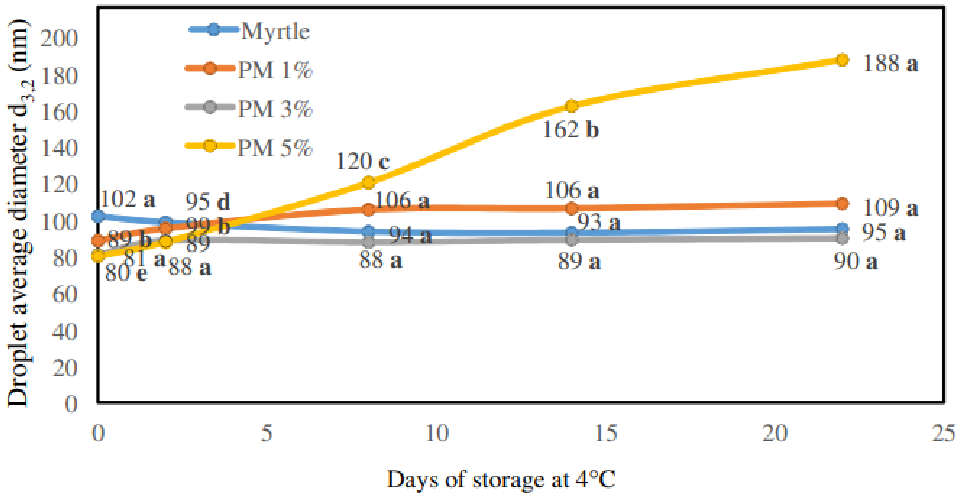


**Figure 3.** Myrtle Nanoemulsion Reduces Biofilm Formation.

**Table 3.** Time-Kill Assay (Reduction in CFU/mL).

| Time (h) | Control | Antibiotic | ME Nanoemulsion | Combination |
|---|---|---|---|---|
| **0** | $10^7$ | $10^7$ | $10^7$ | $10^7$ |
| **2** | $10^7$ | $10^6$.3 | $10^6$.5 | $10^5$.8 |
| **4** | $10^7$ | $10^5$.7 | $10^6$ | $10^4$.3 |
| **6** | $10^7$ | $10^4$.9 | $10^5$.2 | $10^3$.5 |
| **24** | $10^7$ | $10^3$.8 | $10^4$.6 | <$10^2$ |

The results of the time-kill test show big differences in how well the treatments kill Pseudomonas aeruginosa over 24 hours. The main goal of this test was to see how well Myrtle extract (ME) nanoemulsions work over time, how well antibiotics work by themselves, and how well they work together. At the start (0 hours), all treatments started with about the same amount of bacteria, around 10^5 CFU/mL, to keep the starting conditions consistent. In the control group that did not get any treatment, the number of bacteria stayed steady at 10 CFU/mL for the entire 24 hours, showing that P. Aeruginosa was able to keep living and growing even without any antibiotics. Treating with the antibiotic alone showed that the number of bacteria slowly decreased over time[13]. After 2 hours, the amount went down a little to 10. 3 CFU/mL, and after 24 hours, it decreased more to $10^3$. This means that the antibiotic worked better over time, killing almost 99. 9% of the bacteria over 24 hours. On the other hand, the Myrtle extract nanoemulsion by itself also decreased the number of bacteria, but it did so more slowly. After 2 hours, the number dropped to 10. 5 CFU/mL, and after 24 hours, it went down to 10. 6 This shows that the nanoemulsion has some antibacterial properties, but it worked not as well as the antibiotic by itself in lowering the number of bacteria[14].

**Table 4.** Biofilm Inhibition (% Reduction).

| Treatment | Biofilm Reduction (%) |
|---|---|
| **Control** | 0 |
| **Myrtle Nanoemulsion** | 42.3 |
| **Ciprofloxacin** | 58.6 |
| **Ciprofloxacin + Nanoemulsion** | 87.1 |

The results in Table 4 show how effective different treatments are at decreasing the formation of biofilm by Pseudomonas aeruginosa. This is important because the biofilm helps the bacteria resist and survive. In the control group, no blocking was seen, which shows that P. can form a strong biofilm. Pseudomonas aeruginosa without the presence of antibiotics. This shows that we need good methods to fight infections caused by biofilms. Using Myrtle nanoemulsion by itself led to a 42. 3% decrease in the growth of biofilm. This shows that the nanoemulsion has its own ability to fight against biofilms. The reason for this reduction probably has to do with the active compounds in Myrtle extract, like phenolics and terpenes[15]. These compounds can disrupt quorum sensing and the sticking of bacteria, which are important steps in the formation of biofilms. Ciprofloxacin, a type of antibiotic, was able to stop 58. 6% of biofilm growth. Although this level is higher than just the nanoemulsion by itself, it is still not enough. This matches what we know about the limits of antibiotics, which often can't fully break through and break apart mature biofilms[16]. The mixture of Ciprofloxacin and Myrtle nanoemulsion led to a much greater effect, reducing biofilm by 87. 1% This means that the two agents work well together, and the nanoemulsion may help antibiotics get through bacterial membranes and biofilm more easily, improving their effectiveness[17]. Also, the parts of Myrtle extract might affect different targets or processes that are involved in making biofilms. This can cause a greater disruption when these parts are used together.

These results are especially important in a medical setting, as P. A group of germs called aeruginosa form clusters that are very hard to treat with just antibiotics. Using tiny droplets made from plants as helpers can make antibiotics work better and allow for smaller doses. This could help lessen side effects and prevent bacteria from becoming resistant[18].

## 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. 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 microorganism referred to as Pseudomonas aeruginosa. It wished a small amount, zero. Five mg/mL, to stop the microorganism from developing and 1. Zero mg/mL to kill them completely[19].

These outcomes display that the nano emulsion 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 microorganism. Smaller nanoparticles have more floor area compared to their size, which allows them interact higher with bacteria. This lets them get deeper into the microorganism and spoil down their shielding outer layers. The zeta ability of -27. Three mV indicates that the nanoemulsion is strong[20]. This approach it remains powerful for a long term and can keep running nicely with microorganism. Nanoemulsions are better than normal emulsions due to the fact they dissolve extra without problems, work better within the body, and are greater solid. 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[21]. These chemical substances can harm bacterial cell membranes, forestall enzymes from running, and disrupt DNA production, all of which assist kill microorganism, 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 microorganism's protective layer, allowing them to input the microorganism extra efficaciously. 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[22]. Biofilms serve as a protective layer that helps keep bacteria safe from the body's immune system and from medicines that fight infections. 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[15]. This influences the early levels of biofilm improvement. 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[23].

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 Photobacterium violaceus 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. 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 microorganism 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 microorganism[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. 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.

## 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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