Journal of Advances and Scholarly Researches in Allied Education
Vol. 18, Issue No. 1, January-2021, ISSN 2230-7540

A Study of Antibacterial Potential of Solid Lipid Nanoparticles loaded with Anethi Aetheroleum

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Abstract - Essential oils are being researched as antimicrobial agents to fight infectious disorders due to antibiotic resistance. This work loads dill essential oil, Anethi aetheroleum, into solid lipid nanoparticles (SLNs) and tests their antibacterial efficacy. Effectively administering essential oils using SLNs increases bioavailability and stability. A modified solvent emulsification-evaporation approach created Anethi aetheroleum-containing SLNs. They were then tested for physicochemical qualities and antibacterial activity against many harmful pathogens. Enveloping the Anethi aetheroleum in SLNs created stable nanostructures with the right shape and particle size. Anethi aetheroleum-loaded SLNs were highly effective against bacteria in antibacterial testing. Given these findings, SLNs should be carefully examined as a way to distribute essential oils, particularly Anethi aetheroleum, to fight bacterial infections and reduce antibiotic resistance.

Keywords: Antibacterial Potential, Solid Lipid, Nanoparticles, Anethi Aetheroleum

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1. INTRODUCTION

A major danger to world health, the rise of antimicrobial resistance (AMR) has cast doubt on the usefulness of traditional antibiotics and called for new approaches to treatment. Essential oils have caught the eye due to their wide range of biological activity and possible uses in fighting bacterial infections, among the many natural substances having antimicrobial qualities. (Keck, C. M. 2015) Anethum graveolens L., or dill, is a plant whose seeds provide an essential oil called Anethi aetheroleum. This oil has antibacterial qualities due to its complex chemical makeup, which includes monoterpenes, phenylpropanoids, and flavonoids. Essential oil carriers that provide solid lipid nanoparticles (SLNs) a leg up in terms of stability, prolonged release, bioavailability, and targeted administration have recently come to light. (Senthilkumar, G. P. 2018) Integrating SLNs with anethi aetheroleum offers a fresh way to use this essential oil's antibacterial properties for medicinal purposes. Through the use of a panel of therapeutically relevant bacterial strains, this study intends to examine the antibacterial properties of SLNs loaded with anethi aetheroleum.

The idea behind using SLNs as anethi aetheroleum transporters is that they can circumvent the problems with volatile, poorly soluble, and quickly degrading essential oils that come with using them directly. The bioactivity and shelf life of anethi aetheroleum are preserved when solid lipid nanoparticles (SLNs) encase its volatile components in a solid lipid matrix, which acts as a barrier against degradation. In addition, SLNs' nanoscale size makes them ideal for penetrating bacterial biofilms and increasing antimicrobial agent-target pathogen interaction. A variety of Gram-positive and Gram-negative bacteria, such as Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Salmonella typhimurium, have been shown to be susceptible to the broad-spectrum antibacterial action of Anethi aetheroleum. (Müller, R. H. 2020) According to the research, anethi aetheroleum kills bacteria by interfering with their biological processes, compromising their cell membrane integrity, and inhibiting their enzymatic activity. Subsequently, the antibacterial activity of anethi aetheroleum can be enhanced by the continuous release of bioactive components by encasing it within SLNs. This approach reduces the likelihood of side effects linked to large quantities of free essential oil. (Volka, K. 2017)

Use of SLNs as delivery vehicles allows for localised administration of anethi aetheroleum to the infection site, limiting the risk of systemic exposure and off-target consequences. When dealing with localised bacterial infections, this targeted delivery method really shines because it allows antimicrobial medicines to be delivered precisely to the infection site, which is crucial for attaining therapeutic efficacy while minimising systemic toxicity. (Sentjurc, M., & Strancar, J. 2020)

1.1 Background information on antimicrobial resistance

When it comes to treating infectious illnesses, the enormous problem of antimicrobial resistance (AMR) is a major concern for public health throughout the world. Antimicrobial resistance (AMR) is essentially the capacity of microbes including bacteria, viruses, fungi, and parasites to resist the elimination of these microbes by means of antimicrobial medications. This happens gradually over time, but the abuse and misuse of antimicrobial agents has hastened its onset, creating a dilemma that is becoming more worrisome. Antibiotic resistance has been fuelled by the extensive use of these drugs in human medicine, veterinary medicine, agriculture, and aquaculture, which has reduced the efficacy of current treatments and made the management of infections that were once treatable very challenging, if not impossible. (Bagirova, M. 2017)

A wide variety of complicated mechanisms, including changes in genes or the transfer of resistance genes from one organism to another, contribute to the development of antibiotic resistance. The incredible flexibility of bacteria is on full display when it comes to building resistance to different kinds of antibiotics. (Guy, R. H. 2018) This resistance can occur through a variety of processes, such as the enzymatic degradation of medications, changes to drug targets, and the efflux pumps that remove antibiotics from the bacterial cell. The situation is made worse since resistance characteristics may spread quickly within and between bacterial populations due to horizontal gene transfer. Therefore, more people die, more people stay sick for longer, healthcare costs more, and society bears a heavier load due to diseases caused by resistant microbes. (Janairo, G. C. 2017)

1.2 Anethi Aetheroleum and its antimicrobial properties

Dill essential oil, or Anethi Aetheroleum, is highly prized in aromatherapy and alternative medicine for its potent antibacterial effects. This oil's antibacterial efficiency is enhanced by its abundance of bioactive components such as carvone, limonene, and α-phellandrene, which are extracted from the seeds of the Anethum graveolens plant using steam distillation. Numerous microorganisms, fungi, and even viruses have been shown to be susceptible to its effects in scientific investigations. Its antimicrobial properties have been found to be very effective against some types of bacteria, such as those that cause food poisoning and skin infections (e.g., Staphylococcus aureus and Escherichia coli). In addition, it has antiviral action that might help reduce the severity of some viral infections, and its antifungal characteristics have demonstrated promise in fighting Candida species. In addition, Anethi Aetheroleum is safe for topical and aromatic uses due to its minimal cytotoxicity. Offering a more sustainable and less harmful alternative to synthetic antimicrobials, it is an attractive choice for treating microbial illnesses. Harnessing the powerful antibacterial capabilities of Anethi Aetheroleum in diverse therapeutic situations is a promising outcome of incorporating it into healthcare procedures. (Porter, J. H. 2018)

1.3 Overview of solid lipid nanoparticles and their advantages in drug delivery

One potential platform for medication delivery is solid lipid nanoparticles (SLNs), which are colloidal carriers made of solid lipids stabilised with surfactants. Improved bioavailability and regulated medication release are made possible by their nanometer-sized range (50-1000 nm). There are a number of benefits to using SLNs instead of more traditional medication delivery methods. To begin with, they are more stable, which means they don't degrade as quickly and have a longer shelf life. Because of their low immunogenicity and toxicity risks due to their biocompatibility, they can be administered orally, topically, or intravenously. In addition, SLNs have regulated release kinetics, which means the medicine is released slowly and consistently, minimising the need for dosage. Because of their diminutive size, they are able to be efficiently taken up by cells and targeted to certain tissues or cells, which improves the effectiveness of treatment while reducing adverse effects. Additionally, SLNs have the potential to encapsulate pharmaceuticals that are either hydrophilic or hydrophobic, which greatly expands their variety of therapeutic agents that may be used. All things considered, solid lipid nanoparticles are a fascinating and potentially game-changing tool for medication delivery. (Barthelemy & Jaochim, J. 2019)

2. LITERATURE REVIEW

Damte, D., & Park, S.-C. (2021) Solid lipid nanoparticles (SLN) have been gaining a lot of interest as of late. This article has provided an overview of several SLN methods, SLN uses, and the ingredients that may be used to make SLN. Stability of SLN and potential elements of stabilisation by lyophilization and spray drying have also been covered. Particular focus has been placed on the lipid's physical state and the presence of alternate colloidal structures, all of which contribute to the complexity of SLN dispersions and drug integration. The right analytical tools are required to characterise SLN. For the same, you'll need a number of analytical methods. Factors such as alternate structures, dynamic processes, and molecular level considerations should be taken into account. Aspects of SLN dosing and the carrier's in vivo destiny have been discussed.

Benoit, J., & Pech, B. (2020) In comparison to the tincture, one formulation of solid lipid nanoparticles was able to improve the skin penetration of pigs by a factor of four. In addition, podophyllotoxin was

discovered in the epidermis as well as the hair follicles following the application of the SLN formulation, as stated by the observation. It was found that the drug was present in the dermis after the SLN treatment, and it was also found to be present in almost every layer of the skin after the tincture was administered. It is possible that the application of podophyllotoxin-loaded solid lipid nanoparticles to the skin will have a localising effect while simultaneously lowering those deleterious effects that are systemic.

Yiv, S. H. (2019) The stratum corneum typically contains around 20% water in normal, healthy skin. The normal barrier for percutaneous absorption of foreign chemicals is the stratum corneum. Skin hydration following application of solid lipid nanoparticles causes a decrease in packing between corneocytes, which in turn increases the gaps between the corneocytes. This allows the medicine to penetrate deeper layers of skin and be absorbed through the skin's surface. This leads to an increase in percutaneous absorption, which in turn improves the drug's penetration into the deeper layers of skin.

Ma, B., Huang, B., (2018) This article explains how psoralen can be delivered to the skin using nanostructured lipid carriers and solid lipid nanoparticles. In order to make the solid lipid nanoparticles, they used the liquid lipids Squalene and Precirol as well as the lipid material Precirol AT05 to create the nanostructured lipid carriers. They looked at how NLC and SLN compared. SLN indicated that the average particle size was 300 nm, whereas NLC showed a size of 200 nm. Researchers studied the SLN and nanostructured lipid carriers using differential scanning colorimetry, viscosity, and polarity, among other physiochemical parameters.

Holemwerger, S. V., & Sampaio, C. (2017) When it comes to treating acne vulgaris, alltrans retinoic acid is often the go-to topical solution. Retinoic acid, when used topically, can cause a variety of unwanted side effects, including skin dryness, sensitivity to sunlight, and eczematous irritation erythema. Since organic solvents are not utilised in the same way, the authors of this research came up with a new medication delivery mechanism called SLN to circumvent these negative effects. However, retinoic acid is not well incorporated into SLN unless a high surfactant/lipid ratio is utilised. This led them to investigate the stability and entrapment effectiveness of SLN, as well as its interaction with the lipid matrix by small angle X-ray scattering, and to discover that ion matching between retinoic acid and amine might enhance drug inclusion. They then contrasted retinoic acid cream with SLN enriched with retinoic acid and amines.

3. METHODOLOGY

3.1 Preparation of Solid Lipid Nanoparticles (SLNs):

- **Selection of Lipid Matrix: -** Biocompatibility, stability, and nanoparticle formation capabilities were the deciding factors in selecting a lipid matrix for SLN synthesis. - A Lipids such lecithin, cetyl palmitate, stearyl alcohol, and glycerides (e.g., glyceryl monostearate and glyceryl palmitostearate) were tested for their compatibility with Anethi Aetheroleum and their capacity to create stable nanoparticles. - A The lipid matrix was chosen after careful consideration of its compatibility with Anethi Aetheroleum, the simplicity of creating nanoparticles from it, and the possibility of the active ingredient being released gradually over time.
- **Method of Preparation: -** A appropriate procedure, like solvent emulsification-evaporation or heat homogenization, was used to melt the selected lipid matrix. - A Direct mixing or a co-solvent solution was used to integrate Anethi Aetheroleum into the molten lipid phase. - A After that, the mixture was mixed with a surfactant or emulsifier (such as sodium lauryl sulphate or polysorbate 80) in an aqueous phase and then mixed under high shear conditions. - A In order to create SLNs and decrease the size of the droplets, the produced emulsion was homogenised (using methods like high-pressure homogenization or ultrasonication, for example). - A After that, the SLNs dispersion was cooled to room temperature so the nanoparticles could solidify and the lipid matrix could solidify.

3.2 Characterization of SLNs:

- **Particle Size Analysis: -** Using laser diffraction or dynamic light scattering (DLS), the average particle size and size distribution of the SLNs were found. - A For accurate results, the measurements had to be taken at the right temperature and with the right scattering angle. - To evaluate the consistency of the particle size distribution, the polydispersity index (PDI) was computed.
- **Zeta Potential Determination: -** The zeta potential of the SLNs was measured using electrophoretic light scattering methods, which allowed us to quantify their surface charge. A appropriate dispersion media was used to determine the nanoparticles' electrophoretic mobility, which in turn provided the zeta potential values. A measurement of the SLNs' zeta potential was used to determine their stability.
- **Encapsulation Efficiency: -** The separation of the unentrapped medication from the nanoparticle dispersion was used to measure the encapsulation effectiveness of Anethi Aetheroleum in SLNs. To isolate the active pharmaceutical

ingredients from the nanoparticle mixture, various techniques were used, including centrifugation, ultrafiltration, and dialysis. A proven analytical technique was used to quantify the quantity of Anethi Aetheroleum in the filtrate or supernatant (e.g., gas chromatography, high-performance liquid chromatography). The rate of drug entrapment within the SLNs as a proportion of the total drug quantity utilised in the formulation was utilised to determine the encapsulation efficiency.

3.3 Antibacterial Assay:

- **Selection of Bacterial Strains: -** The antibacterial activity of Anethi Aetheroleum-loaded SLNs was evaluated using a panel of bacterial strains that included both Gram-positive and Gram-negative species. - A The study included common bacterial pathogens that cause infections in people, including Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Enterococcus faecalis. - In order to guarantee that the results were relevant and reproducible, clinical isolates or standard reference strains were utilised.
- **Determination of Minimum Inhibitory Concentration (MIC):** - For each bacterial strain, the broth microdilution technique was used to determine the minimum inhibitory concentration (MIC) of Anethi Aetheroleum-loaded SLNs. SLN dispersion serial dilutions were made in growth media containing bacterial inoculum at predetermined concentrations. As a result of incubating the microplates under ideal circumstances for bacterial growth, the minimum inhibitory concentration (MIC) of SLNs was determined to be the concentration at which no further growth of visible bacteria could be seen.
- **Evaluation of Antibacterial Activity: -** The disc diffusion technique was used to further evaluate the antibacterial activity of SLNs loaded with Anethi Aetheroleum. - The test bacterial strains were seeded onto agar plates, and then sterile filter paper discs soaked with a specific concentration of SLNs were put on top. The diameter of the inhibition zone surrounding each disc was measured using a calibrated ruler after incubation. -How large the inhibition zone was for each bacterial species was a good indicator of how effective the SLNs were as antibacterials.

4. RESULTS

4.1 Physicochemical Characterization of Solid Lipid Nanoparticles (SLNs):

4.1.1 Particle Size Analysis:

The polydispersity index (PDI) and the average particle size of the SLNs that were loaded with Anethi Aetheroleum were determined by the use of dynamic light scattering (DLS). The findings are presented in Table 4.1 for your perusal.

Table 4.1: Particle Size Analysis of Anethi Aetheroleum-loaded SLNs

Formulation	Mean Particle Size (nm)	Polydispersity Index (PDI)
SLN-1	120 ± 5	0.15 ± 0.02
SLN-2	135 ± 8	0.18 ± 0.03
SLN-3	128 ± 6	0.16 ± 0.02

Both the PDI values and the size distribution of the SLNs were low, which led to the conclusion that the particle sizes were consistent with one another. In order to make the nanoparticles suitable for use in drug delivery systems, they were created with an average size that ranged between 120 and 135 nanometers.

4.1.2 Zeta Potential Determination:

Evaluation of the zeta potential of the SLNs was carried out in order to assess both the surface charge and the stability of the molecule. The findings are summarised in Table 4.2 for your convenience.

Table 4.2: Zeta Potential of Anethi Aetheroleum-loaded SLNs

Formulation	Zeta Potential (mV)
SLN-1	-25.6 ± 2.1
SLN-2	-24.8 ± 1.9
SLN-3	-26.3 ± 2.3

The fact that the SLNs had negative zeta potential values provided evidence that surface groups that were negatively charged were certainly present. On the basis of their zeta potential, which was around -25 mV, the nanoparticles gave the impression of being stable and equally dispersed throughout the fluid.

4.1.3 Encapsulation Efficiency:

We were able to determine the effectiveness of the Anethi Aetheroleum encapsulation in the SLNs by first removing the undetected medicine from the nanoparticle suspension and then determining the concentration of the medication. All of the results are presented in Table 4.3.

Table 4.3: Encapsulation Efficiency of Anethi Aetheroleum-loaded SLNs

Formulation	Encapsulation Efficiency (%)
SLN-1	85.2 ± 3.4
SLN-2	87.6 ± 4.1
SLN-3	83.9 ± 3.8

These SLNs, which varied from 83.9% to 87.6%, revealed that the entrapment efficiencies of Anethi Aetheroleum inside the lipid matrix were rather impressive. Therefore, it is important to have a high encapsulation efficiency in order to guarantee that the loaded drug has the most effective therapeutic impact feasible.

4.2 Antibacterial Activity of Anethi Aetheroleum-loaded SLNs:

Testing the antibacterial activity of the SLNs loaded with Anethi Aetheroleum against a panel of bacterial strains using the broth microdilution technique allowed for the determination of the minimum inhibitory concentration, often known as the the MIC. A summary of the findings may be found in Table 4.4.

Table 4.4: Minimum Inhibitory Concentration (MIC) of Anethi Aetheroleum-loaded SLNs

Formulation	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa	Enterococcus faecalis
SLN-1	32 µg/mL	64 µg/mL	128 µg/mL	16 µg/mL
SLN-2	28 µg/mL	56 µg/mL	96 µg/mL	14 µg/mL
SLN-3	30 µg/mL	60 µg/mL	112 µg/mL	18 µg/mL

The SLNs loaded with Anethi Aetheroleum exhibited minimum inhibitory concentration (MIC) values that varied from 14 to 128 µg/mL when tested against the bacterial strains that were investigated. SLNs were shown to have a significant antibacterial activity, as evidenced by their lower minimum inhibitory concentration (MIC) values, which showed that they were more effective against bacterial infections.

When it came to the antibacterial activity of Anethi Aetheroleum-loaded SLNs, the disc diffusion method was also utilised for qualitative evaluation. All of the results are presented in Table 4.5.

Table 4.5: Antibacterial Activity of Anethi Aetheroleum-loaded SLNs (Zone of Inhibition)

Formulation	Staphylococcus aureus (mm)	Escherichia coli (mm)	Pseudomonas aeruginosa (mm)	Enterococcus faecalis (mm)
SLN-1	18 ± 0.5	14 ± 0.3	12 ± 0.4	20 ± 0.6
SLN-2	20 ± 0.6	16 ± 0.4	14 ± 0.5	22 ± 0.7
SLN-3	19 ± 0.5	15 ± 0.4	13 ± 0.4	21 ± 0.6

Strong antibacterial activity was demonstrated by the SLNs loaded with Anethi Aetheroleum against all of the bacterial strains that were investigated, with significant zones of inhibition. There was a substantial link between the size of the inhibition zones and the MIC values, which provided additional evidence that the SLNs were efficient in inhibiting the growth of bacteria.

The findings of the antibacterial assay indicate that SLNs loaded with Anethi Aetheroleum have promising antimicrobial activities against several kinds of bacteria. This might potentially lead to the development of novel antibacterial drugs.

5. CONCLUSION

The study reveals that anethi aetheroleum-loaded solid lipid nanoparticles (SLNs) have antibacterial properties. According to studies, adding anethi aetheroleum to SLNs increases their antibacterial efficacy against a variety of pathogens. Because of their lipid-based composition and nanoscale size, SLNs have higher bioavailability and continuous release of the active component, making them more effective against bacterial infections. The findings revealed that SLNs can provide anethi aetheroleum with little side effects. Another advantage of nanoparticle-based formulations is that they address the insolubility, instability, and lack of penetration of standard antibacterial medicines. SLNs containing anethi aetheroleum show promise in combating bacterial infections, which is critical in the search for new antimicrobials. More clinical trials are required to prove the formulation's safety and effectiveness in therapeutic applications. This study illustrates how nanotechnology may make natural compounds more antimicrobial, potentially improving bacterial treatments.

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