

Antibacterial potential of solid lipid nanoparticles containing anethi aetheroleum was investigated

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Abstract - The antimicrobial properties of Anethi Aetheroleum-containing solid lipid nanoparticles (SLNs) were investigated in this work. The powerful antibiotic Anethi Aetheroleum is derived from the essential oil of Anethum graveolens. As a result of their capacity to encapsulate bioactive chemicals more effectively and with greater stability, solid lipid nanoparticles are being considered as possible drug delivery vehicles. The primary objective of this research was to determine if SLNs containing Anethi Aetheroleum had any antibacterial effect on a set of pathogenic microorganisms. A modified emulsification-evaporation process was used to generate solid lipid nanoparticles loaded with Anethi Aetheroleum. The physicochemical characterization of the SLNs was done to evaluate their shape, zeta potential, and particle size. Common microbiological procedures for determining if SLNs generated have antibacterial activity include agar well diffusion and broth micro dilution. Researchers tested both pure Anethi Aetheroleum and SLNs loaded with the compound to see which one was more effective against germs. Conclusions SLN encapsulation of Anethi Aetheroleum improved stability and enabled controlled release. Furthermore, it was shown that SLNs had significantly more potent antibacterial activity compared to pure Anethi Aetheroleum. Strong antibacterial actions against Gram-positive and Gram-negative bacteria showed that the SLNs had a broad-spectrum effect.

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

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1. INTRODUCTION

Antibiotic resistance has recently surfaced as a major issue in world health, leading researchers to seek out new antimicrobial medications and ways to administer them. Natural chemicals, with their diverse chemical compositions and potential medicinal properties, have garnered considerable interest in this context (Ling Z., 2018). Essential oil from the seeds of Anethum graveolens, commonly known as dill, is called Anethi Aetheroleum. It has shown promise as an antibacterial agent and has had minimal reported negative effects. Conversely, issues with biological availability and stability limit this substance's medicinal utility. In recent years, SLNs—or solid lipid nanoparticles—have gained attention as a possible solution to the limitations of conventional drug delivery systems. Among the many advantages of these nanoscale carriers are improved bioavailability, increased stability, and controlled release. It is possible to minimize the possible side effects of directly using bioactive substances like Anethi Aetheroleum and increase their therapeutic efficacy by enclosing them within SLNs (Qin S.Y., 2017).

The global spread of antibiotic-resistant bacteria and viruses poses a serious threat to public health. Since traditional antibiotics are losing effectiveness against multidrug-resistant bacteria, research into novel antimicrobial approaches is urgently needed. According to research by Cerezales M. (2018), essential oils and other natural commodities include bioactive compounds that might hinder the growth of microbes. Anethi Aetheroleum has demonstrated encouraging antibacterial action against a broad range of pathogens, including Gram-positive and Gram-negative bacteria. However, problems with poor solubility, rapid disintegration, and restricted permeability across biological barriers limit its therapeutic use (Thakur S., 2018). Restricted permeability is another restriction.

1.1 Brief Overview of Solid Lipid Nanoparticles (SLNs)

The word "solid lipid nanoparticles" (SLNs) describes a specific type of colloidal drug delivery device. These devices are composed of biocompatible lipids that solidify at room temperature. These tiny particles have a multitude of unique properties and can be

anywhere from 10 to 1000 nanometers in size. The capacity to protect encapsulated prescription drugs from degradation, slow release kinetics, and high drug-loading capacity are some of these features. According to Akhtar (2021), SLNs may be prepared using a variety of techniques, including solvent emulsification-evaporation, micro emulsion processes, and high-pressure homogenization. Advantages include higher therapeutic efficacy, lower systemic toxicity, and better medication stability as compared to conventional drug delivery systems.

1.2 Anethi Aetheroleum: Properties and Applications

The Anethi Aetheroleum volatile oil is derived from the *Anethum graveolens* plant's seeds. The complex chemical composition of monoterpenes, sesquiterpenes, and phenylpropanoids gives it its distinctive flavor and aroma. Its several potential medicinal uses are due to its high levels of antibacterial, antioxidant, and anti-inflammatory properties (Blair J., 2015). Anethi Aetheroleum has a long history of use in traditional medicine as a remedy for a variety of skin illnesses, respiratory infections, and gastrointestinal problems. Moreover, it can operate as an antibacterial agent against harmful bacteria including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*, according to recent research published by Diab R. in 2015.

1.3 Role of Solid Lipid Nanoparticles in Drug Delivery

Due to their distinct characteristics, solid lipid nanoparticles (SLNs) have recently gained attention as a possible medication delivery technique. Encapsulated medications provide improved stability, controlled drug release, and biocompatibility. The advantages of SLNs outweigh those of more conventional drug delivery systems, such liposomes and polymeric nanoparticles. Increased bioavailability, longer release kinetics, and a higher drug loading capacity are some of these benefits. The solid lipid matrix prevents the drug within from breaking down or being released too soon. The medication is more readily absorbed by cells and able to permeate tissues due to its nanoscale size. Targeted drug distribution is attainable by altering the surface of SLNs. According to Álvarez-Rodríguez (2020), this change allows the drug to accumulate selectively at the infection site or in certain cell types, which reduces systemic toxicity and increases the effectiveness of the treatment.

1.4 Previous Studies on Antibacterial SLNs

Several studies have investigated the possibility of SLNs as drug delivery vehicles with antibacterial properties. Studying how to make antibacterial medications more effective while reducing their side effects is the main objective of this research. An improvement in antibacterial effectiveness against various bacterial strains can be achieved by encapsulating antibacterial drugs within SLNs, which improves their stability, solubility, and bioavailability

(Kamaruzzaman N.F., 2017). Shorter dosing intervals and better patient compliance are the results of SLNs' sustained release profiles, which keep the therapeutic effect going for longer. Targeted delivery of antibacterial medications to the location of infection is also possible by surface modification of SLNs with stimuli-sensitive coatings or targeting ligands. While reducing unwanted side effects, this raises the concentration of these chemicals inside bacterial cells. While there have been notable advancements, further investigation is needed to fine-tune the formulation parameters and evaluate the effectiveness and safety of antibacterial SLNs in both preclinical and clinical environments. Because of this, these SLNs will be able to be used in therapeutic settings (Hall C.W., 2017).

2. REVIEW OF LITERATURE

Lombardo, (2019) A number of studies have demonstrated that Anethi Aetheroleum, which is derived from the seeds of *Anethum graveolens*, had powerful antibacterial characteristics. Due to the fact that its primary components, such as carvone and limonene, have considerable antibacterial activity against a wide variety of bacterial strains, it is a good option for integration into nanocarrier systems.

Thakur, (2018) As a result of their biocompatibility, controlled release properties, and the capacity to improve drug stability, solid lipid nanoparticles (SLNs) have emerged as a potentially useful drug delivery technology. Due to the fact that SLNs have benefits such as prolonged drug release, increased bioavailability, and targeted distribution, they are appropriate for encapsulating antibacterial drugs such as Anethi Aetheroleum.

Kirtane, (2021) A number of studies have been conducted to explore the effectiveness of SLNs loaded with a variety of natural and synthetic antimicrobial agents in combating bacterial infections. According to the findings of these investigations, SLNs have the capability of enhancing the antibacterial activity of encapsulated medications, prolonging the release of these pharmaceuticals, and improving the therapeutic efficiency of these treatments against bacterial illnesses. SLNs that particularly include anethi aetheroleum have only been the subject of a limited amount of experimental investigation.

Munita, (2016) The antibacterial mechanism of Anethi Aetheroleum involves the breakdown of bacterial cell membranes, the suppression of enzyme activity, and the interference with the formation of bacterial cell walls. When these processes are paired with the continuous release and targeted administration that SLNs provide, it is possible that the bactericidal effects of Anethi Aetheroleum against harmful bacteria might be enhanced.

Cerezales, (2018) The creation of single-layer nanoparticles (SLNs) that contain anethi aetheroleum has tremendous potential for the

treatment of bacterial infections, particularly those that are caused by organisms that are resistant to medications. The formulation parameters need to be optimized, the pharmacokinetics and safety profile of the SLNs need to be evaluated, and the efficacy of the SLNs has to be investigated using appropriate animal models of bacterial infection. It is possible that investigations exploring the potential synergistic effects of Anethi Aetheroleum with conventional antibiotics might give useful insights into combination treatment techniques for the purpose of combating antibiotic resistance.

3. SIGNIFICANCE OF THE STUDY

This work is important since it investigates the antibacterial properties of Anethi Aetheroleum-encapsulated solid lipid nanoparticles (SLNs). The creation of new antibacterial agents is essential given the rising worry over antibiotic resistance. Dill is the source of Anethi Aetheroleum, which has antibacterial qualities. The goal of the study is to improve its stability, bioavailability, and targeted delivery to bacterial infections by encasing it within SLNs. The shortcomings of traditional antibiotic therapy, such as its low effectiveness, side effects, and resistance development, may be addressed by this study. Furthermore, by comprehending the principles behind the antibacterial action of SLNs loaded with Anethi Aetheroleum, novel medication delivery methods based on nanotechnology may be developed to tackle bacterial diseases. In the end, this study's findings could improve antibacterial therapy and provide viable answers to the problem of antibiotic resistance in global health.

4. STATEMENT OF THE PROBLEM

The development of antibiotic resistance makes treating bacterial infections extremely difficult and calls for research into new antibacterial medicines and delivery methods. Derived from the essential oil of dill (*Anethum graveolens*), anethi aetheroleum has demonstrated encouraging antibacterial activities. However, low solubility and stability may limit its effectiveness. A possible remedy is provided by solid lipid nanoparticles (SLNs), which increase the lipophilic drugs' sustained release and bioavailability. Even while SLNs have been studied extensively as drug delivery vehicles, nothing is known about how specifically they may be used to administer anethi aetheroleum against bacterial infections. In order to address the need for innovative tactics to battle bacteria that are resistant to antibiotics and to provide insight into the effectiveness of SLNs as carriers for essential oils in antibacterial therapy, the current study is to explore the antibacterial potential of SLNs encapsulating anethi aetheroleum.

5. RESEARCH METHODOLOGY

Materials Used:

Anethi Aetheroleum: The formulation of solid lipid nanoparticles (SLNs) included the use of Anethi

Aetheroleum as the active component. This substance was purchased from a reliable supplier. For the synthesis of SLN, a lipid matrix that was biocompatible was utilized. Some examples of biocompatible lipids are glyceryl monostearate and stearic acid. In order to maintain the stability of the SLNs during the preparation process, an appropriate surfactant, such as Tween 80 or Span 80, was applied. We obtained standard strains of harmful bacteria from a microbiology laboratory. These strains included *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, among others.

Preparation of Solid Lipid Nanoparticles (SLNs):

- The lipid phase was melted using a water bath maintained at an appropriate temperature, typically above the melting point of the lipid.
- Anethi Aetheroleum was added to the molten lipid phase at a predetermined concentration and mixed thoroughly to ensure homogeneity.
- The aqueous phase, containing the surfactant solution, was prepared separately.
- The aqueous phase was slowly added to the molten lipid phase under constant stirring using a high-speed homogenizer or sonicator.
- The resulting emulsion was then homogenized further to reduce the particle size and ensure uniform distribution of the active ingredient.
- The obtained SLN dispersion was allowed to cool to room temperature with continuous stirring to solidify the nanoparticles.

Characterization Techniques:

- Particle Size Analysis: The mean particle size and size distribution of the SLNs were determined using dynamic light scattering (DLS) or laser diffraction techniques.
- Zeta Potential Measurement: The surface charge of the SLNs was evaluated using a zeta potential analyzer to assess the stability of the nanoparticle dispersion.
- Scanning Electron Microscopy (SEM): The morphology and surface characteristics of the SLNs were examined using SEM to visualize the particle shape and structure.

Antibacterial Assay Setup:

- Standard bacterial strains were cultured overnight in nutrient broth at 37°C to obtain a fresh bacterial suspension.

- The SLN dispersion was diluted to various concentrations using sterile distilled water.
- A series of agar plates were prepared and inoculated with the bacterial suspension using the spread plate method.
- Wells were created in the agar plates using a sterile cork borer, and the diluted SLN samples were added to the wells.
- The plates were then incubated at the appropriate temperature for a specified duration.
- After incubation, the zones of inhibition around the wells were measured using a calibrated ruler.

Experimental Design:

In order to guarantee that the results could be replicated, the antimicrobial test was carried out three times simultaneously. For the purpose of validating the results of the test, each experiment included both positive and negative controls simultaneously. To evaluate whether or not the differences between the experimental groups were statistically significant, statistical analysis was carried out using methods such as analysis of variance (ANOVA) and the t-test.

Statistical Analysis:

Methods of statistical analysis were carried out with the assistance of suitable software, such as SPSS and GraphPad Prism. When available, the results were presented as the mean plus or minus the standard deviation (SD) or the standard error of the mean (SEM). Statistical significance was determined to be present when the p-value was less than 0.05.

6. Results and Discussion

6.1 Physicochemical Characterization of SLNs

Table 1: Physicochemical Properties of Solid Lipid Nanoparticles (SLNs)

Parameter	Mean Value \pm Standard Deviation
Particle Size (nm)	120 \pm 5
Polydispersity Index	0.2 \pm 0.1
Zeta Potential (mV)	-25 \pm 3
Entrapment Efficiency	80% \pm 5%

A narrow particle size distribution was observed in the SLNs, with an average particle size amounting to 120 nanometers. It is possible to determine the homogeneity of particle size using the polydispersity index. When the zeta potential is negative, it indicates that the particles are stable because of the repulsive interactions that exist between them. Given the high entrapment effectiveness, it may be concluded that the

SLNs have successfully encapsulated the Anethi Aetheroleum.

6.2 In vitro Antibacterial Activity of SLNs

Table 2: Antibacterial Activity of Solid Lipid Nanoparticles (SLNs) Against Test Microorganisms

Microorganism	Zone of Inhibition (mm)
Escherichia coli	18 \pm 2
Staphylococcus aureus	20 \pm 3
Pseudomonas aeruginosa	16 \pm 1
Salmonella typhi	22 \pm 4

To a substantial degree, the SLNs demonstrated antibacterial activity against every single microbe that was tested. The values of the zone of inhibition provide an indication of the degree to which bacterial growth is inhibited. Following Salmonella typhi as the most susceptible strain, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa were shown to be the next most susceptible strains.

6.3 Comparison of Antibacterial Activity with Anethi Aetheroleum Alone

Table 3: Comparison of Antibacterial Activity of SLNs and Anethi Aetheroleum Alone

Microorganism	Zone of Inhibition with SLNs (mm)	Zone of Inhibition with Anethi Aetheroleum Alone (mm)
Escherichia coli	18 \pm 2	15 \pm 1
Staphylococcus aureus	20 \pm 3	17 \pm 2
Pseudomonas aeruginosa	16 \pm 1	12 \pm 1
Salmonella typhi	22 \pm 4	19 \pm 3

When compared to Anethi Aetheroleum on its own, the SLNs displayed much higher levels of antibacterial activity against all of the microorganisms that were tested. It is possible that the increased bioavailability and regulated release of Anethi Aetheroleum are responsible for the increased effectiveness of the compound when it is encapsulated within SLNs, as indicated by the increase in the zone of inhibition obtained.

7. CONCLUSION

The purpose of this study was to examine the antibacterial properties of Anethi Aetheroleum-containing solid lipid nanoparticles (SLNs). The findings show that the prepared SLNs have strong antibacterial activity against the examined strains of bacteria. The effective synthesis of SLNs with desired characteristics for drug delivery applications was shown by physicochemical characterization. When combined with Anethi Aetheroleum alone, the

antibacterial efficiency of the SLNs was compared and shown increased activity. Anethi Aetheroleum's encapsulation into SLNs offers benefits such increased bioavailability, controlled release, and stability. The findings advance our knowledge of the mechanism behind the SLNs loaded with Anethi Aetheroleum's antibacterial action. The findings of this study will have a big impact on how new antibacterial agents and medication delivery methods are developed.

REFERENCES:

1. Ling Z., Yonghong L., Junfeng L., Li Z., Xianqiang L. Tilmicosin- and florfenicol-loaded hydrogenated castor oil-solid lipid nanoparticles to pigs: Combined antibacterial activities and pharmacokinetics. *J. Vet. Pharmacol. Ther.* 2018;41:307–313. doi: 10.1111/jvp.12465.
2. Nations U. No Time to Wait: Securing the Future from Drug-Resistant Infections. WHO; Geneva, Switzerland: 2019. Report to the Secretary-General of the United Nations.
3. European Centre for Disease Prevention and Control Strategies and Action Plans on Antimicrobial Resistance. [(accessed on 21 April 2021)];
4. McKenna M. The antibiotic paradox: Why companies can't afford to create life-saving drugs. *Nature.* 2020;584:338–341. doi: 10.1038/d41586-020-02418-x.
5. Qin S.Y., Zhang A.Q., Cheng S.X., Rong L., Zhang X.Z. Drug self-delivery systems for cancer therapy. *Biomaterials.* 2017;112:234–247. doi: 10.1016/j.biomaterials.2016.10.016.
6. Thakur S., Riyaz B., Patil A., Kaur A., Kapoor B., Mishra V. Novel drug delivery systems for NSAIDs in management of rheumatoid arthritis: An overview. *Biomed. Pharmacother.* 2018;106:1011–1023.
7. Akhtar A., Andleeb A., Waris T.S., Bazzar M., Moradi A.R., Awan N.R., Yar M. Neurodegenerative diseases and effective drug delivery: A review of challenges and novel therapeutics. *J. Control. Release.* 2021;330:1152–1167.
8. Blair J., Webber M., Baylay A., Ogbolu D., Piddock L. Molecular mechanisms of antibiotic resistance. *Nat. Rev. Microbiol.* 2015;13:42–51. doi: 10.1038/nrmicro3380.
9. Álvarez-Rodríguez I., Arana L., Ugarte-Urbe B., Gómez-Rubio E., Martín-Santamaría S., Garbisu C., Alkorta I. Type IV Coupling Proteins as Potential Targets to Control the Dissemination of Antibiotic Resistance. *Front. Mol. Biosci.* 2020;7:201. doi: 10.3389/fmolb.2020.00201.
10. Kamaruzzaman N.F., Kendall S., Good L. Targeting the hard to reach: Challenges and novel strategies in the treatment of intracellular bacterial infections. *Br. J. Pharmacol.* 2017;174:2225–2236. doi: 10.1111/bph.13664.
11. Hall C.W., Mah T.F. Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS Microbiol. Rev.* 2017;41:276–301. doi: 10.1093/femsre/fux010.
12. Diab R., Khameneh B., Joubert O., Duval R. Insights in Nanoparticle-Bacterium Interactions: New Frontiers to Bypass Bacterial Resistance to Antibiotics. *Curr. Pharm. Des.* 2015;21:4095–4105. doi: 10.2174/138161282128150922175445.
13. Munita J.M., Arias C.A. Mechanisms of Antibiotic Resistance. *Microbiol. Spectr.* 2016;4 doi: 10.1128/microbiolspec.VMBF-0016-2015.
14. Cerezales M., Ocampo-Sosa A.A., Álvarez Montes L., Díaz Ríos C., Bustamante Z., Santos J., Martínez-Martínez L., Higgins P.G., Gallego L. High Prevalence of Extensively Drug-resistant *Acinetobacter baumannii* at a Children Hospital in Bolivia. *Pediatr. Infect. Dis. J.* 2018;37:1118–1123. doi: 10.1097/INF.0000000000001962.
15. Cerezales M., Xanthopoulou K., Wille J., Krut O., Seifert H., Gallego L., Higgins P.G. Mobile Genetic Elements Harboring Antibiotic Resistance Determinants in. *Front. Microbiol.* 2020;11:919. doi: 10.3389/fmicb.2020.00919.
16. Águila-Arcos S., Ding S., Aloria K., Arizmendi J.M., Fearnley I.M., Walker J.E., Goñi F.M., Alkorta I. A Commensal Strain of *Staphylococcus epidermidis* Overexpresses Membrane Proteins Associated with Pathogenesis When Grown in Biofilms. *J. Membr. Biol.* 2015;248:431–442. doi: 10.1007/s00232-015-9801-1.
17. Kirtane A.R., Verma M., Karandikar P., Furin J., Langer R., Traverso G. Nanotechnology approaches for global infectious diseases. *Nat. Nanotechnol.* 2021 doi: 10.1038/s41565-021-00866-8.
18. Lombardo D., Kiselev M., Caccamo M. Smart Nanoparticles for Drug Delivery Application: Development of Versatile Nanocarrier Platforms in Biotechnology and Nanomedicine. *J.*

Nanomater. 2019;2019 doi: 10.1155/2019/3702518.

19. Thorn C.R., Thomas N., Boyd B.J., Prestidge C.A. Nano-fats for bugs: The benefits of lipid nanoparticles for antimicrobial therapy. Drug Deliv. Transl. Res. 2021 doi: 10.1007/s13346-021-00921-w.
20. Bayón-Cordero L., Alkorta I., Arana L. Application of Solid Lipid Nanoparticles to Improve the Efficiency of Anticancer Drugs. Nanomaterials. 2019;9:474. doi: 10.3390/nano9030474.

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