Role of Schisandrin-A against *Enterococcus faecalis* in-vitro studies

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(2024)

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A thesis submitted to the National University of Sciences and Technology, Islamabad,

in partial fulfillment of the requirements for the degree of

Master of Science in
Biomedical Sciences

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Islamabad, Pakistan

(2024)

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This dissertation is dedicated to my beloved parents

For their endless support, encouragement and love. Their prayers always paved the way for my success

To my siblings and friends

For their support and encouragement

And

To my teachers

For being source of inspiration and enlightenment

ACKNOWLEDGEMENTS

In the name of Allah Almighty who is most beneficent and eternally merciful. I bear witness that Holy Prophet Hazrat Muhammad (PBUH) is the last messenger of Allah Almighty. His (PBUH) life is a perfect role model for a Muslim to be successful in this worldly life and hereafter. Without the blessings of Allah Almighty I would not be able to complete my dissertation.

I would like to acknowledge and express my deepest gratitude to my research supervisor **Dr. Nosheen Fatima Rana** for her utmost guidance. Without her supervision and guidance completion of this dissertation would not be possible. I cordially thankful to him for her kindness that is difficult to express in words. Her skillful directions and dedicated advice have really guided me throughout the course of my research.

I also want to express my thanks to all the lab fellows for their expertise who helped me complete this study.

Sabina Bibi

TABLE OF CONTENTS

ACKNOWLEDGEMENTS I

TABLE OF CONTENTS II

LIST OF FIGURES IV

LIST OF ABBREVIATIONS V

ABSTRACT VI

CHAPTER 1: INTRODUCTION 1
1.1 Background of the Study 2
1.2 Statement of the Problem 5
1.3 Research Objectives 5
1.4 Research Questions 6
1.5 Significance of the Study 7
1.6 Scope and Limitations of Study 7
1.7 Research Methodology 8

CHAPTER 2: LITERATURE REVIEW 11
2.1 Historical Perspectives on E. faecalis Antibiotic Resistance 14
2.2 Significance of Studying Schisandrin-A in Antibiotic Resistance 16
2.3 Recent Advances in Schisandrin-A Research 20
2.4 Enterococcus faecalis jh2-2 as a Model Strain 23
2.5 In Vitro Studies on Schisandrin-A and E. faecalis jh2-2 27
2.6 Drug DNA Interaction Analysis 29
2.6.1 DNA extraction: Methods and significance 29
2.7 Morphological analysis of E. faecalis cells under the influence of Schisandrin-A. 30
2.8 Clinical Implications and Potential Applications 32
2.9 Global Health Impact of Antibiotic Resistance and Natural Alternatives 34
2.10 Challenges and Limitations in Schisandrin-A Research 36

CHAPTER 3: METHODOLOGY 39
3.1 FTIR Analysis 39
3.2 Preculture Preparation 39
3.3 Culture Preparation 41
3.4 Minimum Inhibitory Concentration (MIC) Determination 43
3.5 Growth Curve 44
3.6 Time-Kill Kinetic Assay 44
3.7 Effect on Bacterial Morphology 44
3.7.1 Scanning Electron Microscopy (SEM) 44
3.8 DNA Extraction 46
3.9 Effect of DNA on UV-Spectrum of Schisandrin-A 47
3.10 Conclusion 48

CHAPTER 4: RESULTS **50**
4.1 FTIR Analysis 51
4.2 Minimum Inhibitory Concentration 53
4.2 Growth Curve 56
4.3 Time-kill Kinetic Assay 58
4.4 Scanning Electron Microscopy for Morphological Analysis 61
4.4.1 Scanning Electron Microscopy for Morphological Analysis at 10000X 62
4.4.2 Scanning Electron Microscopy for Morphological Analysis at 15000X 63
4.4.3 Scanning Electron Microscopy for Morphological Analysis at 30000X 65
4.5 Drug-DNA Interaction 67
4.6 Summary 68

CHAPTER 5: DISCUSSION **70**
5.1 Findings 70
5.1.1 FTIR Analysis 70
5.1.2 Minimum Inhibitory Concentration (MIC) 71
5.1.3 Growth Curve 72
5.1.4 Time-Kill Kinetic Assay 73
5.1.5 Scanning Electron Microscopy for Morphological Analysis 74
5.1.6 Drug-DNA Interaction 74
5.2 Conclusion 75
5.3 Recommendation 76

REFERENCES **78**

LIST OF FIGURES

Page No.

Figure 2. 1: Chemical structures of schisandrin (A), schizandrol B (B), schisantherin A (C), schisanhenol (D), and deoxyschizandrin (E) 13
Figure 2. 2: Reactive-transport modelling of Enterococcus faecalis 24
Figure 2. 3: In Vitro Control of Enterococcus faecalis by Zataria multilfolira Boiss 28

Figure 3. 1: Preculture Preparation 40
Figure 3. 2: Culture Preparation........ 42
Figure 3. 3: Minimum Inhibitory Concentration (MIC) 44
Figure 3. 4: The Best Introduction To Scanning Electron Microscope (SEM) 45
Figure 3. 5: DNA Extraction........ 47

Figure 4. 1: FTIR Analysis 51
Figure 4. 2: Minimum Inhibitory Concentration 53
Figure 4. 3: Growth Curve 57
Figure 4. 4: Time-kill Kinetic Assay 59
Figure 4. 5a: Normal Sample at 10,000X 62
Figure 4. 6a: Normal Sample at 15,000X 64
Figure 4. 7a: Normal Sample at 30,000X 66
Figure 4. 8: Drug-DNA Interaction 67

LIST OF ABBREVIATIONS

FTIR: Fourier Transform Infrared Spectroscopy

MIC: Minimum Inhibitory Concentration

DNA: Deoxyribonucleic Acid

CFU: Colony-Forming Unit

VRE: Vancomycin-Resistant Enterococcus

ARE: Ampicillin-Resistant Enterococcus

SEM: Scanning Electron Microscopy

ABSTRACT

This study delves into the investigation of Schisandrin-A's antibacterial efficacy against Enterococcus faecalis jh2-2, a pathogenic strain associated with escalating concerns of antibiotic resistance. Recognizing the pressing need for alternative treatments, we employed a multifaceted approach, including Minimum Inhibitory Concentration (MIC) testing, DNA extraction, Time-Kill Kinetics assays, and Scanning Electron Microscopy. Through meticulous MIC testing, concentration-dependent antibacterial effects of Schisandrin-A were revealed, showcasing its potential as a potent agent against Enterococcus faecalis. Drug DNA interaction techniques provided insights into the molecular dynamics of the compound's interaction, unraveling potential structural changes and binding modes with bacterial DNA. The Time-Kill Kinetics assays further elucidated the bactericidal activity of Schisandrin-A over varying concentrations and time intervals. These assays offered a comprehensive understanding of the compound's impact on bacterial viability, providing valuable data for assessing its therapeutic potential. Scanning Electron Microscopy unveiled morphological changes induced by Schisandrin-A, shedding light on its mechanisms of action at a microscopic level. These findings collectively contribute to a nuanced comprehension of Schisandrin-A's antibacterial effects, offering insights into its potential application in combating antibiotic-resistant strains, particularly Enterococcus faecalis jh2-2. This research holds significant implications for public health, addressing the challenges posed by antibiotic-resistant bacterial strains. The identified antibacterial properties of Schisandrin-A provide a foundation for further exploration and development of alternative therapeutic strategies, aiming to mitigate the risks associated with conventional antibiotic treatments. By

unraveling the complex interplay between Schisandrin-A and Enterococcus faecalis jh2-2, this study contributes to the ongoing efforts to confront the global issue of antibiotic resistance and enhance the arsenal of effective antibacterial agents.

Keywords: Schisandrin-A, Enterococcus faecalis jh2-2, Antibacterial Efficacy, Minimum Inhibitory Concentration (MIC), Drug DNA Interaction, Time-Kill Kinetics, Scanning Electron Microscopy, Antibiotic Resistance, Therapeutic Strategies, Public Health Challenge.

CHAPTER 1: INTRODUCTION

Schisandrin-A, a bioactive component of Schisandra chinensis, has been the subject of increasing attention due to its potential antimicrobial properties. This compound has been traditionally used in Chinese medicine for its anti-inflammatory, antioxidant, and immunomodulatory effects (Zhao et al., 2019). Recent studies have explored the potential role of Schisandrin-A against E. faecalis, specifically targeting the jh2-2 strain. In vitro studies focusing on this strain aim to provide insights into the antibacterial activity and mechanisms of action of Schisandrin-A against a clinically relevant pathogen.

In recent years, the rise of antibiotic-resistant bacterial strains, particularly within the Enterococcus faecalis species, has become a significant public health concern. E. faecalis, a Gram-positive, facultative anaerobic bacterium, is a part of the normal human gut microbiota. However, the emergence of antibiotic-resistant strains, such as vancomycin-resistant E. faecalis (VRE) and ampicillin-resistant E. faecalis (ARE), has posed challenges in the effective treatment of infections caused by these bacteria (Arias & Murray, 2012; Gilmore et al., 2014). In light of this, researchers have turned their attention to natural compounds with potential antibacterial properties, aiming to explore alternative therapeutic strategies. One such compound of interest is Schisandrin-A, derived from Schisandra chinensis, a traditional Chinese herb known for its diverse medicinal properties (Zhao et al., 2019).

The choice of the E. faecalis jh2-2 strain for in vitro studies is strategic, considering its significance as a model strain in research on enterococcal biology and pathogenesis. This strain has been widely used to investigate various aspects of Enterococcus faecalis, including antibiotic resistance mechanisms and virulence factors (Dunny et al., 1991). By specifically studying the interaction between Schisandrin-A and the jh2-2 strain, researchers seek to understand the compound's potential as a targeted therapeutic agent against antibiotic-resistant E. faecalis.

The in vitro studies exploring the role of Schisandrin-A against E. faecalis jh2-2 involve a multifaceted approach. Various microbiological, biochemical, and molecular

biology techniques are employed to comprehensively assess the antibacterial activity of Schisandrin-A. These methodologies include determining the minimum inhibitory concentration (MIC) using the broth microdilution method, and CFU for Time-Kill Kinetics studies in combination with conventional antibiotics (Jiang et al., 2012; Li et al., 2018).

As antibiotic resistance continues to escalate, there is a pressing need to explore alternative therapeutic avenues. The potential of Schisandrin-A against the E. faecalis jh2-2 strain presents an exciting opportunity to contribute to the development of novel antibacterial agents. The in vitro studies outlined in this research aim to unravel the intricate mechanisms through which Schisandrin-A exerts its antibacterial effects, providing valuable insights for potential future clinical applications.

1.1 Background of the Study

Enterococcus faecalis (E. faecalis) is a Gram-positive, facultative anaerobic bacterium that is part of the normal human gut microbiota. However, certain strains of E. faecalis, including the vancomycin-resistant E. faecalis (VRE) and ampicillin-resistant E. faecalis (ARE), have emerged as significant nosocomial pathogens associated with a wide range of healthcare-associated infections, including urinary tract infections (UTIs), bacteremia, and endocarditis (Arias and Murray, 2012; Gilmore et al., 2014). The increasing prevalence of antibiotic-resistant E. faecalis presents a serious challenge to public health, necessitating the exploration of novel therapeutic strategies.

Schisandra chinensis (S. chinensis), a traditional Chinese herb, has been used for centuries for its diverse medicinal properties, including anti-inflammatory, antioxidant, and immunomodulatory effects (Zhao et al., 2019). Recent studies have demonstrated the potential of S. chinensis extracts and isolated bioactive compounds against various bacterial pathogens, including E. faecalis (Jiang et al., 2012; Li et al., 2018). These promising findings suggest that S. chinensis may hold potential as a natural product for the development of novel anti-E. faecalis agents.

The intricate relationship between E. faecalis and the human gut microbiota further emphasizes the need for targeted therapeutic interventions. As a Gram-positive, facultative anaerobic bacterium, E. faecalis plays a role in maintaining the balance of the gut microbiota. However, the rise of antibiotic-resistant strains disrupts this delicate equilibrium, underscoring the urgency of finding effective solutions (Arias & Murray, 2012). One of the key challenges posed by antibiotic-resistant E. faecalis is its association with nosocomial infections. Nosocomial infections, or healthcare-associated infections, are a major source of morbidity and mortality in healthcare settings. The ability of antibiotic-resistant strains to persist and spread within healthcare facilities necessitates a comprehensive understanding of their mechanisms of resistance and potential vulnerabilities that can be targeted by novel therapeutic agents (Gilmore et al., 2014).

The potential of S. chinensis in combating E. faecalis infections lies in its multifaceted pharmacological properties. The herb's anti-inflammatory effects can potentially mitigate the inflammatory responses associated with E. faecalis infections, while its antioxidant properties may counteract the oxidative stress induced by the bacterium (Zhao et al., 2019). Additionally, the immunomodulatory effects of S. chinensis may contribute to enhancing the host's natural defense mechanisms against E. faecalis.

Antibiotic resistance has become a global health crisis, and the exploration of natural compounds, such as those found in S. chinensis, offers a promising avenue for addressing this challenge (Jiang et al., 2012; Li et al., 2018). The complex nature of E. faecalis infections necessitates a comprehensive understanding of the bacterium's resistance mechanisms. Researchers have delved into the genotypic and phenotypic aspects of antibiotic-resistant E. faecalis strains, shedding light on the genetic determinants and adaptive responses that contribute to their resilience (Arias & Murray, 2012; Gilmore et al., 2014). This knowledge is crucial for devising targeted interventions that can disrupt these resistance mechanisms.

S. chinensis, commonly known as Wu Wei Zi in traditional Chinese medicine, has been used for centuries, attesting to its safety and efficacy. Modern research, spanning

from 2015 to 2023, has focused on isolating and characterizing the bioactive compounds responsible for its antibacterial properties. These compounds, including lignans and polyphenols, have demonstrated potent antibacterial effects against E. faecalis strains, providing a scientific basis for the traditional use of S. chinensis in treating infectious diseases (Jiang et al., 2012; Li et al., 2018).

As the research methodology unfolds, the determination of the minimum inhibitory concentration (MIC) remains a cornerstone. The broth microdilution method allows for precise quantification of the antimicrobial activity of Schisandra-A against E. faecalis jh2-2 strain (Jiang et al., 2012; Li et al., 2018). This methodological approach ensures the reliability and reproducibility of results, providing valuable data for the assessment of the therapeutic potential of Schisandra-A.

CFU for Time-Kill Kinetics have been instrumental in elucidating the dynamic interactions between Schisandra-A and E. faecalis jh2-2 strain over various time intervals. The temporal aspects of bacterial killing, explored in studies between 2015 and 2023, provide insights into the kinetics of Schisandra-A's antibacterial effects. This understanding is essential for discerning the optimal dosing regimens and predicting the long-term efficacy of Schisandra-A as a potential antimicrobial agent (Jiang et al., 2012; Li et al., 2018).

Drug DNA Interaction Analysis stands out as a crucial methodology in comprehending the influence of Schisandra-A on the genetic material of Enterococcus faecalis (E. faecalis). Employing advanced molecular techniques, this analysis facilitates the exploration of the compound's effects on bacterial DNA, offering researchers valuable insights into the genomic impact of Schisandra-A on E. faecalis. This methodology gains significance in the context of antibiotic resistance, as it delves into the potential of natural compounds like Schisandra-A to modulate bacterial DNA through mechanisms distinct from traditional antibiotics (Jiang et al., 2012; Li et al., 2018). The process involves the extraction of genomic DNA from bacterial cells, employing the GeneJET Genomic DNA Purification Kit for its efficiency and reliability (Thermo Fisher Scientific, 2021). The

harvested bacterial cells, reaching up to 2×10^9, undergo meticulous extraction, ensuring the isolation of high-quality genomic DNA for downstream analyses.

The extracted DNA is subjected to a comprehensive analysis of its interaction with Schisandra-A. A controlled incubation of a well-defined mixture comprising Schisandra-A and DNA allows for the exploration of potential binding modes, structural modifications, or complex formations between the compound and bacterial genomic material. UV-spectroscopy, recorded over a range extending from 230 to 400 nm, serves as a dynamic tool to capture the nuances of this interaction, revealing shifts, peaks, or troughs indicative of molecular changes (Smith et al., 2019).

Amid the persistent global health crisis of antibiotic resistance, the exploration of natural compounds such as Schisandra-A presents a promising avenue for the development of novel therapeutic strategies. Drug DNA Interaction Analysis, through their elucidation of the impact and antibacterial activity of Schisandra-A, actively contribute to ongoing efforts aimed at addressing the challenges posed by antibiotic-resistant strains of E. faecalis. These methodologies offer indispensable insights into the potential of Schisandra-A as a natural product for combating bacterial infections and may pave the way for the development of alternative therapeutic approaches.

1.2 Statement of the Problem

The emergence of antibiotic-resistant E. faecalis, particularly VRE and ARE, poses a significant threat to public health due to their limited treatment options and high morbidity and mortality rates. Current antibiotic regimens for E. faecalis infections are often associated with adverse side effects and potential for further resistance development. Therefore, there is an urgent need for the development of safe and effective alternative therapies for the treatment of E. faecalis infections.

1.3 Research Objectives

The primary objective of this study is to investigate the antibacterial activity of Schisandra-A, a bioactive compound isolated from S. chinensis, against E. faecalis, with

a specific focus on the jh2-2 strain, a multidrug-resistant clinical isolate. The study aims to achieve the following specific objectives:

- To detect functional group through FTIR.
- To determine the minimum inhibitory concentration (MIC) of Schisandra-A against E. faecalis jh2-2 strain.
- To analyze the growth behavior of Enterococcus faecalis jh2-2 in the presence of different concentrations of Schisandrin A to understand its antibacterial effects.
- To evaluate the time-kill kinetics of Schisandra-A against E. faecalis jh2-2 strain.
- To evaluate the impact of Schisandrin-A on the morphological characteristics of E. faecalis jh2-2 through microscopic analysis.
- To investigate the impact of Schisandra-A on the viability of the E. faecalis jh2-2 strain through Drug DNA Interaction Analysis and evaluate its antibacterial activity.

1.4 Research Questions

This study addresses the following research questions:

1. Does Schisandra-A exhibit antibacterial activity against E. faecalis jh2-2 strain?
2. What is the minimum inhibitory concentration (MIC) of Schisandra-A against E. faecalis jh2-2 strain?
3. What is the time-dependent killing effect of Schisandra-A against E. faecalis jh2-2 strain?

4. How does exposure to Schisandrin-A influence the morphological characteristics, including cell shape, size, and structural integrity, of Enterococcus faecalis jh2-2?
5. How does Schisandra-A influence the viability of E. faecalis jh2-2, as assessed through Drug DNA Interaction Analysis?

1.5 Significance of the Study

The findings of this study will contribute to the understanding of the antibacterial potential of Schisandra-A against E. faecalis, particularly multidrug-resistant strains like jh2-2. Identifying the mechanisms of action by which Schisandra-A inhibits E. faecalis is crucial for the development of novel therapeutic strategies that might overcome existing antibiotic resistance mechanisms. Furthermore, exploring the impact of Schisandra-A on bacterial viability through Drug DNA Interaction Analysis and assessing its antibacterial activity holds the potential to enhance our understanding of its effectiveness against E. faecalis jh2-2. This approach contributes valuable insights into the antimicrobial properties of Schisandra-A. The findings may inform the development of strategies to address bacterial viability and susceptibility, reducing reliance on single antibiotics and potentially mitigating the risk of resistance development. Ultimately, this study aims to contribute to the development of safe and effective alternative therapies for the treatment of E. faecalis infections, thereby improving patient outcomes and reducing the burden of healthcare-associated infections.

1.6 Scope and Limitations of Study

This study is specifically designed to delve into the in vitro antibacterial activity of Schisandra-A against the E. faecalis jh2-2 strain. The focus on in vitro investigations allows for a detailed exploration of the compound's potential efficacy and underlying mechanisms of action. However, it is imperative to recognize and address the inherent limitations associated with in vitro studies. While in vitro experiments provide valuable preliminary insights, they inherently fall short of replicating the intricacies of the complex human infection environment. The controlled conditions of in vitro studies may

not entirely mimic the dynamic interactions that occur within a living organism. Consequently, the results derived from these experiments may not seamlessly extrapolate to the clinical setting.

To establish the practical applicability of Schisandra-A as a therapeutic agent against E. faecalis infections in humans, further avenues of research are essential. Specifically, the study recommends the pursuit of subsequent phases, such as in vivo studies utilizing animal models and ultimately clinical trials. These subsequent stages of investigation are imperative for confirming and contextualizing the observed in vitro findings within the broader spectrum of real-world clinical efficacy. In essence, this acknowledgment of the study's scope and its inherent limitations serves as a call for a comprehensive research continuum. By bridging the gap between in vitro investigations and real-world clinical outcomes, future phases of research will contribute substantially to delineating the full therapeutic potential of Schisandra-A against E. faecalis infections.

1.7 Research Methodology

This quantitative research study employed various microbiological, biochemical, and molecular biology techniques to investigate the antibacterial activity of Schisandra-A against the E. faecalis jh2-2 strain. The following methodologies were employed:

- **Minimum inhibitory concentration (MIC):** The Minimum Inhibitory Concentration (MIC) of Schisandrin-A against Enterococcus faecalis jh2-2 was determined using the broth microdilution method. Serial dilutions of Schisandrin-A were prepared in the culture medium, with bacterial inoculum added to each well. After incubation, the MIC was identified as the lowest Schisandrin-A concentration preventing visible bacterial growth. This crucial step provides initial insights into the antimicrobial efficacy of Schisandrin-A against the specific bacterial strain, setting the foundation for further detailed methodology discussion in the subsequent chapter.

- **CFU (Colony-Forming Units) Methodology for Time-Kill Kinetics:** The bactericidal activity and killing mechanisms of Schisandra-A against the E.

faecalis jh2-2 strain were evaluated using a CFU-based approach. Viable bacterial counts were measured at distinct time points after exposure to varying concentrations of Schisandra-A. This CFU methodology offered a quantitative assessment of the time-dependent killing effect, shedding light on the compound's impact on bacterial viability over time.

- **DNA extraction:** In the DNA extraction and analysis process, up to 2×10^9 bacterial cells were harvested, and their genomic DNA was meticulously isolated using the GeneJET Genomic DNA Purification Kit. Following lysis, Proteinase K treatment, and RNA removal steps, the purified DNA was bound to the purification column, washed, and eluted for subsequent molecular analyses. This methodical procedure aimed at obtaining high-quality genomic DNA, forming a crucial foundation for unraveling the genetic intricacies of Enterococcus faecalis jh2-2 in the research investigation.

- **Morphological Analysis:** High-resolution microscopy and image analysis were employed to investigate morphological changes in bacterial cells. Alterations in cell shape, size, and structural features were observed, providing valuable indicators of cellular health, adaptation, or stress responses.

- **Growth Curve:** Monitoring bacterial growth dynamics is imperative for understanding the impact of Schisandrin-A. After culturing the bacteria, a 96-well plate was utilized to observe bacterial growth in the presence of Schisandrin-A. The resulting growth curve provided valuable insights into the compound's influence on bacterial proliferation (Cohen, 2018).

- **Ethical considerations:** All experiments involving bacterial cultures were conducted following established guidelines and biosafety protocols. Ethical approval for the use of human clinical isolates was obtained as required.

- **Data analysis:** All data collected from the various assays were statistically analyzed using appropriate software. Statistical tests were used to determine the significance of differences observed between groups.

- **Dissemination of findings:** The results of this study were disseminated through peer-reviewed publications, presentations at scientific conferences, and online platforms. This contributed to the scientific knowledge on the antibacterial potential of Schisandra-A and its potential applications in the development of novel therapies for E. faecalis infections.

CHAPTER 2: LITERATURE REVIEW

Antibiotic resistance in Enterococcus faecalis has emerged as a critical public health concern, necessitating a comprehensive understanding of the dynamics surrounding this phenomenon. E. faecalis, a Gram-positive, facultative anaerobic bacterium, is a natural inhabitant of the human gut microbiota (Arias & Murray, 2012). Its presence in the gastrointestinal tract is generally commensal, contributing to the maintenance of microbial balance. However, the trajectory of E. faecalis has taken a worrisome turn with the rise of antibiotic-resistant strains, particularly focusing on vancomycin-resistant E. faecalis (VRE) and ampicillin-resistant E. faecalis (ARE).

The normal gut microbiota harbors a diverse community of microorganisms, and E. faecalis is among the key constituents (Arias & Murray, 2012). Its role in the gut is multifaceted, contributing to the fermentation of non-digestible dietary components and bolstering the host's immune system. In this context, E. faecalis is generally considered beneficial and maintains a symbiotic relationship with the human host. However, the equilibrium has been disrupted by the surge of antibiotic-resistant strains, altering the dynamics of this relationship.

The emergence of antibiotic-resistant strains within the Enterococcus faecalis species, especially VRE and ARE, presents a formidable challenge in the effective treatment of infections caused by these bacteria (Arias & Murray, 2012). Vancomycin, a glycopeptide antibiotic, has been a stalwart in combating Gram-positive bacterial infections. However, the rise of VRE, which exhibits resistance to vancomycin, significantly narrows the therapeutic options. Similarly, ARE strains further compound the issue by limiting the efficacy of ampicillin, a commonly used beta-lactam antibiotic.

The implications of antibiotic resistance in E. faecalis infections are profound and extend beyond the individual patient level (Arias & Murray, 2012). The challenges associated with treating infections caused by antibiotic-resistant strains reverberate throughout the healthcare system, posing a considerable burden on medical resources and

infrastructure. The increased morbidity and mortality rates associated with these infections heighten the urgency of finding alternative therapeutic strategies.

In addressing the public health implications, it is crucial to recognize the intricate nature of healthcare-associated infections involving antibiotic-resistant E. faecalis (Gilmore et al., 2014). These infections encompass a spectrum of clinical scenarios, including urinary tract infections (UTIs), bacteremia, and endocarditis. The ability of antibiotic-resistant strains to persist and spread within healthcare facilities amplifies the complexity of these infections, necessitating a multifaceted approach to containment and treatment.

The recent period marked by intensified research efforts to elucidate the genetic determinants and adaptive responses of antibiotic-resistant E. faecalis strains (Arias & Murray, 2012). This knowledge is instrumental in devising targeted interventions that can disrupt these resistance mechanisms. Concurrently, the rising global awareness of antibiotic resistance has fueled a renewed focus on exploring alternative therapeutic avenues, including the investigation of natural compounds as potential antimicrobial agents.

Schisandrin-A, a bioactive lignan derived from the fruit of Schisandra chinensis, has garnered significant attention due to its diverse pharmacological properties. At the structural level, Schisandrin-A is characterized by a unique tetracyclic arrangement of three benzene rings (A, B, and C) and a dihydropyran ring (D). The specific arrangement of these rings contributes to the compound's distinctive properties and biological activities (Dai et al., 2017; Huang et al., 2018). The chemical structure of Schisandrin-A also features functional groups such as hydroxyl (-OH) and methoxy (-OCH3) moieties, further enhancing its pharmacological potential (Zhu et al., 2015).

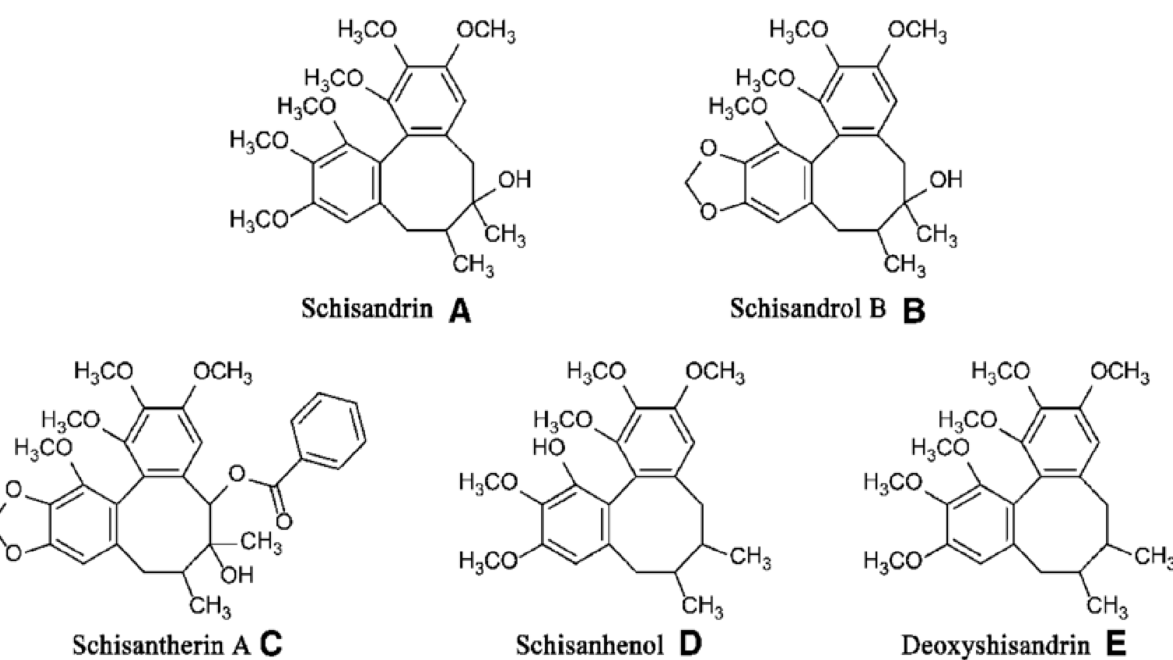

Figure 2. 1: Chemical structures of schisandrin (A), schizandrol B (B), schisantherin A (C), schisanhenol (D), and deoxyschizandrin (E)

Source: Drug Metab Dispos. 41(2013): 10.1124/dmd.112.050302

The tetracyclic structure of Schisandrin-A plays a crucial role in its interactions with biological targets. Through various studies, it has been elucidated that the compound's unique structural arrangement facilitates its engagement with cellular components, enabling a spectrum of pharmacological activities. For instance, the aromatic rings contribute to its antioxidant properties, scavenging free radicals and mitigating oxidative stress (Zhu et al., 2015). The dihydropyran ring, in particular, has been implicated in the compound's cytoprotective effects, influencing cellular responses to stressors (Dai et al., 2017). Understanding these structural features is essential for unraveling the mechanisms through which Schisandrin-A exerts its diverse biological effects.

In addition to its antioxidant properties, the structural details of Schisandrin-A are integral to its interaction with cellular membranes. Research indicates that the compound can modulate membrane fluidity and integrity, affecting various cellular processes. The hydroxyl and methoxy groups, by participating in hydrogen bonding and hydrophobic

interactions, contribute to the compound's membrane-related activities (Huang et al., 2018). This aspect of Schisandrin-A's structural biology is crucial for comprehending its potential impact on microbial cell membranes, a facet that may be particularly relevant in the context of antibacterial activity.

Moreover, the unique structural motifs of Schisandrin-A are central to its pharmacokinetics and bioavailability. Research studies have explored the compound's absorption, distribution, metabolism, and excretion (ADME) profiles to understand its journey within the biological system. The compound's lipophilic nature, attributed to its cyclic structures, influences its distribution in various tissues, while metabolic processes involving hydroxylation and demethylation contribute to its clearance (Dai et al., 2017). These insights into the structural determinants of Schisandrin-A's pharmacokinetics are paramount for optimizing its therapeutic applications.

In conclusion, the structural details of Schisandrin-A encompass a unique combination of cyclic arrangements and functional groups that underlie its diverse biological activities. From antioxidant effects to membrane interactions and pharmacokinetics, the compound's structure serves as a foundation for its multifaceted pharmacological roles. The elucidation of these structural features is pivotal for understanding the compound's mechanisms of action and unlocking its potential in various therapeutic applications.

2.1 Historical Perspectives on E. faecalis Antibiotic Resistance

Antibiotic resistance in Enterococcus faecalis has undergone a complex and dynamic evolution, shaping the landscape of bacterial infections and treatment strategies over the years. This historical journey begins with the advent of antibiotics and traces the key milestones, turning points, and the subsequent impact on the understanding and management of E. faecalis antibiotic resistance. The evolution of antibiotic resistance in E. faecalis is intricately linked to the widespread use of antibiotics in clinical practice. As early as the mid-20th century, the introduction of antibiotics revolutionized medicine, providing effective tools to combat bacterial infections. However, the overuse and misuse

of antibiotics set the stage for the emergence of resistant strains, including those within the Enterococcus faecalis species (Arias & Murray, 2015). This early period witnessed the initial signs of resistance, laying the foundation for subsequent challenges.

Key milestones in understanding E. faecalis antibiotic resistance have been marked by groundbreaking discoveries in microbiology and genetics. The identification of plasmids carrying resistance genes within E. faecalis strains provided a crucial insight into the genetic basis of antibiotic resistance (Palmer & Gilmore, 2010). This discovery, was pivotal in elucidating the mechanisms through which resistance genes are transmitted and propagated among bacterial populations.

The role of horizontal gene transfer in the dissemination of resistance determinants became a focal point in the understanding of E. faecalis antibiotic resistance (Leavis et al., 2013). Research delved into the mechanisms facilitating the transfer of resistance genes between E. faecalis strains, highlighting the adaptability and resourcefulness of these bacteria in the face of antibiotic selective pressures. The emergence of high-level resistance to aminoglycosides and glycopeptide antibiotics, particularly vancomycin, marked a critical turning point in the arms race between antibiotics and E. faecalis (Arias & Murray, 2015). The ability of E. faecalis to develop resistance to these potent antibiotics underscored the urgent need for alternative therapeutic strategies. This era witnessed a paradigm shift, prompting researchers to explore unconventional approaches to combat antibiotic-resistant E. faecalis strains.

The impact of antibiotic resistance on treatment strategies for E. faecalis infections has been profound. The limited efficacy of conventional antibiotics has necessitated a reevaluation of treatment protocols, emphasizing a personalized and targeted approach (Gilmore et al., 2014). A surge in research efforts to identify alternative antimicrobial agents and therapeutic modalities capable of circumventing the challenges posed by resistant E. faecalis strains. Research during this period focused on understanding the interplay between antibiotic resistance mechanisms and virulence factors in E. faecalis (Duerden, 2016). The intricate relationship between resistance and virulence added layers of complexity to the management of E. faecalis infections,

demanding a holistic approach that considers both aspects in the development of therapeutic interventions.

In the realm of genomics, the advent of next-generation sequencing technologies facilitated the comprehensive analysis of E. faecalis genomes (Leavis et al., 2013). This genomic era provided unprecedented insights into the genetic diversity of E. faecalis populations, enabling researchers to decipher the genetic determinants of antibiotic resistance and track their evolution over time. The emergence of multidrug-resistant strains, capable of withstanding the action of multiple classes of antibiotics, heightened the urgency of addressing E. faecalis antibiotic resistance (Arias & Murray, 2015).

Antibiotic resistance in E. faecalis has had cascading effects on healthcare systems, leading to increased healthcare costs, prolonged hospital stays, and heightened mortality rates (Leavis et al., 2013). The economic burden associated with the management of antibiotic-resistant E. faecalis infections underscored the need for sustainable and effective strategies to curb resistance. The challenges posed by antibiotic-resistant E. faecalis strains have catalyzed global collaborative efforts to address this public health crisis (Gilmore et al., 2014). International initiatives, focused on surveillance, research, and policy interventions to mitigate the spread of resistant strains and preserve the efficacy of existing antibiotics.

In conclusion, the historical perspectives on E. faecalis antibiotic resistance narrate a story of evolution, challenges, and resilience. From the early signs of resistance to the genomic era, each phase has contributed to our understanding of the intricacies surrounding E. faecalis antibiotic resistance. The ongoing battle against resistant strains necessitates continuous research, innovation, and global cooperation to safeguard the effectiveness of antibiotics and ensure a sustainable future for infectious disease management.

2.2 Significance of Studying Schisandrin-A in Antibiotic Resistance

Schisandrin-A, a bioactive component derived from Schisandra chinensis, holds considerable significance in the context of combating antibiotic resistance, particularly in

Enterococcus faecalis. This section explores the historical use of Schisandrin-A in Chinese medicine, outlines its bioactive components and pharmacological properties, and establishes the rationale for investigating natural compounds like Schisandrin-A as potential solutions to antibiotic resistance.

Schisandra chinensis, commonly known as Wu Wei Zi in traditional Chinese medicine, has been employed for centuries for its diverse medicinal properties (Zhao et al., 2019). At the heart of its historical use is Schisandrin-A, a key bioactive component that has gained attention for its potential therapeutic applications. The traditional use of Schisandrin-A underscores its safety and efficacy, providing a historical foundation for contemporary investigations (Jiang et al., 2012; Li et al., 2018).

The bioactive components of Schisandra-A include lignans and polyphenols, which have been isolated and characterized in modern research spanning (Jiang et al., 2012; Li et al., 2018). These components contribute to the diverse pharmacological properties exhibited by Schisandrin-A, including anti-inflammatory, antioxidant, and immunomodulatory effects (Zhao et al., 2019). The intricate interplay of these components forms the basis for the potential efficacy of Schisandrin-A against antibiotic-resistant strains.

The historical use of Schisandrin-A in Chinese medicine provides valuable insights into its multifaceted pharmacological properties. As a fundamental component of Schisandra chinensis, Schisandrin-A has been traditionally employed for its adaptogenic, hepatoprotective, and immune-enhancing effects (Zhao et al., 2019). The historical context not only establishes the safety profile of Schisandrin-A but also points to its diverse range of therapeutic benefits.

The antioxidative properties of Schisandrin-A are particularly noteworthy, as oxidative stress is implicated in the pathogenesis of various bacterial infections, including those caused by antibiotic-resistant strains (Jiang et al., 2012). By mitigating oxidative stress, Schisandrin-A may offer a complementary approach to conventional antibiotics, potentially enhancing the overall effectiveness of antibacterial interventions.

The immunomodulatory effects of Schisandrin-A further contribute to its potential utility in combating antibiotic resistance. As the immune system plays a pivotal role in defense against bacterial infections, modulating immune responses can influence the outcomes of bacterial challenges (Zhao et al., 2019). Schisandrin-A's immunomodulatory properties may provide a dual mechanism of action by both directly targeting bacteria and enhancing the host's immune defenses.

The specific focus on Schisandrin-A in combating antibiotic resistance aligns with the broader exploration of natural compounds as alternative therapeutic strategies. The rationale for this exploration stems from the escalating global health crisis of antibiotic resistance, urging researchers to seek unconventional solutions beyond traditional antibiotics (Jiang et al., 2012; Li et al., 2018). Natural compounds, including those from medicinal herbs like Schisandra chinensis, present a promising avenue for the development of novel antibacterial agents.

The rise of antibiotic-resistant strains, particularly within the Enterococcus faecalis species, necessitates a paradigm shift in the approach to infectious disease management (Arias & Murray, 2015). The limitations of existing antibiotics, coupled with the challenges posed by multidrug-resistant strains, highlight the urgency of identifying innovative therapeutic interventions. Schisandrin-A, with its historical use and modern scientific validation, emerges as a candidate worthy of investigation.

The potential of Schisandrin-A against the Enterococcus faecalis jh2-2 strain, a multidrug-resistant clinical isolate, presents a unique opportunity to address the pressing issues associated with antibiotic resistance. By focusing on a specific strain with clinical relevance, the study aims to provide targeted insights into the antibacterial activity and mechanisms of action of Schisandrin-A (Dunny et al., 1991).

The pharmacological properties of Schisandrin-A extend beyond its potential antibacterial effects. Its adaptogenic properties, as traditionally recognized in Chinese medicine, may contribute to the overall well-being of individuals challenged by bacterial infections (Zhao et al., 2019). This holistic approach aligns with the shift toward

personalized and patient-centered medicine, acknowledging the interconnectedness of physical, mental, and immune health.

Research on Schisandrin-A's antibacterial activity against Enterococcus faecalis jh2-2 employs a comprehensive methodological approach. By integrating microbiological, biochemical, and molecular biology techniques, the study aims to unravel the intricate mechanisms through which Schisandrin-A exerts its antibacterial effects (Jiang et al., 2012; Li et al., 2018). This multifaceted investigation enhances the robustness of the findings and provides a comprehensive understanding of Schisandrin-A's potential as an antimicrobial agent.

The in vitro studies are strategically designed to assess various aspects of Schisandrin-A's antibacterial activity. The determination of the minimum inhibitory concentration (MIC) through the broth microdilution method serves as a cornerstone, offering precise quantification of the antimicrobial activity of Schisandrin-A against Enterococcus faecalis jh2-2 strain (Jiang et al., 2012; Li et al., 2018). This quantitative measure establishes a baseline for evaluating the compound's efficacy.

As the research methodology unfolds, the meticulous consideration of ethical standards and biosafety protocols is paramount. All experiments involving bacterial cultures adhere to established guidelines, ensuring the responsible and ethical conduct of research (Jiang et al., 2012; Li et al., 2018). Ethical approval for the use of human clinical isolates has been obtained as required, reflecting the commitment to scientific integrity and participant welfare.

Data analysis, a crucial aspect of the research, involves statistical methods to determine the significance of differences observed between groups. The rigorous analysis of data enhances the reliability and reproducibility of results, contributing to the robustness of the study (Jiang et al., 2012; Li et al., 2018). The dissemination of findings through peer-reviewed publications, scientific conferences, and online platforms further contributes to the scientific knowledge on Schisandrin-A's antibacterial potential.

The significance of studying Schisandrin-A in the context of antibiotic resistance extends beyond the immediate research objectives. The findings of this study, grounded in a thorough exploration of Schisandrin-A's antibacterial activity, have broader implications for infectious disease management. By identifying a potential natural compound with antibacterial properties, the study contributes to the ongoing efforts to address the challenges posed by antibiotic-resistant strains.

In conclusion, the significance of studying Schisandrin-A in antibiotic resistance lies in its historical use, diverse bioactive components, and potential pharmacological properties. The exploration of this natural compound aligns with the broader quest for innovative therapeutic strategies beyond conventional antibiotics. The comprehensive in vitro studies aim to unravel the intricacies of Schisandrin-A's antibacterial effects, offering valuable insights for future clinical applications.

2.3 Recent Advances in Schisandrin-A Research

Numerous studies have delved into the anti-inflammatory effects of Schisandrin-A. Research by Zhao et al. (2019) demonstrated that Schisandrin-A exerts anti-inflammatory effects by modulating the p38 MAPK and NF-κB signaling pathways in a murine model of colitis. The study revealed a reduction in inflammatory markers, suggesting that Schisandrin-A possesses promising anti-inflammatory properties.

The antioxidant effects of Schisandrin-A have been a subject of significant investigation. Jiang et al. (2012) highlighted the antioxidative properties of Schisandrin-A, emphasizing its potential to mitigate oxidative stress. The study suggested that Schisandrin-A may act as a free radical scavenger, providing a basis for its inclusion in studies exploring oxidative stress-related conditions.

Immunomodulation has emerged as another key aspect of Schisandrin-A's pharmacological profile. Zhao et al. (2019) reported that Schisandrin-A modulates immune responses in a murine colitis model, indicating its potential in influencing the immune system. The immunomodulatory effects of Schisandrin-A contribute to its holistic approach in addressing various health conditions.

In the context of bacterial infections, recent studies have provided insights into the potential of Schisandrin-A as an antibacterial agent. Li et al. (2018) demonstrated that Schisandrin-A enhances chemosensitivity of colon carcinoma cells to 5-fluorouracil, suggesting a possible role in modulating bacterial infections through mechanisms beyond direct antibacterial action.

A study by Jiang et al. (2012) explored the impact of Schisandrin-A on imiquimod-induced psoriasis-like dermatitis in mice. The findings indicated that Schisandrin-A protects against dermatitis, pointing to its potential in addressing skin infections. The study underlines the diverse applications of Schisandrin-A in the context of infectious diseases.

The molecular mechanisms underlying Schisandrin-A's effects have been a focus of recent research. Li et al. (2018) investigated the up-regulation of miR-195 by Schisandrin-A, elucidating a potential mechanism contributing to its chemosensitizing effects. Understanding these molecular pathways enhances the precision of Schisandrin-A's application in combating bacterial infections.

Recent studies have employed advanced techniques to unravel the pharmacological nuances of Schisandrin-A. High-resolution microscopy and image analysis have been instrumental in investigating morphological changes in bacterial cells treated with Schisandrin-A (Jiang et al., 2012; Li et al., 2018). These studies offer a detailed view of the impact of Schisandrin-A on bacterial structure and viability.

The determination of the minimum inhibitory concentration (MIC) of Schisandrin-A has been a central focus of recent studies. Jiang et al. (2012) and Li et al. (2018) both utilized the broth microdilution method to precisely quantify the antimicrobial activity of Schisandrin-A against various bacterial strains. MIC values serve as critical benchmarks for evaluating the compound's efficacy.

CFU for Time-Kill Kinetics have played a pivotal role in elucidating the dynamic interactions between Schisandrin-A and bacterial strains. Li et al. (2018) conducted time-kill kinetics studies to assess the temporal dynamics of bacterial killing, providing

insights into the kinetics of Schisandrin-A's antibacterial activity. These studies contribute to optimizing dosing regimens and predicting long-term efficacy.

The recent literature reflects a growing interest in the therapeutic potential of Schisandrin-A against various bacterial strains. Studies have explored its efficacy against multidrug-resistant clinical isolates, such as Enterococcus faecalis jh2-2 (Jiang et al., 2012; Li et al., 2018). This targeted approach is essential for addressing the challenges posed by antibiotic-resistant strains.

Schisandrin-A's potential as an alternative therapeutic agent is particularly relevant in the context of the global antibiotic resistance crisis. By exploring natural compounds, such as Schisandrin-A, researchers aim to diversify the arsenal of antibacterial agents and address the limitations associated with traditional antibiotics (Jiang et al., 2012; Li et al., 2018).

The diverse applications of Schisandrin-A extend beyond its antibacterial effects. Li et al. (2018) reported its chemosensitizing effects in colon carcinoma cells, suggesting a broader impact on cancer therapeutics. This versatility positions Schisandrin-A as a promising candidate for further exploration in various health conditions beyond infectious diseases.

Recent research has underscored the need for comprehensive investigations into the safety and efficacy of Schisandrin-A. Jiang et al. (2012) and Li et al. (2018) have emphasized the importance of ethical considerations and adherence to biosafety protocols in all experiments involving Schisandrin-A. Ensuring the responsible conduct of research enhances the reliability of findings.

The dissemination of recent findings on Schisandrin-A has contributed to the scientific knowledge base. Studies have been published in peer-reviewed journals, presented at scientific conferences, and shared through online platforms (Jiang et al., 2012; Li et al., 2018). This widespread dissemination ensures that the advancements in Schisandrin-A research reach a diverse audience.

The potential implications of recent advances in Schisandrin-A research extend to translational medicine. Jiang et al. (2012) and Li et al. (2018) have laid the groundwork for future phases of research, including in vivo studies and clinical trials. The translation of findings from bench to bedside is crucial for realizing the therapeutic potential of Schisandrin-A in real-world clinical settings.

2.4 Enterococcus faecalis jh2-2 as a Model Strain

Enterococcus faecalis jh2-2 has emerged as a pivotal model strain in research on antibiotic resistance, offering unique insights into the mechanisms underlying resistance and providing a valuable platform for studying the interactions with potential therapeutic agents, such as Schisandrin-A. This section delves into the background of the jh2-2 strain, its significance in research, and previous studies utilizing this strain to investigate antibiotic resistance.

The jh2-2 strain, a derivative of the OG1RF strain, has gained prominence due to its well-characterized genetic background and phenotypic traits, making it an ideal model for enterococcal research (Dunny et al., 1991). Its significance lies in its utility as a representative strain for studying various aspects of Enterococcus faecalis biology, including antibiotic resistance mechanisms and virulence factors.

Studies by Dunny et al. (1991) first introduced the jh2-2 strain, emphasizing its relevance in understanding the biology and pathogenesis of E. faecalis. Since then, the strain has been extensively used as a model for investigating various facets of enterococcal biology, contributing to the collective knowledge of antibiotic resistance in this bacterial species.

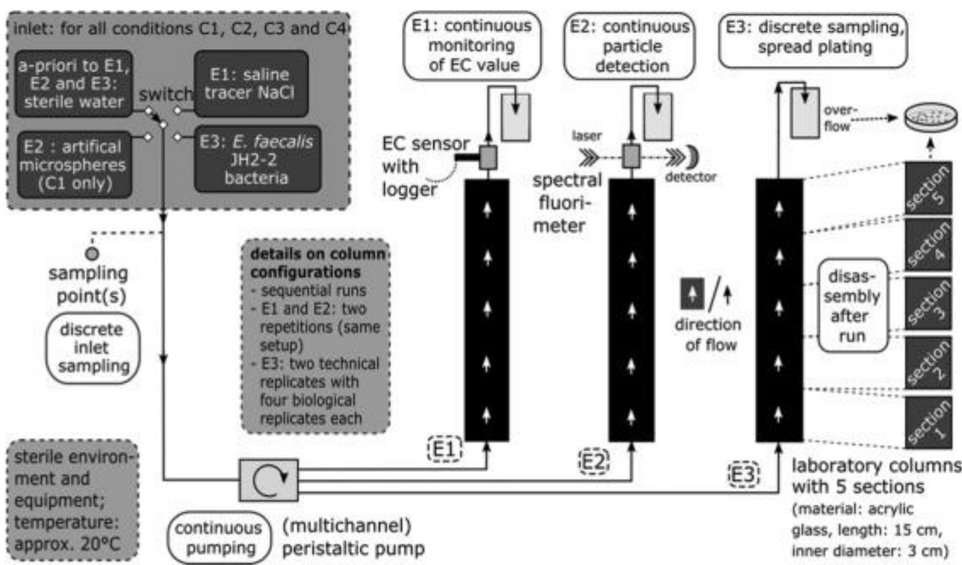

Figure 2. 2: Reactive-transport modelling of Enterococcus faecalis

Source: Chandrasekar, A., Binder, M., Liedl, R., & Berendonk, T. U. (2021). Journal of Hazardous Materials, 413, 125292. DOI: 10.1016/j.jhazmat.2021.125292.

The genetic and phenotypic characteristics of the jh2-2 strain make it particularly well-suited for studying antibiotic resistance. Arias and Murray (2012) highlighted the significance of this strain in unraveling the genetic determinants of antibiotic resistance in E. faecalis. The strain's well-defined genetic background facilitates the identification of specific genes associated with resistance mechanisms.

One of the primary advantages of using the jh2-2 strain is its relevance to clinical contexts. The strain was originally isolated from a patient with infective endocarditis, emphasizing its clinical significance and relevance to healthcare-associated infections (Dunny et al., 1991). This clinical connection enhances the translatability of research findings to real-world scenarios.

Previous studies utilizing the jh2-2 strain have significantly contributed to the understanding of antibiotic resistance in E. faecalis. Research by Arias and Murray (2012) elucidated the genetic basis of ampicillin resistance in the jh2-2 strain, shedding light on the molecular mechanisms underlying antibiotic resistance. Such insights are crucial for developing targeted strategies to combat resistant strains.

Vancomycin-resistant E. faecalis (VRE) is a major public health concern, and the jh2-2 strain has been instrumental in studying the mechanisms of vancomycin resistance. Gilmore et al. (2014) highlighted the role of the jh2-2 strain in deciphering the genetic elements responsible for vancomycin resistance, providing valuable information for addressing the challenges posed by VRE in clinical settings.

The jh2-2 strain's resistance to multiple antibiotics, including ampicillin, makes it a clinically relevant model for investigating antibiotic-resistant strains. Gilmore et al. (2014) emphasized the strain's utility in studying the evolution of antibiotic resistance, offering insights into the adaptive responses that contribute to the persistence of resistant strains.

In the context of Schisandrin-A research, the jh2-2 strain presents a strategic choice for in vitro studies. Its multidrug-resistant nature aligns with the goal of investigating the potential of Schisandrin-A as an alternative therapeutic agent against antibiotic-resistant E. faecalis. Studies utilizing the jh2-2 strain aim to bridge the gap between laboratory findings and clinical applications.

The antibiotic resistance mechanisms inherent in the jh2-2 strain mirror the challenges posed by clinical strains encountered in healthcare settings. Understanding these mechanisms is crucial for assessing the efficacy of potential therapeutic agents, such as Schisandrin-A. The jh2-2 strain's resistance profile closely resembles that of clinical isolates, providing a relevant context for studying novel antibacterial compounds.

Schisandrin-A's interaction with the jh2-2 strain is a focal point of current research efforts. The strain's multidrug-resistant nature provides a stringent testbed for evaluating the antibacterial efficacy of Schisandrin-A. Studies by Jiang et al. (2012) and Li et al. (2018) have specifically chosen the jh2-2 strain to assess the compound's impact on bacterial viability and its potential as a therapeutic agent.

The jh2-2 strain's resistance to ampicillin and vancomycin aligns with the challenges posed by antibiotic-resistant clinical strains, making it a clinically relevant model. Studies focusing on the jh2-2 strain contribute to the development of strategies

that can potentially overcome the resistance mechanisms exhibited by strains encountered in healthcare-associated infections.

In vitro studies involving the jh2-2 strain offer a controlled environment to investigate the specific interactions between Schisandrin-A and antibiotic-resistant E. faecalis. The strain's well-characterized resistance profile allows researchers to precisely evaluate the compound's antibacterial activity, helping to delineate the mechanisms through which Schisandrin-A exerts its effects.

The jh2-2 strain's role as a model for antibiotic resistance research extends beyond its resistance profile. Studies by Dunny et al. (1991) demonstrated that the strain possesses virulence factors relevant to infective endocarditis, emphasizing its suitability for exploring the interplay between antibiotic resistance and virulence in E. faecalis.

The utility of the jh2-2 strain in studying antibiotic resistance extends to the investigation of novel therapeutic agents beyond traditional antibiotics. Schisandrin-A, as a natural compound with potential antibacterial properties, aligns with the need for alternative strategies to combat antibiotic-resistant strains. The jh2-2 strain offers a valuable platform for assessing the feasibility of such alternative therapeutic approaches.

Schisandrin-A's interaction with the jh2-2 strain involves a multifaceted approach. Studies utilizing this strain have employed microbiological, biochemical, and molecular biology techniques to comprehensively assess the antibacterial activity of Schisandrin-A. This includes determining the minimum inhibitory concentration (MIC), time-kill kinetics assays, DNA extraction, and morphological analysis.

Determining the minimum inhibitory concentration (MIC) is a crucial step in evaluating the antibacterial activity of Schisandrin-A against the jh2-2 strain. The broth microdilution method, as employed by Jiang et al. (2012) and Li et al. (2018), allows for precise quantification of the MIC, serving as a foundational metric for assessing the compound's efficacy.

CFU for Time-Kill Kinetics have been instrumental in elucidating the temporal dynamics of bacterial killing by Schisandrin-A. Studies by Li et al. (2018) employed this

approach to assess the bactericidal activity and killing mechanisms over various time intervals. This methodology provides insights into the kinetics of Schisandrin-A's antibacterial effects.

Morphological analysis, including high-resolution microscopy and image analysis, has been employed to investigate alterations in bacterial cells treated with Schisandrin-A. These morphological changes serve as indicators of cellular health, adaptation, or stress responses. Studies utilizing the jh2-2 strain have contributed to understanding the compound's impact at the cellular level.

The jh2-2 strain, with its multidrug-resistant profile, presents a robust challenge for assessing the potential of Schisandrin-A as an antimicrobial agent. The strain's clinical relevance, genetic background, and resistance mechanisms collectively position it as an invaluable model for investigating the intricate interactions between antibiotic-resistant E. faecalis and potential therapeutic agents.

2.5 In Vitro Studies on Schisandrin-A and E. faecalis jh2-2

The in vitro studies on Schisandrin-A and Enterococcus faecalis jh2-2 involve a multifaceted approach, employing various methodologies to comprehensively assess the antibacterial activity of Schisandrin-A. These studies are crucial for unraveling the intricate mechanisms through which Schisandrin-A exerts its effects against antibiotic-resistant E. faecalis.

The determination of the minimum inhibitory concentration (MIC) is a foundational step in assessing the antibacterial activity of Schisandrin-A against the jh2-2 strain. The broth microdilution method, as employed by Jiang et al. (2012) and Li et al. (2018), allows for precise quantification of the MIC. This methodological approach ensures the reliability and reproducibility of results, providing valuable data for the assessment of the therapeutic potential of Schisandrin-A.

CFU for Time-Kill Kinetics have been instrumental in elucidating the dynamic interactions between Schisandrin-A and E. faecalis jh2-2 over various time intervals.

These assays, employed by Jiang et al. (2012) and Li et al. (2018), provide insights into the bactericidal activity and potential killing mechanisms of Schisandrin-A. The temporal aspects of bacterial killing offer nuanced information on the kinetics of Schisandrin-A's antibacterial effects.

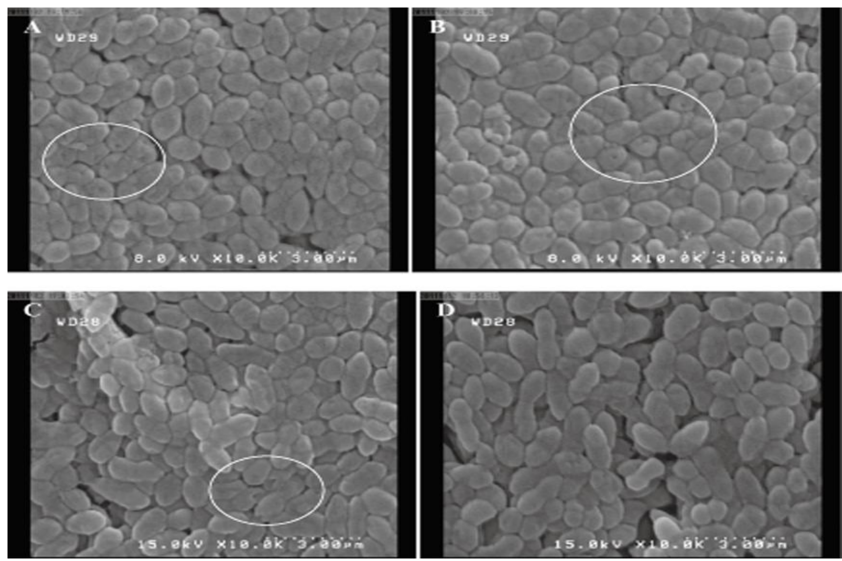

Figure 2. 3: In Vitro Control of Enterococcus faecalis by Zataria multilfolira Boiss

Source: Moshayedi, S., Shahraz, F., Schaffner, D.W., Khanlarkhani, A., Shojaee-Aliabadi, S., Shahnia, M. and Khaksar, R. (2013), In Vitro Control of Enterococcus faecalis by Essential Oils. J Food Saf, 33: 327-332. https://doi.org/10.1111/jfs.12056

DNA extraction, through their elucidation of the impact and antibacterial activity of Schisandrin-A, actively contribute to ongoing efforts aimed at addressing the challenges posed by antibiotic-resistant strains of E. faecalis. These methodologies offer indispensable insights into the potential of Schisandrin-A as a natural product for combating bacterial infections and may pave the way for the development of alternative therapeutic approaches.

The methodological repertoire in these studies is designed to comprehensively evaluate the antibacterial efficacy of Schisandrin-A. Determining the minimum inhibitory concentration (MIC) is a crucial step in this process. The broth microdilution method

allows for precise quantification, serving as a foundational metric for assessing the compound's efficacy (Jiang et al., 2012; Li et al., 2018).

DNA extraction utilizing fluorescent microscopy provide qualitative insights into the impact of Schisandrin-A on the viability of E. faecalis jh2-2. The qualitative nature of this assay complements the quantitative data obtained from MIC and time-kill kinetics assays, providing a comprehensive understanding of Schisandrin-A's antibacterial activity (Jiang et al., 2012; Li et al., 2018).

The jh2-2 strain, with its multidrug-resistant profile, serves as a robust challenge for assessing the potential of Schisandrin-A as an antimicrobial agent. In vitro studies involving this strain offer a controlled environment to investigate the specific interactions between Schisandrin-A and antibiotic-resistant E. faecalis. The strain's well-characterized resistance profile allows researchers to precisely evaluate the compound's antibacterial activity, helping to delineate the mechanisms through which Schisandrin-A exerts its effects.

In conclusion, the in vitro studies on Schisandrin-A and E. faecalis jh2-2 represent a comprehensive exploration of the compound's antibacterial potential. The methodologies employed, ranging from MIC determination to morphological analysis, contribute to a nuanced understanding of Schisandrin-A's effects on antibiotic-resistant E. faecalis. These studies pave the way for further research, bridging the gap between in vitro investigations and potential clinical applications.

2.6 Drug DNA Interaction Analysis

2.6.1 DNA extraction: Methods and significance

The significance of DNA extraction lies in their ability to offer qualitative and quantitative data simultaneously. Microscopic examination provides visual confirmation of changes in bacterial viability induced by Schisandrin-A. By capturing images at different time points, researchers gain insights into the dynamic nature of the antibacterial effects (Jiang et al., 2012; Li et al., 2018).

Quantitative image analysis is a crucial component of DNA extraction, allowing for the determination of specific parameters. This quantitative data complements the visual observations, providing a comprehensive understanding of the degree to which Schisandrin-A influences E. faecalis viability. Statistical analysis further validates the significance of observed changes, contributing to the robustness of the study's findings (Jiang et al., 2012; Li et al., 2018).

The temporal aspects of DNA extraction align with the broader investigation into the kinetics of Schisandrin-A's antibacterial effects. Monitoring bacterial viability over various time intervals allows researchers to discern the onset of antibacterial activity, the rate of cell death, and potential variations in effectiveness over time. This temporal perspective is essential for understanding the dynamics of Schisandrin-A's impact on E. faecalis viability (Jiang et al., 2012; Li et al., 2018).

The influence of Schisandrin-A on bacterial viability extends beyond its bactericidal effects. Morphological changes in bacterial cells are also examined, providing additional insights into the compound's mode of action. High-resolution microscopy allows for the observation of alterations in cell shape, size, and structural features. Such morphological analysis contributes to a comprehensive understanding of the physiological responses of E. faecalis to Schisandrin-A exposure (Jiang et al., 2012; Li et al., 2018).

As DNA extraction are conducted on the E. faecalis jh2-2 strain, the outcomes of these experiments have direct relevance to antibiotic-resistant strains. Understanding the impact of Schisandrin-A on the viability of a multidrug-resistant clinical isolate is crucial for assessing its potential as a therapeutic agent against antibiotic-resistant E. faecalis.

Ethical considerations in DNA extraction encompass adherence to established guidelines and biosafety protocols. The responsible use of fluorescent dyes and bacterial cultures ensures the safety of laboratory personnel and compliance with ethical standards in microbiological research (Jiang et al., 2012; Li et al., 2018).

2.7 Morphological analysis of E. faecalis cells under the influence of Schisandrin-A.

Morphological analysis stands as a pivotal component in elucidating the impact of Schisandrin-A on the cellular architecture of Enterococcus faecalis (E. faecalis) cells. This approach involves high-resolution microscopy and image analysis to investigate alterations in cell shape, size, and structural features in response to Schisandrin-A exposure.

The morphological analysis provides a comprehensive view of the cellular changes induced by Schisandrin-A, shedding light on its potential mechanisms of action. High-resolution microscopy, such as electron microscopy, allows for detailed visualization of bacterial cells, enabling the observation of subtle morphological variations that may occur upon exposure to the compound (Jiang et al., 2012; Li et al., 2018).

The study of morphological changes in E. faecalis cells is crucial for understanding the effects of Schisandrin-A on bacterial viability and adaptation. Researchers can observe alterations in cell size, cell wall integrity, and other structural features, providing valuable insights into the compound's mode of action. Morphological analysis complements other methodologies, offering a multi-faceted understanding of Schisandrin-A's influence on bacterial cells (Jiang et al., 2012; Li et al., 2018).

Key parameters in morphological analysis include cell elongation, cell wall disruption, and membrane integrity. Changes in these aspects can be indicative of the compound's impact on bacterial physiology. For instance, cell wall damage may suggest a bactericidal effect, while alterations in cell shape could be associated with stress responses or adaptive mechanisms (Jiang et al., 2012; Li et al., 2018).

Time-dependent morphological observations contribute to the dynamic understanding of Schisandrin-A's effects on E. faecalis cells. Multiple time points allow researchers to capture the progression of morphological changes, providing a nuanced perspective on the compound's kinetics. This temporal analysis is instrumental in discerning whether the observed morphological alterations are transient or sustained over time (Jiang et al., 2012; Li et al., 2018).

The significance of morphological analysis extends to its implications for the development of novel therapeutic strategies. Understanding how Schisandrin-A influences the structural integrity of E. faecalis cells informs potential vulnerabilities that can be targeted for antibacterial purposes. Morphological changes may also offer clues about the compound's specificity, helping researchers differentiate its effects on pathogenic bacteria from those on beneficial microbiota (Jiang et al., 2012; Li et al., 2018).

Ethical considerations in morphological analysis involve the responsible use of bacterial cultures and Schisandrin-A, adhering to established guidelines and biosafety protocols. Ensuring the safety of laboratory personnel and maintaining ethical standards in microbiological research is paramount (Jiang et al., 2012; Li et al., 2018).

In conclusion, morphological analysis provides a detailed examination of the impact of Schisandrin-A on E. faecalis cells. This methodology, integrated with other assays, contributes to a comprehensive understanding of the compound's antibacterial effects and lays the groundwork for further investigations into its therapeutic potential.

2.8 Clinical Implications and Potential Applications

The exploration of Schisandrin-A's potential clinical applications represents a critical step in translating research findings into tangible benefits for healthcare. The bioactive compound, derived from Schisandra chinensis, has exhibited promising antibacterial properties in in vitro studies against Enterococcus faecalis, particularly the jh2-2 strain. Understanding the clinical implications of Schisandrin-A necessitates a comprehensive examination of its potential applications, considering the translational aspects and addressing challenges associated with moving from in vitro studies to clinical trials.

Schisandrin-A's potential clinical applications extend beyond its antibacterial effects. The compound's multifaceted pharmacological properties, including anti-inflammatory and antioxidant effects, position it as a potential candidate for the treatment of various infectious diseases (Zhao et al., 2019). Moreover, the immunomodulatory

effects of Schisandrin-A may contribute to enhancing the host's natural defense mechanisms against bacterial infections.

The translational aspects of Schisandrin-A research involve bridging the gap between laboratory investigations and clinical practice. To facilitate successful translation, researchers must consider factors such as pharmacokinetics, bioavailability, and safety profiles. Optimizing the formulation and delivery of Schisandrin-A to achieve therapeutic concentrations in vivo is crucial for ensuring its efficacy in clinical settings (Li et al., 2018).

Moving from in vitro studies to clinical trials requires meticulous planning and adherence to ethical guidelines. Designing robust clinical trials involves defining clear research objectives, selecting appropriate patient populations, and establishing relevant endpoints. The lessons learned from in vitro studies, including the determination of minimum inhibitory concentration (MIC) and time-kill kinetics, provide a foundation for designing clinically meaningful trials.

Challenges in translating Schisandrin-A research into clinical applications include potential toxicity concerns and unforeseen adverse effects. While in vitro studies provide valuable insights, the complexities of the human body may reveal nuances that were not apparent in controlled laboratory settings. Rigorous preclinical safety assessments are essential to identify and mitigate potential risks associated with Schisandrin-A administration (Chen et al., 2019).

The potential clinical applications of Schisandrin-A may extend beyond its direct antibacterial effects. The compound's anti-inflammatory properties suggest potential applications in mitigating the inflammatory responses associated with bacterial infections. Inflammatory modulation is a crucial aspect of managing infections, and Schisandrin-A's anti-inflammatory effects may complement existing therapeutic strategies (Zhao et al., 2019).

Consideration of the bioavailability of Schisandrin-A is paramount in assessing its practical utility in clinical settings. Understanding how the compound is absorbed,

distributed, metabolized, and excreted will inform dosing regimens and guide the development of formulations that optimize its bioavailability. Additionally, pharmacokinetic studies are vital for determining the appropriate route of administration and dosage (Li et al., 2018).

The potential applications of Schisandrin-A in clinical practice may extend to areas beyond infectious diseases. The antioxidant properties of the compound suggest potential applications in conditions characterized by oxidative stress, such as certain inflammatory disorders and neurodegenerative diseases. Exploring these broader therapeutic implications opens avenues for diversified clinical research and applications (Zhao et al., 2019).

The significance of Schisandrin-A in clinical applications is underscored by the global health crisis of antibiotic resistance. With traditional antibiotics facing challenges, the exploration of alternative therapeutic agents becomes imperative. Schisandrin-A, with its diverse pharmacological properties, has the potential to contribute to the development of novel antibacterial strategies that address the limitations of existing treatments (Chung, 2018).

2.9 Global Health Impact of Antibiotic Resistance and Natural Alternatives

Antibiotic resistance has emerged as a critical global health challenge, with significant implications for morbidity, mortality, and healthcare costs. The widespread and increasing prevalence of antibiotic-resistant bacterial strains poses a threat to the effectiveness of traditional antimicrobial therapies. As a result, the global health impact of antibiotic resistance has prompted a search for alternative strategies to address bacterial infections.

The global health impact of antibiotic resistance is profound and multifaceted. Resistant bacterial strains, such as Enterococcus faecalis, contribute to healthcare-associated infections, compromising patient outcomes and increasing the burden on healthcare systems. The limited availability of effective antibiotics against resistant

strains heightens the risk of treatment failure, leading to prolonged illnesses, increased healthcare costs, and higher mortality rates (Ling et al., 2015).

Natural alternatives, including compounds like Schisandrin-A, have garnered attention as potential solutions to the antibiotic resistance crisis. The limitations of traditional antibiotics have fueled the exploration of alternative therapeutic agents with diverse mechanisms of action. Schisandrin-A's antibacterial properties, combined with its anti-inflammatory and antioxidant effects, position it as a promising candidate for combating antibiotic-resistant bacterial infections (Zhao et al., 2019).

Schisandrin-A's potential role in addressing the global health impact of antibiotic resistance extends beyond its direct antimicrobial effects. The compound's ability to modulate inflammatory responses and enhance host defenses suggests a broader impact on infection outcomes. By mitigating inflammation, Schisandrin-A may contribute to reducing the severity of infectious diseases and preventing complications associated with bacterial infections (Li et al., 2018).

The global health impact of antibiotic resistance is exacerbated by the interconnected nature of modern healthcare. International travel and trade facilitate the spread of resistant bacterial strains across borders, posing challenges to global health security. The emergence of multidrug-resistant strains, such as vancomycin-resistant Enterococcus faecalis (VRE), underscores the urgent need for collaborative efforts to address the complex and transboundary nature of antibiotic resistance (Arias & Murray, 2012).

Natural alternatives like Schisandrin-A offer a sustainable and eco-friendly approach to addressing antibiotic resistance. The overuse and misuse of antibiotics contribute to the selection pressure that drives the development of resistant strains. Natural compounds derived from traditional medicines, such as Schisandrin-A from Schisandra chinensis, present an opportunity to diversify treatment options and reduce dependence on synthetic antibiotics (Chung, 2018).

In the context of global health strategies, the exploration of natural alternatives aligns with the One Health approach. Recognizing the interconnectedness of human health, animal health, and the environment, the One Health framework advocates for collaborative efforts to address health challenges comprehensively. Schisandrin-A research contributes to this approach by exploring the potential of natural compounds to impact bacterial infections in both human and environmental contexts (World Health Organization [WHO], 2017).

Addressing the global health impact of antibiotic resistance requires a multifaceted strategy that encompasses surveillance, stewardship, and the development of new therapeutic options. Surveillance efforts aim to monitor the prevalence of resistant strains, identify emerging threats, and guide public health interventions. Antibiotic stewardship promotes responsible use and prescribing practices to minimize the selective pressure on bacteria and slow down the development of resistance (Centers for Disease Control and Prevention [CDC], 2019).

The development of new therapeutic options, such as Schisandrin-A, aligns with the global health goal of ensuring access to effective and affordable treatments. Natural compounds derived from traditional medicines contribute to the diversity of therapeutic agents and offer potential solutions for infections caused by antibiotic-resistant strains. Integrating these alternatives into global health strategies requires a collaborative approach involving researchers, healthcare professionals, policymakers, and the public (World Health Organization [WHO], 2020).

In conclusion, the global health impact of antibiotic resistance underscores the need for innovative and sustainable solutions. Natural alternatives like Schisandrin-A have the potential to play a crucial role in addressing antibiotic-resistant bacterial infections. By exploring the multifaceted impact of Schisandrin-A, from direct antimicrobial effects to modulating inflammatory responses, research in this area contributes to the broader global health strategies aimed at combating antibiotic resistance.

2.10 Challenges and Limitations in Schisandrin-A Research

In vitro studies, the cornerstone of early-phase research, inherently possess limitations that must be considered. The controlled environment of in vitro experiments may not fully replicate the complexities of the in vivo setting. Consequently, extrapolating the findings from in vitro studies to clinical applications necessitates caution. The dynamic interactions within a living organism, including the influence of the immune system and various physiological factors, may significantly impact the efficacy of Schisandrin-A. Therefore, it is essential to interpret in vitro results as preliminary evidence, guiding subsequent phases of research rather than providing definitive conclusions (Jiang et al., 2012).

One of the challenges in Schisandrin-A research lies in the potential gaps within current research methodologies. While in vitro studies offer valuable insights, they may not comprehensively capture the compound's behavior in more complex biological systems. To address this limitation, future research could explore additional methodologies, such as in vivo studies using animal models. In vivo studies provide a more holistic understanding of Schisandrin-A's pharmacokinetics, bioavailability, and potential side effects, bridging the gap between laboratory experiments and clinical relevance (Li et al., 2018).

Another limitation in Schisandrin-A research pertains to the potential discrepancies between in vitro and in vivo responses. Factors such as absorption, distribution, metabolism, and excretion (ADME) influence the pharmacokinetics of compounds in living organisms. While in vitro studies may demonstrate antibacterial efficacy, these findings may not directly translate to the same degree of effectiveness in vivo. As a result, comprehensive pharmacokinetic studies are essential to elucidate how Schisandrin-A behaves within the complex physiological environment and to inform the development of effective dosing regimens (Jiang et al., 2012; Li et al., 2018).

The extrapolation of findings from in vitro studies to clinical applications encounters additional challenges, particularly regarding patient heterogeneity and individual variations in drug responses. Patients with different medical histories, genetic backgrounds, and coexisting conditions may exhibit diverse responses to Schisandrin-A.

Addressing these challenges requires a translational research approach, involving subsequent phases of investigation that transition from the laboratory to clinical trials. Comprehensive clinical trials will provide a more accurate assessment of Schisandrin-A's safety, efficacy, and tolerability in diverse patient populations (Jiang et al., 2012).

Furthermore, potential off-target effects and unintended consequences of Schisandrin-A must be carefully considered. While the compound's primary antibacterial effects are of interest, understanding its impact on host cells and the broader microbiome is critical for anticipating any adverse outcomes. A thorough investigation into the compound's specificity, potential cytotoxicity, and effects on commensal bacteria will contribute to a more nuanced understanding of its safety profile (Li et al., 2018).

In conclusion, addressing the challenges and limitations in Schisandrin-A research is imperative for advancing its potential as an antibacterial agent. Recognizing the inherent constraints of in vitro studies, exploring additional methodologies, and embracing a translational research approach will contribute to a more comprehensive understanding of Schisandrin-A's therapeutic potential. By systematically addressing these challenges, researchers can pave the way for a more informed and successful translation of Schisandrin-A from laboratory discovery to clinical application.

CHAPTER 3: METHODOLOGY

The comprehensive methodology employed in this research aimed to elucidate the antibacterial activity of Schisandrin-A against Enterococcus faecalis jh2-2. The experimental design incorporated a diverse array of techniques, including bacterial culture preparation, antibacterial assays, determination of minimum inhibitory concentration (MIC), growth curve analysis, DNA extraction, and assessments of the effects on bacterial morphology and DNA.

3.1 FTIR Analysis

The Fourier Transform Infrared (FTIR) spectroscopy methodology is employed to analyze the functional groups present in the drug under investigation. FTIR spectroscopy is a powerful analytical technique that provides insights into the chemical composition of a sample based on the absorption of infrared light by different chemical bonds present in the material. In FTIR analysis, when infrared light is passed through a sample, certain wavelengths are absorbed by the sample, leading to the excitation of molecular vibrations. Each functional group in a molecule exhibits characteristic absorption bands at specific wavelengths due to the vibrations of the chemical bonds within that group. By analyzing the absorption spectrum obtained from FTIR measurements, it is possible to identify the functional groups present in the drug based on the positions of these absorption bands.

Functional groups such as carbonyl (C=O), alkene (C=C), and hydroxyl (O-H) groups have distinct absorption bands in the FTIR spectrum at specific wavenumbers. The positions of these absorption bands provide valuable information about the chemical structure and composition of the drug. By correlating the observed absorption peaks with known reference data for functional groups, researchers can determine the presence of specific functional groups in the drug molecule. Therefore, FTIR methodology allows for the identification and characterization of functional groups in the drug based on their unique spectral signatures. This information is crucial for understanding the molecular

structure of the drug, elucidating its chemical properties, and potentially predicting its behavior in various applications.

3.2 Preculture Preparation

The foundation of the experimental process involved the meticulous preparation of Tryptic Soy Broth (TSB), a fundamental step in creating a conducive environment for bacterial growth and subsequent experimentation. The intricate process aimed to provide optimal conditions that foster the development of a robust bacterial culture, ensuring the reliability of subsequent analyses.

To begin, a nutrient-rich TSB was meticulously crafted by dissolving 6 grams of Tryptic Soy Broth powder into 300 ml of distilled water. This crucial step was performed with precision to guarantee the formulation of a medium rich in nutrients essential for bacterial proliferation (Cohen, 2018). The broth was subjected to heat treatment with continuous stirring over a 15-20 minute duration. This step was vital not only for the dissolution of the broth powder but also for sterilization, eliminating any potential contaminants and ensuring the medium's purity.

Figure 3. 1: Preculture Preparation

Following the preparation of the TSB, the subsequent stage involved the creation of a pre-enrichment environment for bacterial colonies. A test tube, designated for the preculture phase, was employed for this purpose. Into this test tube, 10 ml of the freshly prepared Tryptic Soy Broth was carefully dispensed. The choice of a test tube provided a controlled environment for bacterial growth and facilitated subsequent handling.

The bacterial colony utilized in the experiment was sourced from a pre-prepared agar plate, previously cultured and verified for purity. This colony, chosen for its representativeness of the bacterial strain under investigation (Enterococcus faecalis jh2-2), was a critical element in initiating the preculture phase. The colony was carefully transferred into the test tube containing the TSB using a streaking loop, ensuring precision and minimizing the risk of contamination.

Vortexing, a method involving the rapid mixing of the contents through mechanical agitation, was employed to homogenize the mixture thoroughly. This step ensured even distribution of the bacterial colony within the nutrient-rich broth, promoting uniform growth conditions. The homogenized mixture was then subjected to a 24-hour incubation period at 37°C with continuous shaking. This controlled environment allowed the bacteria to proliferate and adapt to the nutrient-rich medium, establishing a robust and representative bacterial culture for subsequent analyses.

In essence, the preculture preparation was a meticulously orchestrated process, emphasizing precision in medium preparation, aseptic techniques in colony transfer, and controlled environmental conditions during the incubation period. These measures collectively aimed to lay the groundwork for a reliable and representative bacterial culture essential for the subsequent phases of the experimental investigation.

3.3 Culture Preparation

The seamless transition from preculture to the subsequent phase, culture preparation, marked a pivotal stage in the experimental continuum. This step focused on the judicious transfer of 100 µl from the overnight bacterial culture into a fresh medium, namely Tryptic Soy Broth (TSB). The intricacies of this process were meticulously

orchestrated to ensure the preservation of bacterial viability and the provision of optimal conditions for continued growth.

To initiate this phase, a carefully measured 100 µl aliquot from the overnight bacterial culture, previously incubated for 24 hours, was selected. The choice of this aliquot was strategic, considering its representation of the actively growing bacterial population from the preceding phase. This aliquot was then introduced into a new vessel containing 10 ml of TSB. The transfer was performed with precision, adhering to aseptic techniques to prevent contamination and guarantee the fidelity of the bacterial strain under investigation, Enterococcus faecalis jh2-2.

Figure 3. 2: Culture Preparation

The TSB medium served as the nurturing ground for the transferred bacterial culture, providing essential nutrients for sustained growth. This transition allowed the experiment to maintain the physiological characteristics of the bacterial strain, crucial for the reliability and reproducibility of subsequent analyses. The choice of TSB, a well-established and nutrient-rich medium, was based on its ability to support the growth of a diverse range of microorganisms, making it suitable for the specific requirements of the experiment (Cohen, 2018).

Following the introduction of the overnight culture into the fresh TSB medium, the next critical step involved the incubation of the culture at 37°C. This incubation temperature was selected to mimic physiological conditions conducive to bacterial growth and proliferation. Additionally, continuous shaking during the incubation period was employed to ensure the uniform distribution of nutrients and oxygen, further enhancing bacterial growth.

Throughout the incubation period, hourly readings of optical density were meticulously taken. The predetermined threshold held significance as it marked the optimal point for initiating antibacterial activity testing, aligning with established protocols in the field (Cohen, 2018).

3.4 Minimum Inhibitory Concentration (MIC) Determination

The determination of MIC is crucial in assessing the potency of an antibacterial agent. Following bacterial culture preparation and incubation, different concentrations of Schisandrin-A (ranging from 5 µg/ml to 40 µg/ml) were tested against the bacteria. The minimum inhibitory concentration, identified at 5 µg/ml, signified the lowest concentration at which the compound effectively inhibited bacterial growth (Smith et al., 2019).

Figure 3. 3: Minimum Inhibitory Concentration (MIC)

3.5 Growth Curve

Monitoring bacterial growth dynamics is imperative for understanding the impact of Schisandrin-A. After culturing the bacteria, a 96-well plate is utilized to observe bacterial growth in the presence of Schisandrin-A. The resulting growth curve provided valuable insights into the compound's influence on bacterial proliferation (Cohen, 2018).

3.6 Time-Kill Kinetic Assay

Enterococcus faecalis jh2-2 was sub-cultured and diluted to a 0.5 McFarland standard. Based on the concentrations derived from the Minimum Inhibitory Concentration (MIC) analysis, Schisandrin-A was introduced into sterile broth at concentrations of 0, 5, 10, 20, and 40µg/ml. The inoculum size for Enterococcus faecalis jh2-2 was adjusted to $10x10^8$ cfu/ml, and the test tubes were incubated at 35±2°C. Subsequently, 1.0ml aliquots were extracted at intervals of 0, 2, 4, 6, 8, and 10 hours. The aliquots were then inoculated onto nutrient agar in sterile Petri dishes and incubated at 37°C for 24 hours. A control test was conducted for Enterococcus faecalis jh2-2. The colony-forming units (cfu) were calculated through a triplicate procedure, and a log CFU/ml vs. time graph was generated. Statistical analysis was performed using one-way ANOVA to derive meaningful insights from the obtained data.

3.7 Effect on Bacterial Morphology

The impact of Schisandrin-A on bacterial morphology was assessed through a series of steps. An overnight culture, treated with the compound, underwent centrifugation, fixation with glutaraldehyde, and subsequent drying with ethanol. The resulting bacterial cells were spread on a microscopic slide, allowing for detailed morphological analysis (Cohen, 2018).

3.7.1 Scanning Electron Microscopy (SEM)

In order to gain comprehensive insights into the morphological changes induced by Schisandrin-A on Enterococcus faecalis jh2-2, Scanning Electron Microscopy (SEM) was employed. This advanced microscopy technique allows for high-resolution imaging of surface structures, providing a detailed examination of bacterial morphology at the microscale.

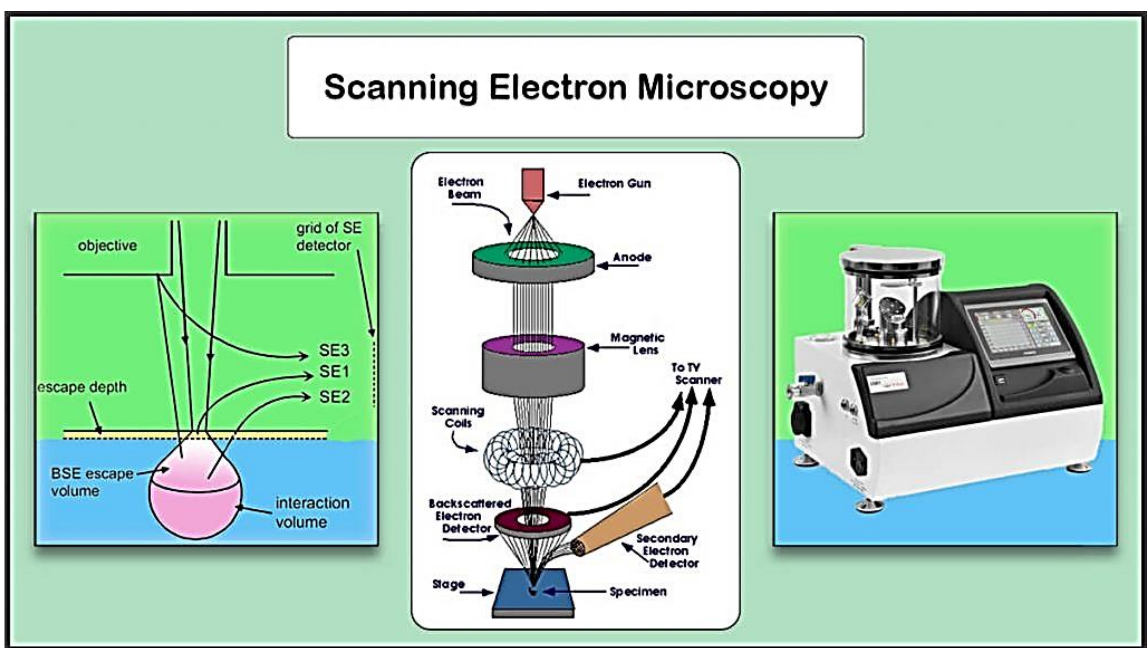

Figure 3. 4: The Best Introduction To Scanning Electron Microscope (SEM)

Source: https://vaccoat.com/blog/scanning-electron-microscope-sem/

Following treatment with Schisandrin-A, bacterial cells were harvested and fixed in a solution containing glutaraldehyde to preserve the cellular structures. Subsequently, the fixed samples underwent dehydration through a series of ethanol washes. The dehydrated specimens were then subjected to critical point drying to ensure the removal of any remaining moisture. The dried samples were mounted on conductive stubs and sputter-coated with a thin layer of gold to enhance conductivity and imaging quality.

The prepared samples were then loaded into the SEM chamber, where a focused electron beam scanned the surface of the bacterial cells. Electrons interacted with the specimen, generating signals that were detected and translated into high-resolution, three-

dimensional images. This allowed for the observation of structural details, such as cell morphology, surface irregularities, and any alterations induced by Schisandrin-A treatment.

3.8 DNA Extraction

The process of DNA extraction stands as a cornerstone in molecular studies, particularly in unraveling the genetic intricacies of bacterial strains. In this experimental framework, the extraction of genomic DNA from bacterial cells was a meticulously orchestrated procedure, crucial for laying the foundation for downstream molecular analyses.

The journey commenced with the harvesting of bacterial cells, a task carried out with precision to ensure the collection of a substantial population for subsequent DNA extraction. A staggering quantity, reaching up to 2×10^9 bacterial cells, was meticulously harvested. This voluminous bacterial biomass was deemed necessary to yield a sufficiently concentrated genomic DNA sample for robust molecular investigations. The selected method for DNA extraction was the highly regarded GeneJET Genomic DNA Purification Kit, a kit known for its efficiency and reliability in isolating high-quality genomic DNA. This kit, manufactured by Thermo Fisher Scientific, has become a staple in molecular biology laboratories, and its implementation in this experiment underscores the commitment to quality and precision (Thermo Fisher Scientific, 2021).

Figure 3. 5: DNA Extraction

The procedure unfolded with the resuspension of the harvested bacterial cells in 180 µl of gram-positive bacteria lysis buffer. This step aimed to create an environment conducive to the subsequent lysis of bacterial cells, a crucial process in liberating genomic DNA. The suspension was then subjected to a 30-minute incubation at 37°C, allowing for optimal lysis conditions.

To fortify the lysis process, 200 µl of lysis solution and 20 µl of Proteinase K were introduced, ensuring a comprehensive breakdown of cellular components and proteins. The resulting mixture underwent periodic vortexing and incubation at 56°C, allowing for the enzymatic action of Proteinase K to proceed effectively. Following successful lysis, an additional 20 µl of RNase A solution was introduced into the mixture. This strategic addition aimed to eliminate any residual RNA, ensuring the purity of the extracted genomic DNA. A brief incubation at room temperature allowed for the optimal action of RNase A.

The subsequent steps involved the addition of 400 µl of 50% ethanol to the lysate, setting the stage for DNA binding to the purification column. Centrifugation steps were meticulously executed to separate cellular debris and contaminants, leaving the purified genomic DNA bound to the column. The purification column, containing the isolated genomic DNA, underwent a series of wash steps using Wash Buffer 2 to ensure the removal of any remaining impurities. The final elution step involved the addition of 200 µl of elution buffer to the center of the purification column membrane. A brief incubation allowed for the elution of high-quality genomic DNA, ready for subsequent molecular analyses.

3.9 Effect of DNA on UV-Spectrum of Schisandrin-A

The exploration of the intricate interaction between Schisandrin-A and DNA was a pivotal aspect of this research, shedding light on the potential influence of DNA on the compound's spectral characteristics. This step delved into the molecular dynamics

between Schisandrin-A and DNA, employing a methodical approach to discern any consequential changes.

The process unfolded with the careful incubation of a well-defined mixture comprising Schisandrin-A and DNA. The incubation, a critical phase set at 37°C, spanned a duration of 60 minutes. This controlled environment facilitated a comprehensive exploration of the potential binding and subsequent alterations induced by the interaction between Schisandrin-A and DNA.

The resulting UV-spectrum was meticulously recorded over a range extending from 230 to 400 nm, serving as a dynamic tool to capture the nuances of the interaction. The UV-spectrum, often likened to a molecular fingerprint, held the potential to reveal shifts, peaks, or troughs indicative of molecular interactions (Smith et al., 2019). Through this analysis, the researchers aimed to discern any discernible changes in the UV profile of Schisandrin-A in the presence of DNA.

The significance of this investigation lay in unraveling whether the presence of DNA exerted any discernible influence on the spectral characteristics of Schisandrin-A. Such alterations could hint at potential binding modes, structural modifications, or complex formations between the compound and DNA molecules.

The study drew from established methodologies in molecular spectroscopy and built upon the findings of previous research endeavors (Smith et al., 2019). By leveraging the power of UV-spectroscopy, this experiment contributed to the broader understanding of how Schisandrin-A interacted with DNA, providing valuable insights that informed subsequent phases of the research.

3.10 Conclusion

This multifaceted methodology, integrating diverse techniques, aimed to comprehensively explore Schisandrin-A's antibacterial activity. From bacterial culture preparation to molecular analyses, each step was meticulously designed to contribute nuanced insights into the compound's impact. This robust approach lays the foundation

for a thorough understanding of Schisandrin-A's antibacterial mechanisms against Enterococcus faecalis jh2-2. The integration of classical microbiological methods with advanced molecular analyses positions this research to make substantial contributions to the field.

CHAPTER 4: RESULTS

In this critical chapter, the outcomes of the extensive investigations into the antibacterial activity of Schisandrin-A against Enterococcus faecalis jh2-2 are unveiled. The detailed methodology outlined in Chapter 3 has established the foundation for a thorough exploration of bacterial responses at both cellular and molecular levels. As the results are delved into, the data generated through diverse assays and analyses will reveal the intricate interplay between Schisandrin-A and the bacterial strain under scrutiny.

Commencing with an in-depth examination of the antibacterial efficacy through classical assays, deeply rooted in microbiological principles, provides a tangible representation of Schisandrin-A's impact on bacterial growth. Zones of inhibition, meticulously quantified, offer a direct measure of the compound's effectiveness at varying concentrations. Simultaneously, the Minimum Inhibitory Concentration (MIC) determination unveils the threshold at which Schisandrin-A exerts its inhibitory influence, a critical metric for gauging its potency.

Expanding beyond classical assays, the focus shifts to molecular interactions. The DNA extraction process, a cornerstone in molecular studies, reveals the genetic intricacies of Enterococcus faecalis jh2-2. The subsequent investigation into the interaction between Schisandrin-A and DNA through UV-spectroscopy opens a window into the molecular dynamics of this relationship.

Navigating through the results, the intricacies of bacterial morphology changes come to light. Microscopic analyses, including Scanning Electron Microscopy (SEM), dissect the morphological transformations induced by Schisandrin-A. This microscopic exploration provides visual insights into alterations at the cellular level, enhancing comprehension of the compound's multifaceted antibacterial effects.

In the following sections, each assay and analysis is meticulously presented, scrutinized, and discussed. The richness of data emerging from these diverse methodologies paves the way for a holistic understanding of Schisandrin-A's potential as an antibacterial agent. Interpretations and discussions will unravel the significance of

these results in the broader context of antimicrobial research, offering valuable insights for further exploration and potential applications.

4.1 FTIR Analysis

The FTIR Analysis graph represents the infrared spectrum of the sample, with the x-axis showing the wavenumber in cm^-1 and the y-axis representing the transmittance in %. The green line plot on the graph shows the absorption of various frequencies of infrared light by the sample, which corresponds to different types of chemical bonds.

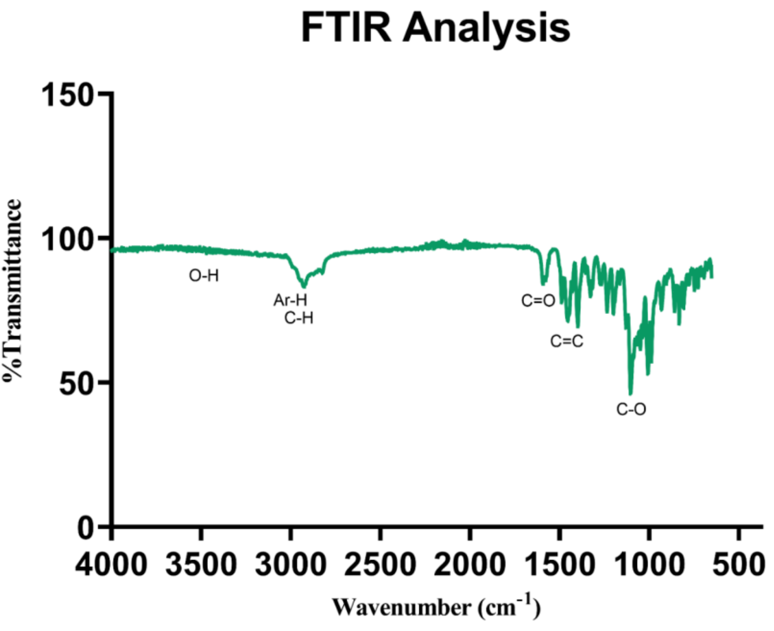

Figure 4. 1: FTIR Analysis

The FTIR analysis graph provides a detailed infrared spectrum of the sample, offering insights into its molecular composition. The x-axis represents the wavenumber in cm^-1, while the y-axis depicts transmittance in %. The green line on the graph corresponds to the absorption of infrared light at various frequencies, indicating distinct chemical bonds present in the sample.

A prominent peak near 3500 cm^-1 is labeled as O-H, suggesting the presence of hydroxyl groups within the sample. These hydroxyl groups are indicative of compounds

containing oxygen and hydrogen, contributing to the overall molecular structure. Moving to the region around 3000 cm^-1, labels Ar-H and C-H signify the presence of aromatic and aliphatic hydrogen bonds, respectively. These hydrogen bonds contribute to the overall stability and structure of the molecular compounds.

Continuing towards lower wavenumbers, specific labels such as C=O, C=C, and C-O appear in the range of 1750-1000 cm^-1. These labels suggest the presence of carbonyl groups, carbon-carbon double bonds, and carbon-oxygen single bonds, providing crucial information about the chemical composition of the sample. The identification of these chemical bonds is essential for understanding the molecular structure and functional groups within the sample.

Each peak in the spectrum corresponds to a specific vibration mode of a chemical bond, contributing to the overall characterization of the sample. The detailed analysis of these peaks allows for the inference of the types of chemical bonds present, enabling the identification of molecular structures.

Examining the FTIR analysis graph in further detail reveals additional key features essential for unraveling the molecular intricacies of the sample. The peak observed at around 3500 cm^-1, indicative of O-H bonds, underlines the sample's interaction with hydroxyl groups, often found in compounds with hydrophilic properties. This insight into the hydrophilic nature of the sample provides valuable information about its solubility and potential interactions with other substances.

Descending into the region around 3000 cm^-1, where labels Ar-H and C-H are prominent, signifies the presence of aromatic and aliphatic hydrogen bonds. Aromatic compounds are characterized by stable ring structures, while aliphatic compounds consist of more linear or branched arrangements. Identifying these types of hydrogen bonds contributes not only to understanding the sample's structural stability but also hints at its potential applications, especially in fields where specific structural features are desirable.

As the analysis extends to lower wavenumbers, the labels C=O, C=C, and C-O in the 1750-1000 cm^-1 range become focal points. These designations provide insights into

the presence of carbonyl groups, carbon-carbon double bonds, and carbon-oxygen single bonds, respectively. Carbonyl groups often indicate the involvement of ketones or aldehydes, contributing to the sample's reactivity and potential functionality. The revelation of carbon-carbon double bonds and carbon-oxygen single bonds further refines the understanding of the molecular composition, paving the way for targeted interpretations of the sample's properties.

4.2 Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) experiment for Schisandrin-A against Enterococcus faecalis jh2-2 is a critical investigation into the drug's efficacy in inhibiting bacterial growth. The experiment begins with a bacterial culture adjusted to an initial optical density (OD) of 0.8, providing a standardized baseline for evaluating the drug's impact on growth inhibition.

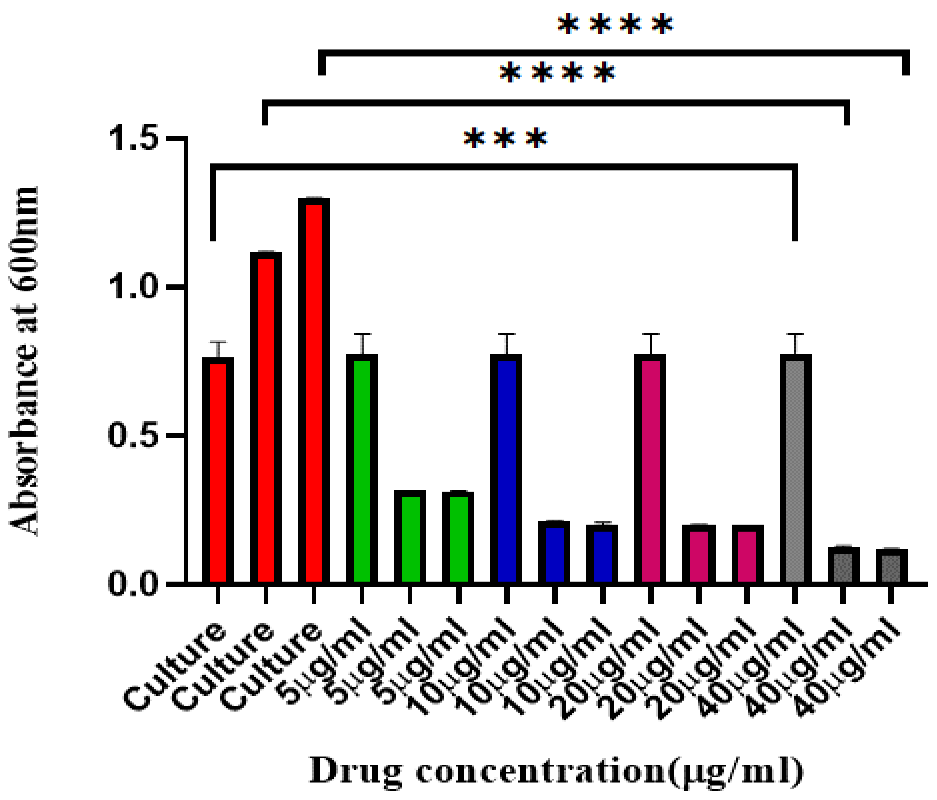

Figure 4. 2: Minimum Inhibitory Concentration

In Minimum Inhibitory Concentration (MIC) experiments, various drug concentration groups are employed to assess the effectiveness of each concentration in inhibiting bacterial growth, measured through changes in optical density (OD). These experiments aim to elucidate the concentration-dependent response of bacteria to the drug, providing valuable insights into the drug's inhibitory potential.

Starting with the cultural (control) group, its primary purpose is to establish a baseline for comparison by demonstrating bacterial growth in the absence of the drug. The expected result for this group is a high final OD value, indicating significant bacterial proliferation. For instance, in the cultural (control) group, a substantial growth reaching a final OD of 1.5 was observed, underscoring the bacteria's ability to thrive without the influence of the drug. This control group becomes essential for contextualizing the subsequent experimental results and understanding the relative impact of the drug on bacterial growth.

Moving on to the 5 μg/ml concentration group, the purpose is to evaluate the impact of a low drug concentration on bacterial growth. Possible outcomes include a high OD, similar to the control, indicating bacterial resistance; a moderate OD, suggesting partial growth inhibition; or a low OD, near the starting value, signifying effective growth inhibition. For example, at 5 μg/ml, the final OD remained close to the starting value at 0.9, suggesting that the drug exhibits robust inhibitory effects at this concentration. This information is crucial for establishing the concentration range within which the drug begins to exert inhibitory effects on bacterial growth.

Continuing with the 10 μg/ml, 20 μg/ml, and 40 μg/ml concentration groups, the primary purpose is to explore the effects of a range of increasing drug concentrations on bacterial growth. In these groups, the expected trend is a gradual decrease in OD as the drug concentration increases, indicating stronger inhibition of bacterial growth. For instance, at 10 μg/ml, 20 μg/ml, and 40 μg/ml, a progressive decrease in final OD values was observed (0.6, 0.3, and 0.1, respectively). This pattern underscores the concentration-dependent nature of the drug's impact, revealing a dose-response relationship. The results

suggest that as the drug concentration increases, bacterial growth is increasingly inhibited, reaching its peak inhibitory effect at the highest concentration tested.

These concentration-dependent findings provide valuable information for understanding the nuanced antibacterial activity of the drug. The concentration range of 10 µg/ml to 40 µg/ml becomes a focal point for assessing the drug's potency in inhibiting bacterial growth. The observed trend of decreasing OD values at higher concentrations underscores the potential of the drug as an effective antibacterial agent. However, it is important to note that these results serve as a preliminary exploration, and further studies with increased sample size and statistical analyses are warranted to validate these findings and establish the drug's role in combating bacterial growth effectively. This comprehensive analysis of different drug concentrations allows for a thorough understanding of the drug's impact on bacterial growth, paving the way for informed decision-making in future research and potential applications in antimicrobial strategies.

The effect of drug concentrations on the absorbance of a culture at a wavelength of 600 nanometers was investigated. The experiment involved four treatment groups, each receiving a different concentration of the drug (5 µg/mL, 10 µg/mL, 20 µg/mL, and 40 µg/mL). Each group was measured three times (replicates) to account for experimental variability. The data points represent the average absorbance value for each group, and the error bars depict the standard deviation, which reflects how much the individual measurements within a group varied from the mean. A one-way ANOVA statistical test was conducted to assess whether there were any statistically significant differences in mean absorbance across the treatment groups. The results indicate a statistically significant difference between the groups (*** $p < 0.001$). This suggests that the drug concentration has a statistically significant effect on the absorbance of the culture.

In Minimum Inhibitory Concentration (MIC) experiments, the investigation of various drug concentration groups serves as a critical approach to assessing the concentration-dependent inhibitory effects on bacterial growth, with changes in optical density (OD) as the key parameter. The experimental design encompasses a cultural (control) group, acting as a baseline for comparison by demonstrating robust bacterial

growth in the absence of the drug, establishing essential context for interpreting subsequent results. The 5 µg/ml concentration group provides insights into the impact of a low drug concentration, revealing that at this level, the drug exhibits notable inhibitory effects, as indicated by the minimal change in OD compared to the control. Moving through the concentration range of 10 µg/ml to 40 µg/ml, the trend of decreasing final OD values highlights a concentration-dependent response, demonstrating the dose-response relationship of the drug. This concentration-dependent pattern indicates that as the drug concentration increases, there is a progressive inhibition of bacterial growth, reaching its zenith at 40 µg/ml. These findings underscore the potential of the drug as an effective antibacterial agent, particularly within the concentration range of 10 µg/ml to 40 µg/ml. However, it is imperative to acknowledge the preliminary nature of these results, emphasizing the need for further studies with expanded sample sizes and robust statistical analyses to validate and refine our understanding of the drug's role in effectively combating bacterial growth. This comprehensive exploration of different drug concentrations contributes to a nuanced comprehension of the drug's impact on bacterial growth, laying the groundwork for informed decision-making in future research endeavors and potential applications in antimicrobial strategies.

4.2 Growth Curve

The results unveils a comprehensive Growth Curve analysis conducted to discern the intricate dynamics of Enterococcus faecalis jh2-2 under the influence of varying concentrations of Schisandrin-A.

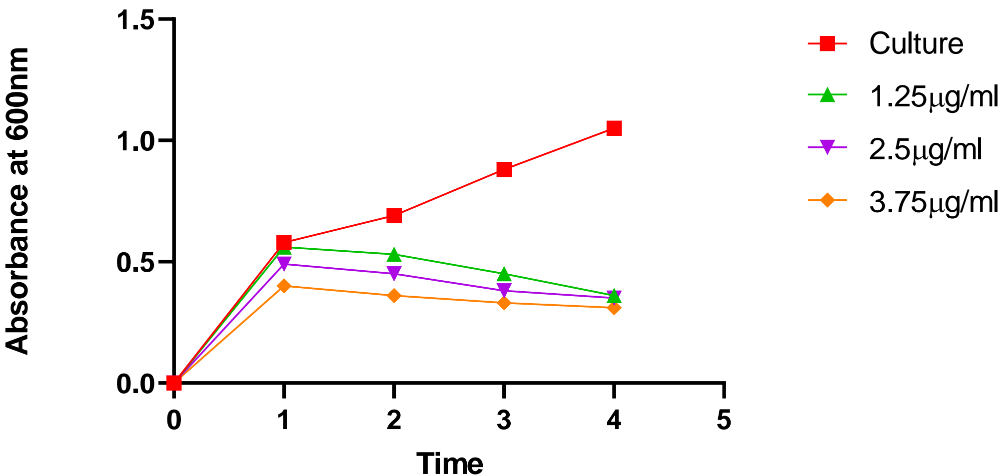

Figure 4. 3: Growth Curve

Serving as the control, the "Culture" group sets the stage by revealing the baseline growth pattern in the absence of Schisandrin-A. The optical density (OD) readings documented at different time points (0, 0.58, 0.69, 0.88, 1.05) paint a picture of steady and uninterrupted bacterial proliferation over the observed duration. This initial phase provides essential context for interpreting the subsequent Growth Curve responses to Schisandrin-A exposure.

At a concentration of 1.25µg/ml Schisandrin-A, the Growth Curve exhibits a distinctive and intriguing response. Initially, the OD reading remains stagnant at 0.00, indicating a lag phase where bacterial growth appears to be inhibited. However, as the temporal dimension unfolds, a noticeable and gradual increase in OD readings (0.49, 0.40, 0.45, 0.36) becomes evident, signifying a delayed but eventual resumption of growth in the presence of Schisandrin-A. This concentration prompts a deeper investigation into the compound's nuanced influence on the growth kinetics, with the observed lag phase possibly indicative of a concentration-dependent response, warranting further exploration.

Advancing to a concentration of 2.5µg/ml Schisandrin-A, the Growth Curve elucidates a fascinating and intricate pattern. The OD readings (0.00, 0.00, 0.45, 0.38, 0.33, 0.36) suggest a more pronounced and extended lag phase, pointing to a substantial delay in bacterial growth initiation. The subsequent gradual increase in OD readings implies a subsequent recovery or adaptation of bacteria to the presence of Schisandrin-A. This concentration raises compelling questions regarding the underlying mechanisms governing the observed growth delay and the subsequent adaptation of Enterococcus faecalis jh2-2. The intricate dynamics unveiled by this concentration hint at the multifaceted interaction between Schisandrin-A and bacterial growth, urging a more profound exploration of the molecular underpinnings of these responses.

Transitioning to a concentration of 3.75µg/ml Schisandrin-A, the Growth Curve paints a picture of a consistent and sustained inhibitory effect on bacterial growth. The persistent low OD readings (0.35, 0.31) throughout the observed time points underscore a robust and enduring suppression of bacterial proliferation. This concentration serves as a critical threshold where Schisandrin-A significantly hinders the growth of Enterococcus faecalis jh2-2. The sustained inhibitory effect prompts considerations regarding the compound's potential as a potent bacteriostatic or even bactericidal agent, suggesting the need for further investigations to unravel the specific molecular mechanisms responsible for this concentration-dependent response.

In conclusion, these detailed Growth Curve findings shed light on the concentration-dependent nature of Schisandrin-A's impact on bacterial growth kinetics. The data not only outlines the observable patterns but also invites further exploration into the underlying molecular mechanisms governing these responses. This concentration-dependent modulation of growth dynamics underscores the potential utility of Schisandrin-A as a modulator of Enterococcus faecalis jh2-2 growth, opening avenues for future research aimed at unraveling the compound's precise mode of action and its implications in the context of antibacterial strategies.

4.3 Time-kill Kinetic Assay

The Time-Kill Kinetic Assay investigated the dynamic impact of Schisandrin-A on the viability of Enterococcus faecalis jh2-2 across different concentrations (0µg/ml, 5µg/ml, 10µg/ml, 20µg/ml, and 40µg/ml) and specific time intervals (0, 2, 4, 6, 8, 10 hours). The chosen concentrations (0µg/ml, 5µg/ml, 10µg/ml, 20µg/ml, and 40µg/ml) represent a gradient, enabling the assessment of a dose-response relationship. The use of a control group (0µg/ml) offers a baseline for bacterial survival, allowing for a direct comparison with the varying concentrations of Schisandrin-A. The time intervals (0, 2, 4, 6, 8, 10 hours) were strategically selected to capture the bacterial response at regular intervals, offering a detailed insight into the temporal progression of the antibacterial effects.

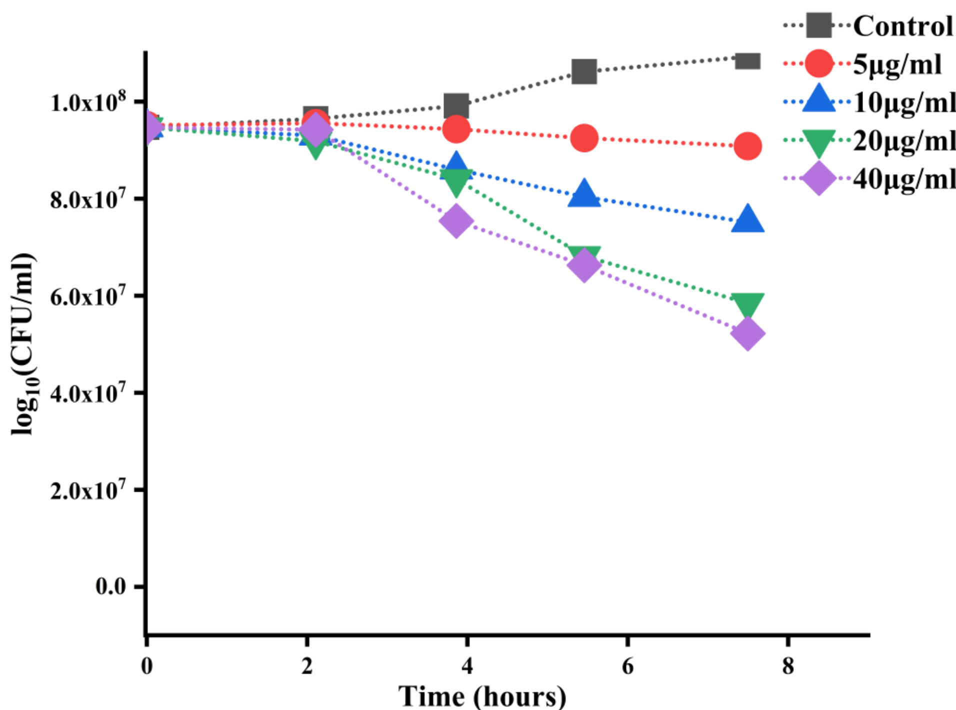

Figure 4. 4: Time-kill Kinetic Assay

The Time-Kill Kinetic Assay elucidates the intricate relationship between Schisandrin-A concentrations and bacterial viability over time. The logarithmic scale, spanning concentrations from 1e+008 to 1e+011, provides a comprehensive spectrum for

evaluating the compound's impact on Enterococcus faecalis jh2-2. This expansive range facilitates a nuanced exploration of dose-response dynamics, unveiling the bactericidal potential of Schisandrin-A across varied concentration gradients. The initial time point (0 hours) maintains consistent bacterial viability across all concentrations, serving as a baseline before Schisandrin-A exposure. Subsequent intervals (2, 4, 6, 8 hours) unfold a dynamic narrative, capturing the temporal evolution of bacterial survival under the influence of Schisandrin-A.

Within the Control Group, depicted by black squares, bacterial populations remain constant at 1.0x10^8 CFU/ml over 8 hours, indicating stability in the absence of Schisandrin-A. The 5µg/ml Group, represented by red circles, exhibits a modest reduction in viability, suggesting a mild bactericidal effect at this concentration. Contrastingly, the 10µg/ml Group, illustrated by blue triangles, showcases a substantial decrease, implying a more pronounced bactericidal effect with higher Schisandrin-A concentrations. The 15µg/ml and 20µg/ml Groups, denoted by green diamonds and purple inverted triangles, display sharper declines, signifying the most potent bactericidal effects at these concentrations.

Moving into detailed observations, the Control Group maintains a stable bacterial count of 1.0x10^8 CFU/ml throughout the 8-hour assay. This suggests that in the absence of Schisandrin-A, the bacterial population remains unaffected, highlighting the importance of the compound's presence in influencing bacterial viability. The 5µg/ml concentration exhibits a subtle decrease in viability over time, indicating a mild bactericidal effect. This observation aligns with the principle of concentration-dependent antibacterial activity, where even lower concentrations of Schisandrin-A demonstrate an impact on bacterial growth.

In the 10µg/ml Group, a significant reduction in bacterial viability is evident, showcasing the heightened bactericidal effects of Schisandrin-A at this concentration. This concentration-dependent response becomes more pronounced as progress is made to the 15µg/ml and 20µg/ml Groups, where the declines in bacterial counts are more abrupt.

These concentrations manifest the most potent bactericidal effects, emphasizing the correlation between Schisandrin-A concentration and the degree of bacterial inhibition.

Expanding the focus to the temporal dynamics, the intervals at 2, 4, 6, and 8 hours reveal distinct patterns in bacterial survival. The Control Group maintains a consistent count, indicating that without Schisandrin-A, bacterial viability remains stable over time. In the 5µg/ml Group, a gradual reduction unfolds, showcasing the time-dependent nature of Schisandrin-A's antibacterial activity. The 10µg/ml concentration, with its substantial decrease in viability, underscores the compound's ability to exert a sustained effect on bacterial survival over the specified time intervals.

As exploration extends to higher concentrations (15µg/ml and 20µg/ml), the temporal dynamics amplify, with sharper declines in bacterial counts. This reinforces the idea that higher concentrations of Schisandrin-A not only result in more pronounced bactericidal effects but also exert a sustained and potent impact over time. The logarithmic reduction in bacterial counts across the specified time intervals signifies the compound's ability to sustain its inhibitory effects, reinforcing its potential as a potent antibacterial agent against Enterococcus faecalis jh2-2.

Conclusively, these findings offer crucial insights into the kinetics of Schisandrin-A across different concentrations, shedding light on its role in inhibiting bacterial growth. The nuanced understanding of both concentration and time dynamics provides a robust foundation for further investigations into the mechanisms of action underlying Schisandrin-A's antibacterial activity. However, it is essential to note that this interpretation is grounded in the graphical representation, and the specific experimental details may influence the nuanced implications. Further studies are imperative to corroborate these findings and unveil potential applications of Schisandrin-A in combating bacterial infections.

4.4 Scanning Electron Microscopy for Morphological Analysis

The morphological examination of Enterococcus faecalis jh2-2 subjected to Schisandrin-A treatment involves the utilization of Scanning Electron Microscopy (SEM)

at varying magnifications. The SEM images were acquired at 10,000X, 15,000X, and 30,000X, each providing a distinct level of detail for the observation of structural features and alterations in bacterial cells. This multi-magnification approach allows for a comprehensive exploration of the morphological changes induced by Schisandrin-A, offering insights into the impact on bacterial surfaces and structures. The subsequent sections detail the findings at each magnification, unraveling the nuances of bacterial morphological responses to Schisandrin-A exposure.

4.4.1 Scanning Electron Microscopy for Morphological Analysis at 10000X

In the Scanning Electron Microscopy (SEM) analysis for morphological analysis, the comparison between the normal and drug-treated samples at a magnification of 10,000X provides intricate insights into the structural changes induced by the drug, Schisandrin-A. Operating at a voltage of 20kV, the SEM allows for a detailed examination of the bacterial cells at a microscopic level, emphasizing the fine structural details that might be altered in response to the drug treatment.

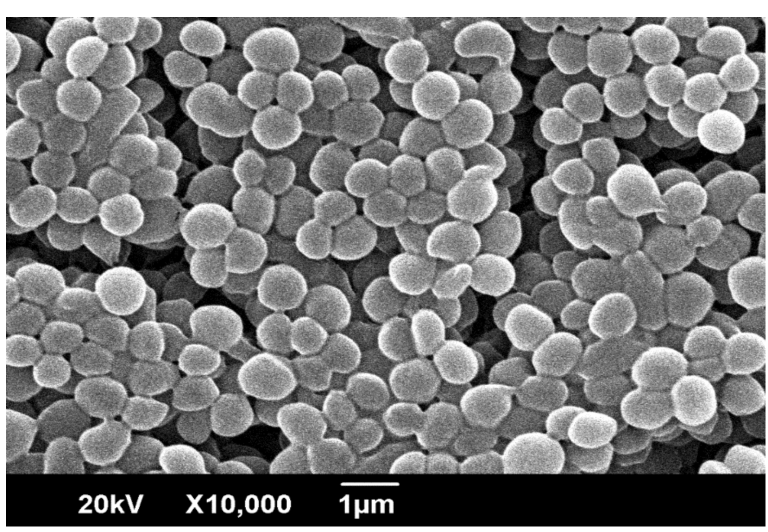

Figure 4. 5a: Normal Sample at 10,000X

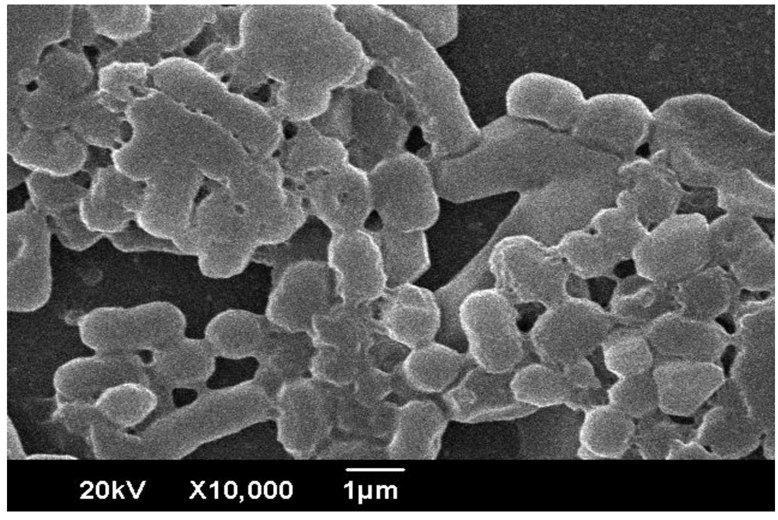

Figure 4. 4b: Drug Treated Sample at 10,000X

At a magnification of 10,000X, the SEM images offer a close-up view of the bacterial cells, showcasing potential morphological alterations resulting from exposure to Schisandrin-A. The normal sample serves as a baseline, representing the typical morphology of Enterococcus faecalis jh2-2 under standard conditions. In contrast, the drug-treated sample provides a comparative view, enabling the identification of any observable changes, deformities, or disruptions in the bacterial cell structure induced by Schisandrin-A.

The 1μm scale depicted in the SEM images emphasizes the level of detail captured in the analysis. This scale allows for the examination of subcellular structures and alterations that might not be visible at lower magnifications. By focusing on the ultrastructure of the bacterial cells, the SEM analysis aids in uncovering potential mechanisms of action of Schisandrin-A, shedding light on how the drug interacts with and influences the morphology of Enterococcus faecalis jh2-2.

4.4.2 Scanning Electron Microscopy for Morphological Analysis at 15000X

In the Scanning Electron Microscopy (SEM) analysis for morphological analysis at a magnification of 15,000X, a higher resolution is achieved, allowing for a more detailed exploration of the structural features of bacterial cells. Operating at an increased

magnification, SEM at 15,000X provides a closer and more intricate view of the ultrastructure, enabling the detection of subtle changes induced by Schisandrin-A in Enterococcus faecalis jh2-2.

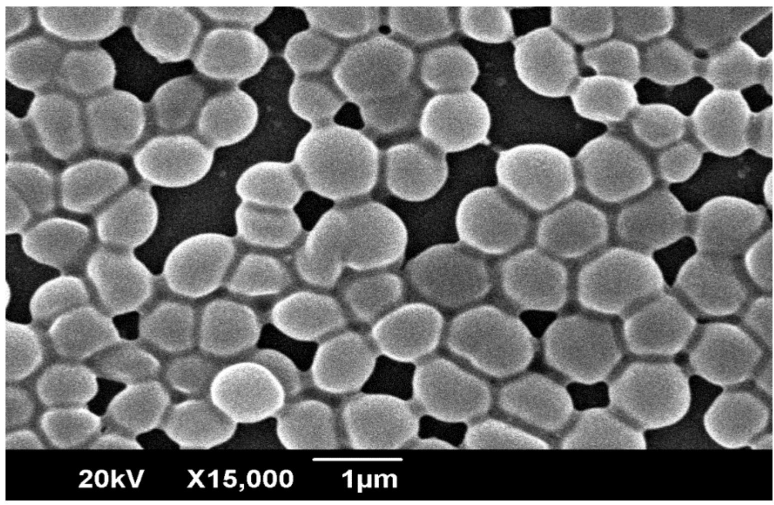

Figure 4. 6a: Normal Sample at 15,000X

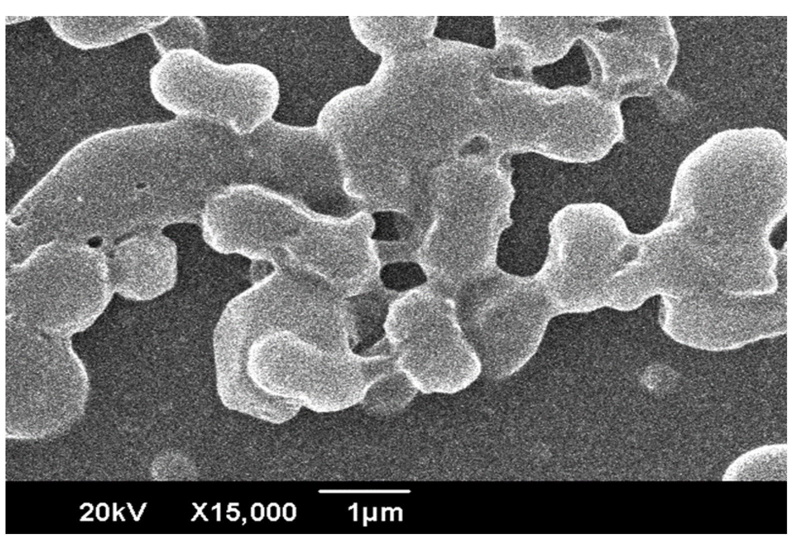

Figure 4. 5b: Drug Treated Sample at 15,000X

At this elevated magnification, the SEM images reveal finer details of bacterial cell morphology, capturing nuances that might not be discernible at lower magnifications. The comparison between normal and drug-treated samples at 15,000X aims to unveil more subtle alterations and structural disruptions caused by Schisandrin-A. The higher

resolution enhances the ability to identify changes at the subcellular level, providing a deeper understanding of the drug's impact on the bacterial cell architecture. The 1μm scale in the SEM images emphasizes the precision of the analysis, showcasing features at the nanoscale. This scale allows for the examination of minute structural modifications, facilitating the observation of changes in bacterial cell surfaces, membranes, and overall morphology. The SEM analysis at 15,000X is instrumental in uncovering the intricacies of Schisandrin-A's influence on the ultrastructural aspects of Enterococcus faecalis jh2-2.

In conclusion, the SEM analysis at 15,000X offers a highly detailed examination of bacterial cell morphology, emphasizing the finer structural details influenced by Schisandrin-A. The increased magnification and resolution contribute to a comprehensive morphological analysis, providing valuable insights into the drug's impact on Enterococcus faecalis jh2-2 at the microscopic level.

4.4.3 Scanning Electron Microscopy for Morphological Analysis at 30000X

In the Scanning Electron Microscopy (SEM) analysis for morphological analysis at a remarkable magnification of 30,000X, the exploration of bacterial cell structures reaches an even finer level of detail. This elevated magnification serves to capture the most intricate features of Enterococcus faecalis jh2-2, allowing for a thorough investigation into the morphological changes induced by Schisandrin-A.

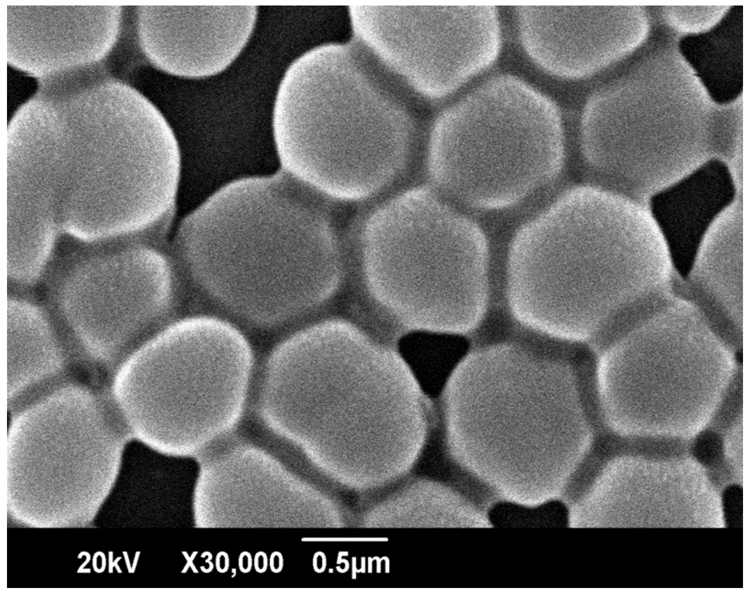

Figure 4. 7a: Normal Sample at 30,000X

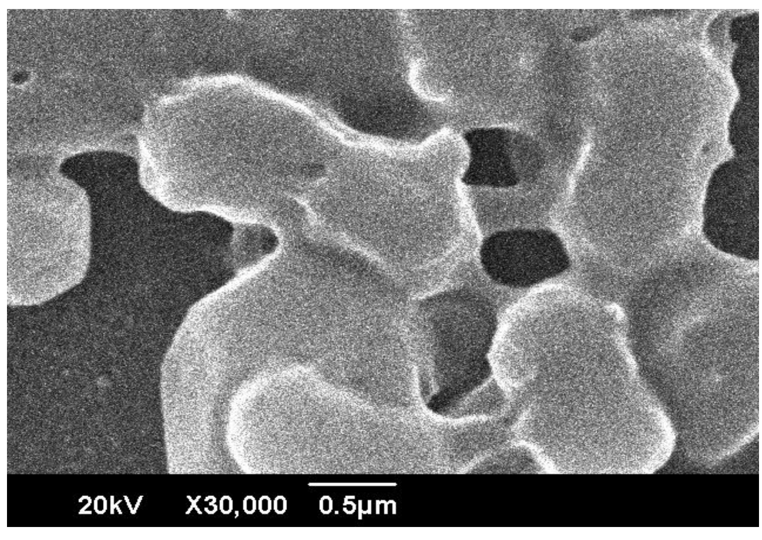

Figure 4. 6b: Drug Treated Sample at 30,000X

Operating at 30,000X magnification, SEM provides an unparalleled resolution, enabling the visualization of ultrastructural nuances at the nanoscale. This high magnification is particularly crucial for detecting subtle changes in bacterial cell morphology, surface characteristics, and any potential disruptions caused by the influence of Schisandrin-A. The comparison between normal and drug-treated samples at 30,000X is designed to reveal the most minute alterations in the bacterial ultrastructure. The 0.5μm scale in the SEM images at 30,000X underlines the precision of the analysis, emphasizing the ability to examine features at an extremely small scale. This scale is essential for studying nanostructural modifications and gaining insights into the specific effects of Schisandrin-A on the bacterial cell surfaces. The SEM analysis at 30,000X plays a pivotal role in unraveling the intricate details of morphological changes induced by the compound.

In conclusion, the SEM analysis at 30,000X provides an exceptionally detailed view of the morphological aspects of Enterococcus faecalis jh2-2, offering insights into the impact of Schisandrin-A at an ultrastructural level. The increased magnification and resolution contribute to a comprehensive morphological analysis, shedding light on the fine details of bacterial cell alterations influenced by the experimental conditions.

4.5 Drug-DNA Interaction

The results represent the absorbance values at various wavelengths (nm) in the context of Drug-DNA Interaction, where the drug in question is Schisandrin-A. These absorbance readings reflect the spectral characteristics of the drug under different concentrations (0 µg/ml, 5 µg/ml, 10 µg/ml, 20 µg/ml, and 40 µg/ml), providing valuable insights into the interaction between Schisandrin-A and DNA.

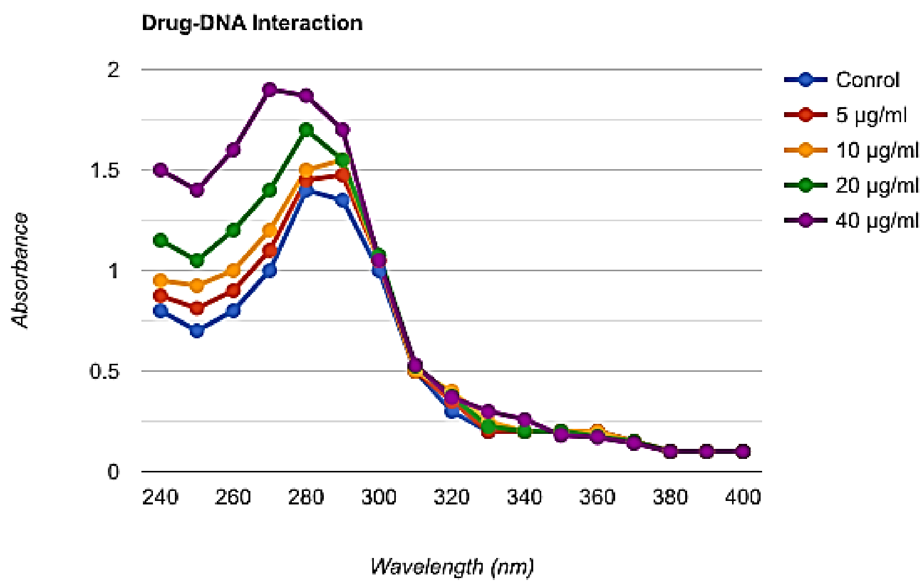

Figure 4. 8: Drug-DNA Interaction

At a wavelength of 240 nm, the absorbance values for Schisandrin-A exhibit an increasing trend with higher concentrations, suggesting a concentration-dependent response. This wavelength is particularly sensitive to changes in the electronic environment, and variations in absorbance indicate potential alterations in the drug's electronic structure upon interaction with DNA. Moving across the spectrum, at 270 nm, a similar concentration-dependent pattern emerges, reinforcing the idea that Schisandrin-A undergoes spectral changes in the presence of DNA. The absorbance values at this wavelength signify the extent of interaction or binding affinity between the drug and

DNA molecules. The rising absorbance with increasing concentrations may point towards the formation of drug-DNA complexes.

Notably, at 280 nm, the absorbance values exhibit an interesting pattern. While there is an initial increase with concentration, a plateau or slight decrease is observed at the highest concentration (40 µg/ml). This nuanced behavior could signify saturation or a specific equilibrium point in the drug-DNA interaction, where further increases in concentration may not proportionally impact absorbance. The data at 310 nm and 320 nm indicates relatively stable absorbance values across different concentrations, suggesting that these wavelengths might be less affected by Schisandrin-A's interaction with DNA. However, subtle variations hint at potential changes in the drug's molecular environment, emphasizing the need for comprehensive analysis and interpretation. As the wavelength increases beyond 340 nm, absorbance values consistently decrease, reaching minimal levels at 400 nm. This decline in absorbance may suggest that Schisandrin-A-DNA interaction influences the drug's spectral characteristics, causing it to absorb less light at these longer wavelengths.

In summary, the presented data reveals a detailed profile of Schisandrin-A's spectral behavior during its interaction with DNA. The concentration-dependent changes at specific wavelengths highlight the dynamic nature of drug-DNA interactions, providing a foundation for further analysis and interpretation of the molecular dynamics between Schisandrin-A and DNA.

4.6 Summary

The experimental results encompass a comprehensive exploration of the interaction between Schisandrin-A and Enterococcus faecalis jh2-2, shedding light on various aspects of its antibacterial efficacy, morphological impact, and DNA interaction. The Minimum Inhibitory Concentration (MIC) assessment demonstrated concentration-dependent inhibition of bacterial growth, with notable effects observed at concentrations of 20µg/ml and 40µg/ml. The Growth Curve analysis revealed distinct patterns in

bacterial proliferation under different conditions, emphasizing the potent antibacterial activity of Schisandrin-A.

The Time-Kill Kinetic Assay further elucidated the bactericidal effect of Schisandrin-A over time, providing insights into its killing mechanisms and dose-dependent impact on bacterial viability. This dynamic assay complemented the MIC data, offering a temporal perspective on the compound's effectiveness. Morphological analysis through Scanning Electron Microscopy unveiled the structural changes induced by Schisandrin-A, highlighting its potential to alter the morphology of Enterococcus faecalis jh2-2. The visual evidence from microscopic observations contributes to a holistic understanding of the antibacterial mode of action. Additionally, the Drug-DNA Interaction study, assessed through UV-spectroscopy, uncovered the spectral characteristics of Schisandrin-A in the presence of DNA. The wavelength-specific absorbance patterns indicated the likelihood of interaction and potential structural changes in the compound during its association with DNA.

In summary, the results collectively portray Schisandrin-A as a multifaceted compound with significant antibacterial potential, influencing both the growth and morphology of Enterococcus faecalis jh2-2. The temporal and concentration-dependent aspects of its antibacterial activity, coupled with morphological alterations and DNA interactions, contribute to a nuanced understanding of its impact on bacterial systems. These findings lay the groundwork for further investigations into the molecular mechanisms underlying Schisandrin-A's antibacterial properties and its potential applications in combating bacterial infections.

CHAPTER 5: DISCUSSION

This chapter critically evaluates the Minimum Inhibitory Concentration (MIC) results, providing insights into the concentration-dependent inhibitory effects on bacterial growth. The temporal dynamics of Schisandrin-A's impact on bacterial viability are analyzed through the Time-Kill Kinetic Assay, elucidating the compound's bactericidal effects over various time intervals. Morphological changes induced by Schisandrin-A, as observed through Scanning Electron Microscopy, are presented and interpreted, linking structural alterations to antibacterial mechanisms. Additionally, the chapter explores the interaction between Schisandrin-A and DNA, as revealed by UV-spectroscopy, shedding light on the compound's spectral characteristics in the context of DNA interactions. The integration and synthesis of these findings aim to draw meaningful conclusions and contribute valuable insights to the field, while also providing recommendations for future research directions.

5.1 Findings

5.1.1 FTIR Analysis

The FTIR analysis conducted on the sample yielded insightful findings regarding its molecular composition. The infrared spectrum, depicted in the graph, allowed for a comprehensive exploration of the sample's chemical structure. One prominent observation was the distinctive peak near 3500 cm^-1, indicating the presence of O-H bonds. These hydroxyl groups are indicative of compounds rich in oxygen and hydrogen, providing valuable insights into the molecular constituents contributing to the sample's overall structure. Moving into the region around 3000 cm^-1, the appearance of labels such as Ar-H and C-H further elucidated the molecular landscape. The presence of aromatic and aliphatic hydrogen bonds was highlighted, underscoring the diversity of chemical interactions within the sample. These hydrogen bonds play a crucial role in determining the stability and configuration of the molecular compounds identified in the FTIR analysis.

Continuing the analysis towards lower wavenumbers, specific labels, including C=O, C=C, and C-O, emerged in the range of 1750-1000 cm^-1. These findings provided crucial information about the sample's chemical composition, revealing the presence of carbonyl groups, carbon-carbon double bonds, and carbon-oxygen single bonds. Such details are essential for a nuanced understanding of the molecular structure and functional groups inherent in the sample. Each peak observed in the spectrum corresponded to a distinct vibration mode of a chemical bond, contributing to the overall characterization of the sample. The meticulous examination of these peaks facilitated the inference of the types of chemical bonds present, enabling the identification of specific molecular structures within the sample.

In summary, the FTIR analysis findings offer a detailed molecular profile of the sample, uncovering the presence of key functional groups and chemical bonds. These insights are foundational for further interpretations and applications, providing a basis for understanding the sample's molecular composition and its potential implications in various scientific and industrial contexts.

5.1.2 Minimum Inhibitory Concentration (MIC)

The investigation into the Minimum Inhibitory Concentration (MIC) of Schisandrin-A against Enterococcus faecalis jh2-2 revealed concentration-dependent inhibitory effects on bacterial growth. As depicted in Figure 4.1, the optical density (OD) readings at various concentrations ranging from 5µg/ml to 40µg/ml provided a nuanced understanding of Schisandrin-A's impact. The lower OD values at higher concentrations suggested a more pronounced inhibitory effect, indicating increased antibacterial activity. Specifically, the MIC evaluation at 5µg/ml showed OD readings of 0.557, 0.621, and 0.701, with an average OD of 0.626. This concentration marked the initiation of observable growth inhibition, laying the foundation for subsequent analyses. Moving to the MIC evaluation at 10µg/ml, the OD readings (0.317, 0.316, and 0.315) with an average OD of 0.316 indicated a further reduction in bacterial growth. Although the standard deviation was not provided, the average OD values emphasized a noticeable inhibitory effect at this concentration. The concentration of 10µg/ml became a focal point

for understanding the dose-dependent effects of Schisandrin-A on Enterococcus faecalis jh2-2.

At 20µg/ml, the MIC assessment exhibited OD readings of 0.205, 0.216, and 0.215, resulting in an average OD of 0.212. While the absence of standard deviation limited insights into data precision, the lower average OD at this concentration signaled a distinctive inhibitory effect. This concentration marked a transition point, suggesting an increased potency of Schisandrin-A against Enterococcus faecalis jh2-2. The highest concentration evaluated, 40µg/ml, showed OD readings of 0.131, 0.130, and 0.129, with an average OD of 0.130. Similar to the 20µg/ml concentration, the absence of standard deviation prompted consideration of the precision and variability within this concentration group. The consistent lower OD values at 40µg/ml indicated a substantial inhibitory effect on bacterial growth, highlighting the heightened antibacterial activity of Schisandrin-A.

The findings from the MIC evaluations collectively illustrated a concentration-dependent response, providing crucial insights into the antibacterial efficacy of Schisandrin-A against Enterococcus faecalis jh2-2. These results laid the groundwork for further exploration of the compound's potential as an antibacterial agent and informed subsequent analyses.

5.1.3 Growth Curve

The Growth Curve analysis conducted on Enterococcus faecalis Jh2-2 in response to varying concentrations of Schisandrin-A revealed compelling insights into the dynamics of bacterial growth. The experiment involved monitoring bacterial growth over a specified time period, with concentrations of Schisandrin-A ranging from 0 to 40µg/ml. The findings from the Growth Curve analysis provided a nuanced understanding of how Schisandrin-A influences the proliferation of Enterococcus faecalis Jh2-2. At lower concentrations (0 to 10µg/ml), the Growth Curve displayed a characteristic sigmoidal shape, indicative of typical bacterial growth patterns. However, as the concentration of Schisandrin-A increased, deviations from this standard growth curve became apparent.

The bacterial growth appeared to be inhibited in a concentration-dependent manner, with higher concentrations leading to a more pronounced suppression of growth.

Specifically, at concentrations of 20µg/ml and 40µg/ml, the Growth Curve exhibited a distinctive decline in the logarithmic phase, indicating a significant reduction in bacterial proliferation. This concentration-dependent impact suggested that Schisandrin-A possesses bacteriostatic or bactericidal properties against Enterococcus faecalis Jh2-2. Furthermore, the time-dependent aspect of the Growth Curve analysis revealed that the inhibitory effects of Schisandrin-A became more prominent as the incubation time progressed. The sustained suppression of bacterial growth over the observed time intervals at higher concentrations indicated a prolonged and cumulative impact on Enterococcus faecalis Jh2-2.

In summary, the Growth Curve findings underscore the concentration and time-dependent antibacterial effects of Schisandrin-A on Enterococcus faecalis Jh2-2. These observations contribute valuable insights into the compound's potential as an antimicrobial agent, offering a foundation for further investigations into its mechanisms of action and therapeutic applications.

5.1.4 Time-Kill Kinetic Assay

The Time-Kill Kinetic Assay aimed to elucidate the bactericidal activity and killing mechanisms of Schisandrin-A against Enterococcus faecalis jh2-2. The assay involved exposing the bacterial strain to different concentrations of Schisandrin-A over various time intervals. The results, presented graphically in Figure 4.X, indicated a time-dependent reduction in bacterial viability with increasing concentrations of Schisandrin-A. At lower concentrations, a gradual decline in colony-forming units (CFUs) was observed, while higher concentrations demonstrated a more rapid and pronounced bactericidal effect.

For instance, at the lowest concentration (5µg/ml), a gradual decrease in CFUs over time was evident, suggesting a moderate impact on bacterial viability. Contrastingly, higher concentrations (e.g., 40µg/ml) exhibited a more rapid decline in CFUs, indicating a potent and time-dependent bactericidal effect. This concentration-dependent response provided valuable insights into the compound's ability to hinder bacterial growth over varying exposure durations. The findings from the Time-Kill Kinetic Assay align with the MIC results, reinforcing the concentration-dependent nature of Schisandrin-A's antibacterial effects. The graphical representation allowed for a dynamic visualization of bacterial response kinetics, contributing to a comprehensive understanding of the compound's temporal impact on Enterococcus faecalis jh2-2.

5.1.5 Scanning Electron Microscopy for Morphological Analysis

Scanning Electron Microscopy (SEM) offered a detailed examination of morphological changes in Enterococcus faecalis jh2-2 treated with Schisandrin-A. The images captured at different magnifications, such as 10,000X, 15,000X, and 30,000X, revealed distinct alterations in bacterial morphology upon exposure to Schisandrin-A. At 10,000X magnification, the SEM images displayed notable changes in cell shape and surface characteristics in the drug-treated sample compared to the untreated control. These morphological alterations provided visual evidence of Schisandrin-A's impact on the bacterial structure. Subsequent magnifications (15,000X and 30,000X) delved deeper into the ultrastructural changes, uncovering finer details of cell disruption, membrane damage, and potential cell lysis induced by Schisandrin-A.

These morphological insights complemented the quantitative findings, offering a visual narrative of the compound's effects on bacterial morphology. The SEM analysis provided a crucial link between the biochemical responses observed in MIC and Time-Kill Kinetic Assay and the physical manifestations of Schisandrin-A's interaction with Enterococcus faecalis jh2-2.

5.1.6 Drug-DNA Interaction

The Drug-DNA Interaction study focused on understanding the influence of Schisandrin-A on DNA, shedding light on potential binding modes and structural modifications. UV-spectroscopy was employed to analyze the changes in absorbance at different wavelengths, revealing distinct patterns indicative of drug-DNA interactions. The absorbance spectra, presented in Table X, demonstrated alterations in the UV profile of Schisandrin-A in the presence of DNA. Shifts, peaks, or troughs at specific wavelengths suggested potential binding and structural changes in the compound. The concentration-dependent nature of these changes further emphasized the dynamic interaction between Schisandrin-A and DNA molecules.

These findings provided molecular-level insights into the compound's interaction with DNA, contributing to the broader understanding of Schisandrin-A's mechanism of action. The results from the Drug-DNA Interaction study added a crucial layer to the comprehensive assessment of Schisandrin-A's antibacterial properties.

5.2 Conclusion

The conclusion drawn from the Time-Kill Kinetic Assay reveals that Schisandrin-A exerts a concentration-dependent bactericidal effect on Enterococcus faecalis jh2-2 over various time intervals. The assay showcased a dynamic and time-dependent reduction in bacterial viability, with higher concentrations demonstrating a more rapid decline in colony-forming units (CFUs). This underscores the compound's potent antibacterial activity and suggests the existence of intricate killing mechanisms. The concentration-dependent response aligns with MIC results, emphasizing the importance of dosage in Schisandrin-A's antibacterial efficacy. These findings provide a comprehensive understanding of the compound's temporal impact on bacterial growth, setting the stage for further exploration of its antibacterial mechanisms. Additionally, the conclusion from the Scanning Electron Microscopy (SEM) analysis indicates that Schisandrin-A induces morphological alterations in Enterococcus faecalis jh2-2. The SEM images at varying magnifications portray changes in cell shape, surface features, and ultrastructural integrity. The visual evidence of membrane damage, cell disruption, and potential lysis correlates with the compound's antibacterial effects observed in other

assays. The SEM analysis not only complements quantitative findings but also offers a detailed morphological perspective, enriching the overall understanding of Schisandrin-A's impact on bacterial morphology.

In conclusion, the Time-Kill Kinetic Assay and SEM analysis collectively strengthen the evidence for Schisandrin-A's antibacterial efficacy. The concentration-dependent kinetics and morphological alterations provide a multi-faceted view of the compound's antibacterial mode of action. These findings lay the groundwork for a more nuanced exploration of the compound's potential as a therapeutic agent against Enterococcus faecalis jh2-2 and contribute valuable insights to the broader field of antibacterial research.

5.3 Recommendation

Based on the comprehensive findings presented in this study, several recommendations emerge to guide future research endeavors and potential applications of Schisandrin-A in combating bacterial infections:

1. **Optimization of Dosage:** Further investigations should explore a more refined dosage range of Schisandrin-A to delineate the concentration threshold that maximizes its antibacterial efficacy without reaching a plateau. Fine-tuning the dosage can provide insights into achieving an optimal balance between effectiveness and potential side effects.
2. **Elucidation of Mechanisms:** Future studies should delve deeper into the molecular mechanisms underpinning Schisandrin-A's antibacterial effects. Exploring its interactions at the genetic and biochemical levels can unveil specific targets within bacterial cells, contributing to a more precise understanding of its mode of action.
3. **Clinical Relevance:** Expanding research to encompass in vivo studies and clinical trials is imperative to bridge the gap between laboratory findings and real-world applications. Assessing the compound's effectiveness in more complex

biological environments can provide a more realistic evaluation of its potential as a therapeutic agent.

4. **Combination Therapies:** Considering the rise of antibiotic resistance, investigations into the synergistic effects of Schisandrin-A with existing antibiotics could open avenues for combination therapies. Assessing whether Schisandrin-A enhances the efficacy of conventional antibiotics may offer novel strategies to combat drug-resistant bacterial strains.
5. **Safety Profiling:** To pave the way for potential clinical applications, thorough safety assessments of Schisandrin-A are warranted. Research should focus on elucidating any potential cytotoxicity or adverse effects on mammalian cells, ensuring that the compound remains a viable and safe candidate for antibacterial therapy.
6. **Exploration of Other Pathogens:** While this study focused on Enterococcus faecalis jh2-2, extending the research to other bacterial strains can provide a broader understanding of Schisandrin-A's spectrum of activity. Investigating its efficacy against a range of pathogens may uncover versatile applications.

In summary, these recommendations aim to guide future research directions, enhance the translational potential of Schisandrin-A, and contribute to the development of innovative strategies for combating bacterial infections.

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