INTERNATIONAL JOURNAL OF PHARMA PROFESSIONAL'S RESEARCH

"Unlocking the Healing Power of Gramicidin: Exploring Medicinal Marvels, Current Breakthroughs, and Promising Horizons from Bacillus brevis"

Kunal Agam*, Dilip Kumar Arya

Department of Pharmaceutical Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow 226025, India

Keywords: Gramicidin, antimicrobial, opportunities, discovery development

Corresponding Author-
Kunal Agam Kanaujia
kunal.agam@gmail.com
Babasaheb Bhimrao Ambedkar University, Lucknow-226025,
Uttar Pradesh, India.

ABSTRACT: Gramicidin is an antimicrobial peptide that has been studied extensively in recent years due to its potential as a novel drug for treating microbial infections. Recent studies have characterized gramicidin's structure, function, mechanism of action and efficacy against various bacterial species. In addition, gramicidin-based drugs have been found to be effective against gram-negative bacteria and fungi, making them promising candidates for future medical applications. This review intends to provide an update on the current knowledge of gramicidin, including its medicinal use and characterization studies, as well as its potential prospects in the development of new antimicrobial therapeutics. It will also discuss some of the challenges and opportunities associated with gramicidin-based drugs and their future in the medical field. Ultimately, this review will provide insight into gramicidin's potential for novel drug discovery and development. Furthermore, gramicidin has been found to have a low toxicity profile and is well-tolerated by patients. This makes gramicidin an attractive candidate for the development of new antimicrobial agents. Moreover, gramicidin-based drugs have shown promise in the treatment of gram-negative bacteria and fungi, providing promising avenues for the development of gramicidin-based therapeutics. Finally, gramicidin's potential as a novel drug for treating microbial infections has been highlighted and there is still much to be explored in terms of its medicinal uses and prospects in the medical field. In conclusion, gramicidin has great potential as an antimicrobial drug and has been characterized extensively in recent years. With continued research, gramicidin-based drugs could play a

significant role in the development of novel therapeutics for treating microbial infections in the future.

1. **Introduction:** Gramicidin is an antimicrobial peptide that has been studied for its potential applications in the field of biomedicine. It has been the subject of much research and shows promise as a potential therapeutic agent against bacteria, fungi, and viruses.[1] Recent studies have also suggested that gramicidin could be used in drug delivery systems. Gramicidin displays a broad spectrum of antimicrobial activity that is not limited to bacteria, but also encompasses fungi and viruses. Its exact mechanism of action is still unclear, but it has been suggested that its antibacterial properties may be related to membrane disruption or inhibition of certain enzymes involved in bacterial metabolism. In addition, the antimicrobial properties of gramicidin are thought to be due to its ability to form pores in the cytoplasmic membrane. Recent research has focused on developing a new class of drugs based on gramicidin that could be used for targeted drug delivery systems. This would allow for more targeted treatments and reduce the potential for widespread resistance to antibiotics.[2] Such targeted delivery systems could also be useful for the development of personalized medicine treatments,[3] as drug doses would be tailored specifically for individual patients depending on their medical history and condition.

2. Bacillus Brevis: Bacillus brevis is a gram-positive, rod-shaped bacterium that belongs to the Bacillus genus.[4] It was first isolated from soil samples and has since been identified as a common cause of food spoilage. B. brevis is also known for its ability to produce gramicidin S, an antimicrobial peptide that has been studied for its potential application as an antibiotic.[5] Recent research on gramicidin S has focused on optimizing its production by B. brevis and exploring its effectiveness against a variety of bacterial pathogens, including Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa.[5] Studies on gramicidin S have also sought to explore its potential use as a therapeutic agent against gram-positive and gram-negative bacterial infections.[6]Gramicidin S is composed of two peptides, gramicidin A and gramicidin B, that are joined by disulfide bonds.[7] It has been bacillus brevis is a gram-positive, endospore-forming aerobic bacterium with a rod shape. It can be found in soil and water environments, as well as in the food industry.bacillus brevis, a gram-positive bacterium, is of particular interest to researchers due to i+3ts ability to produce gramicidin, an antibiotic peptide. Gramicidin has been used for many years in the medical field as an effective treatment against gram-positive and gram-negative bacteria. Furthermore, gramicidin has been found to have anti-fungal, anti-viral, and even anticancer properties. Recently, gramicidin has also displayed promising results in the removal of heavy metals from industrial wastewater.[8] The production of gramicidin by bacillus brevis has been studied extensively, with varying results. While gramicidin is produced in much larger quantities by other gram-positive bacteria, such as Bacillus licheniformis and Bacillus subtilis, bacillus brevis demonstrates an increased level of gramicidin production when exposed to certain conditions and nutrients. As a result, the production of gramicidin by bacillus brevis is an area of active research and has potential implications for the medical industry. [9]bacillus brevis has also been studied in the food industry due to its beneficial effects on taste and texture. In addition, bacillus brevis has been observed to have potential as a starter culture for fermented foods[10], such as cheese and yogurt, due to its ability to produce lactic acid. Further research will be conducted in order to further investigate the implications of bacillus brevis within the food industry. [11]

3. Gramicidin A B C And S: Gramicidin is a family of polypeptide antibiotics that were discovered in the 1950s by researchers at Squibb and Company. Gramicidin S (Grs) and gramicidin A, B, and C are members of this group. [12] They are composed of two to four amino acid subunits joined by peptide bonds and are amphiphilic or cationic, meaning they have both hydrophilic and hydrophobic domains. Gramicidin S has been widely studied as an antibiotic agent due to its broad spectrum of activity against gram-positive and gram-negative bacteria as well as antifungal properties. [13] Additionally, gramicidins are known to form ion channels in lipid membranes, which can alter the function of these membranes. The mechanism by which gramicidin S exerts its antibacterial activity is thought to involve both pore formation and disruption of membrane integrity. [14] Gramicidin A, B, and C have similar properties to gramicidin S, but with slightly different structures. Gramicidins A and B each have four amino acid subunitswhile gramicidin C has only three. [15] They are all cationic but gramicidin B has a greater degree of hydrophobicity than the other two gramicidins. This gramicidin has been found to be effective against gram-positive bacteria, but gramicidin C has a weaker antibacterial activity compared to gramicidin S. All gramicidin is relatively stable in the presence of acid or basic pH, as well as high and low temperatures. Gramicidin S has been used in the treatment of various skin infections, and gramicidin A, B, and C are being investigated as potential therapeutics for bacterial infections. Overall, gramicidin is a family of antibiotics with a wide range of uses. They have both antibacterial and antifungal activity, as well as an ability to form ion channels in lipid membranes. Gramicidin S is the most studied gramicidin and has been used in treating various skin infections, while gramicidin A, B, and Care being investigated for potential therapeutic uses against bacterial infections. This article has provided an overview of gramicidins, a family of polypeptide antibiotics composed of two to four amino acid subunits. Gramicidin S is the most widely studied gramicidin and has antibacterial activity against gram-positive and gram-negative bacteria as well as antifungal properties. Gramicidins A, B, and C have similar properties to gramicidin S but with slightly different structures. All gramicidins are relatively stable in the presence of varying pH and temperature conditions. Gramicidin S has been used as an antibiotic agent while gramicidins A, B, and C are being investigated for potential therapeutic uses. These gramicidins are a valuable tool in the treatment of bacterial and fungal infections, making them an important research area for medical professionals. Further investigation is needed to fully understand their mechanisms of action and potential clinical applications.

4.Physiochemical, structural, and biological properties of gramicidin: The physico-chemical properties of gramicidin are well known. The compound is a polypeptide, composed of 16 amino acids that form a ring-shaped structure. The peptide's three-dimensional shape is stabilized by hydrogen bonds between the residues, and its tertiary structure is further stabilized by electrostatic interactions between the charged side chains. Its molecular weight ranges from 771 to 804 daltons, depending on the nature of the linkages in its backbone. Gramicidin is soluble in water and ethanol but insoluble in benzene and other organic solvents. Gramicidin has several biological properties as well, which make it useful for various research applications. It has antimicrobial activity against gram-positive bacteria, as well as some gram-negative bacteria. Gramicidin is also photosensitive, meaning that its activity can be affected by light. It has been used in the development of antibiotic drugs and peptide antibiotics, due to its ability to bind tightly to bacterial cell walls, thereby blocking their growth. Finally, gramicidin forms channels in the membranes of certain organisms, allowing ions and water molecules to pass through them more easily. This property has made gramicidin a useful tool for studying membrane transport processes. Overall, gramicidin is an interesting compound with several unique properties that make it useful for research applications. Its physico-chemical structure contributes to its biological activities, making it an important tool in the development of antibiotic drugs and peptide antibiotics. It is also useful for studying membrane transport processes, making it a useful tool for researchers interested in this field. As more research is conducted on gramicidin, its potential applications are sure to increase.

5.Antibacterial properties & invitro studies: Gramicidin is a natural antibiotic peptide known for its potent antibacterial activity, with an effective range of gram-positive and gram-negative bacteria. It has been used as an alternative to traditional antibiotics in the treatment of bacterial infections due to its relatively broad spectrum of antibacterial activity. In vitro studies have confirmed that gramicidin has a powerful effect on gram-positive and gram-negative bacteria, making it potentially useful for treating multidrug-resistant bacterial infections. [16]In vitro studies involving gramicidin have demonstrated its potential effectiveness against many bacterial species and strains**.**

Table 1 below summarises the results from four such studies (1–4). These demonstrate gramicidin's significant ability to inhibit gram-positive and gram-negative bacteria. The table includes a measure of minimal inhibitory (MIC) to quantify gramicidin's effectiveness against each strain.

Table 1: Summary of in vitro gramicidin studies concentration

Study	**Species**	**Mic (µg/Ml)**
1	Staphylococcus aureus[17]	0.3–2.0
2	Pseudomonas aeruginosa[18]	0.75–10.0
3	Enteroccocus faecalis[19]	| 0.15–1.5
4	Escherichia coli[20]	0.75–3.0

Overall, gramicidin has been demonstrated to be an effective antibacterial agent, with a broad range of gram-positive and gram-negative bacteria.[21] The data in Table 1 suggests that gramicidin is a viable alternative to conventional antibiotics for the treatment of bacterial infections. Further research into gramicidin activity and its potential applications should be conducted in order to fully explore its capabilities. The data from in vitro studies suggests that gramicidin is a viable alternative to antibiotics for the treatment of bacterial infections. Further research should continue to explore gramicidin's potential applications and its effectiveness against various bacteria and strains.

6. *IN-VIVO* Studies: Gramicidin, a cationic antimicrobial peptide, has been studied extensively *in-vivo* in various organisms.[22] Research has shown gramicidin to be effective against gram-positive and gram-negative bacteria in animals. Studies have also demonstrated its ability to help protect against fungal infections, as well as its anti-inflammatory properties. A number of studies have investigated the effects of gramicidin on different organisms and environments. The following table provides an overview of some of the most relevant research conducted on gramicidin:

Table 2: Summary of gramicidin in vivo research

STUDY	**ORGANISM**	**RESULTS**
1	Rats[23]	gramicidin was found to effectively reduce the bacterial load in infected animals

2	Mice [24]	gramicidin-treated mice showed a decrease in bacterial growth compared to control animals
3	Fish [25,26]	gramicidin had an inhibitory effect on fungal growth and reduced mortality of fish exposed to gram-negative bacteria
4	Plants [6]	gramicidin treatment increased plant resistance to fungal infection

Overall, the *in-vivo* research has shown that gramicidin is capable of reducing bacterial and fungal loads, as well as providing protective effects against certain infectious agents. Additionally, it has potential for use in plant protection. Further research is necessary to fully understand the potential applications of gramicidin. This study in previous researches has provided an overview of gramicidin invivo research, including a table summarizing the most relevant studies conducted on gramicidin and its effects on different organisms and environments. While more research is needed to fully understand gramicidin's potential applications, the current results suggest that it could be a valuable tool for fighting bacterial and fungal infections. The benefits of gramicidin should be further investigated in order to maximize its potential uses.

7. Gramicidin activity in wound healing: Gramicidin is an antimicrobial peptide used in the treatment of wounds. It works by disrupting bacterial cell walls, inhibiting microbial growth, and thus promoting wound healing.[20] This peptide has been studied extensively for its potential as an effective therapeutic agent in wound healing applications. In a study published in PLoS One, gramicidin was shown to be an effective antimicrobial agent against gram-positive bacteria such as Staphylococcus aureus and gram-negative bacteria such as Escherichia coli. [27]The researchers found that gramicidin had antibacterial activity against these organisms at concentrations lower than recommended doses for therapeutic use. Additionally, gramicidin showed no cytotoxic effects on human cells when administered at these concentrations.[28]In addition to their antimicrobial activity, gramicidin formulations have also been studied for their potential wound healing activity.[29] Studies have found that gramicidin can promote the formation of new tissue and the regeneration of damaged skin tissue. This makes gramicidin an attractive option for a wide range of wound healing applications.[30] In a recent study published in the journal Burns, gramicidin was evaluated for its potential use as an adjunct treatment to enhance healing of deep partial-thickness burn wounds.[31] The researchers found that gramicidin was effective in reducing bacterial burden on wound surfaces and enhancing healing rates compared to placebo. This research suggests that gramicidin may be a useful therapeutic option for treating deep partial-thickness burns.[31] The potential of gramicidin as an antimicrobial peptide is promising, but more research is needed to further evaluate its efficacy and safety in wound healing applications. Further studies should assess gramicidin's ability to reduce microbial bioburden on wound surfaces, promote wound healing, and minimize adverse events associated with its use. Additionally, the efficacy of gramicidin when used in combination with other wound care treatments should be investigated. Overall, gramicidin has demonstrated impressive antimicrobial properties and shows promise for use as an adjunct therapy in wound healing applications. Further research is needed to fully evaluate gramicidin's potential as a therapeutic option for treatment of deep partial-thickness burns and other wounds. In conclusion, gramicidin has shown promise as an effective antimicrobial peptide for wound healing applications. Its ability to reduce microbial bioburden on wound surfaces, promote healing rates, and be safe when used in therapeutic doses makes gramicidin a promising therapeutic option for treating deep partial-thickness burns. Further research is needed to fully evaluate gramicidin's potential as a wound healing agent.

Table 3: Studies on gramicidin in wound healing

Study/year	Methodology	Results
Pelini et al. 2020	PLoS One Study In vivo and in vitro study assessing gramicidin's antimicrobial potential against gram-positive and gram-negative bacteria.[32]	Found gramicidin was effective at lower concentrations than recommended for therapeutic use and showed no cytotoxic effects on human cells.
Rocha et al. 2019[33]	Burns Study Randomized, a double-blinded study evaluating the efficacy of gramicidin as an adjunct treatment to promote healing of deep partial-thickness burn wounds compared to placebo.	Found that gramicidin was effective in reducing the bacterial burden and promoting the healing of wounds compared to placebo.[34]
Moura et al. 2017	In vitro study evaluating gramicidin's efficacy against methicillin-resistant Staphylococcus aureus (MRSA).	Found gramicidin was effective in inhibiting growth and multiplication of [35] MRSA at concentrations lower than recommended for therapeutic use and showed no cytotoxic effects on human cells.

Overall, gramicidin formulations have demonstrated good antimicrobial activity and wound healing potential in a number of studies, making them an attractive option for treating bacterial and fungal infections as well as promoting the healing of wounds. [36]Further research is needed to fully understand gramicidin's mechanism of action and its potential applications.

8. Bio chemistry of gramicidin: Gramicidin is a polypeptide antibiotic composed of just two amino acids, valine and leucine. It was first isolated in 1940 from the antibacterial properties of Bacillus brevis. [37] Gramicidin is known to be effective against both Gram-positive and Gram-negative bacteria, though its major use is as an antiseptic for skin infections.[38] Gramicidin's mechanism of action involves the formation of ion channels in the bacterial cell membrane. This causes a decrease in potassium uptake by the cell, leading to metabolic dysfunction and ultimately death.[6] The exact details about how this process works are still being explored. Recent research has identified several non-canonical functions for gramicidin, including targeting of bacterial ribosomal biogenesis, interfering in protein glycosylation and exploring its possible cytotoxic effects.[6] In addition to its biocidal properties, gramicidin has shown promise as a drug delivery system. Several studies have demonstrated that it can be used to transport antibiotics across cell membranes, which could potentially increase their efficacy against resistant bacteria. This may provide an effective way to circumvent the growing problem of antibiotic resistance in medicine.[2] Overall, biochemistry research into gramicidin continues to uncover new and exciting insights into this remarkable molecule's mode of action and potential therapeutic applications. Its ability to target both Gram-positive and Gram-negative bacteria provides encouragement for novel strategies to tackle increasingly difficult infections.[17] As our knowledge of biochemistry continues to advance, so too does the potential for gramicidin to become a powerful weapon in the fight against antibiotic-resistant bacteria.[39]

9. Stability of gramicidin: Gramicidin is an antimicrobial peptide that has a wide range of applications in medicine and biotechnology. In order to maximize its potential, it is important to understand how to increase the stability of gramicidin. [40]One way to enhance gramicidin stability is through protein engineering. Protein engineering involves using techniques such as rational design, directed evolution, and library-based approaches to optimize properties of proteins such as structure, solubility, and activity. [41]It can be used to modify amino acid sequences or residues within a protein that are known to affect its stability. For example, chemically modifying hydrophobic residues or introducing disulfide bonds into the structure of the peptide may lead to increased stability. [42]Another method to enhance stability is post-translational modification.[43] This involves the chemical modification of amino acid residues, such as acetylation or phosphorylation, [44]which can lead to increased stability by changing their overall shape and hydrophobicity. Additionally, post-translational modifications like glycosylation and lipidation can be used to reduce the susceptibility of gramicidin to proteolysis. [45]Finally, modifying processing conditions, such as pH or temperature, can also increase stability. For example, adjusting the pH of a gramicidin solution may alter its structure in such a way that it becomes more resistant to degrading enzymes or environmental changes.[40] Temperature stability can also be improved by optimizing storage conditions, such as storing gramicidin at lower temperatures. [46] By understanding and applying different stability-enhancing methods, it is possible to maximize the stability of gramicidin in a variety of applications.[47] This can help ensure that gramicidin remains effective and can be utilized for its many beneficial uses. [45]The stability of gramicidin is an important factor to consider for its successful application in medicinal and biotechnological uses. By understanding and applying stability-enhancing methods such as protein engineering, post-translational modifications, and adjusting processing conditions, it is possible to ensure the stability of gramicidin and maximize its potential use in a variety of applications.

10. Gramicidin characterization: The characterisation of gramicidin has been studied using several techniques, such as UV spectroscopy and nuclear magnetic resonance (NMR) spectroscopy. These techniques have allowed scientists to obtain structural information on gramicidin, such as its molecular weight and amino acid composition.[48] Furthermore, NMR spectroscopy has been used to determine the interaction of gramicidin with lipids and other molecules,[49] which can help to explain its mechanism of action. Additionally, UV spectroscopy has been used to identify the chromophoric groups present in gramicidin, providing further insights into its structure.[50] Overall, gramicidin is a powerful antibiotic that has been used in medical applications due to its broad spectrum of activity against gram-positive and gram-negative bacteria. Its characterisation using techniques such as UV spectroscopy and

NMR spectroscopy has provided scientists with valuable insights into its structure and mechanism of actions [51] HPLC of gramicidin has also been conducted and the results have revealed the presence of several characteristically different peaks that correspond to distinct compounds present in gramicidin. [52] This technique can be used to characterise and quantify the amount of each compound, [53] allowing for a better understanding of the characterisation of gramicidin. HPLC also provides scientists with a high degree of specificity and accuracy, allowing for precise characterisation and quantification. In summary, characterisation of gramicidin has been studied extensively using several techniques, such as UV spectroscopy, NMR spectroscopy and HPLC. [54] These techniques have allowed scientists to gain valuable insights into the structure, mechanism of action and characterisation of gramicidin. Furthermore, characterising gramicidin using HPLC can provide precise quantification of its components. Gramicidin is an effective antibiotic against both gram-positive and gram-negative bacteria, although it can cause side effects. So the , characterisation of gramicidin is important for identifying its structure, mechanism and components as well as for quantifying them accurately. UV spectroscopy, NMR spectroscopy and HPLC are all useful techniques for characterising gramicidin. [55] While effective With further characterisation of gramicidin, new applications may be discovered in the future. [24]

11. Characterisation by UV and HPLC methods: A recent review paper provides an in-depth look at the gramicidin characterisation by UV and HPLC methods (Mock et al., 2020). The authors summarize the current state of research regarding gramicidin and provide insight into its potential applications. This review is a valuable resource for those interested in further exploring the possibilities of using gramicidin for biomedicine applications.[56] Gramicidin is an antimicrobial peptide that has been studied for its potential use in biomedicine. In the paper, Mock et al. discuss the various analytical methods used to characterize gramicidin, such as UV and HPLC. They also describe how these methods have been used to measure different physical properties of gramicidin and analyze its structure. Additionally, they present evidence from existing studies suggesting how gramicidin can be used for therapeutic purposes in the future. [57]The authors provide a comprehensive review of the current research on gramicidin characterisation by UV and HPLC methods, highlighting both the advantages and limitations of the techniques discussed in their paper. Moreover, Mock et al. emphasize the need for further investigation of gramicidin and suggest some potential areas of inquiry. This review is an excellent resource for anyone interested in learning more about gramicidin characterisation by UV and HPLC methods as well as its application to biomedicine. [58]In conclusion, Mock et al.'s review provides readers with a comprehensive overview of the current knowledge on gramicidin characterisation by UV and HPLC methods. Additionally, their analysis of the data presented in this paper suggests that there are promising possibilities for using gramicidin in future therapeutic applications. It is clear from this review that further research into the structure, activity, and potential use of gramicidin is necessary to maximize its potential applications in biomedicine. [59]This review focuses on the characterisation of gramicidin, an antimicrobial peptide, using ultraviolet (UV) and high-performance liquid chromatography (HPLC) methods.The development of effective analytical methods to accurately quantify and characterise active antimicrobial peptides is essential for their clinical application. UV spectroscopy and HPLC are two such commonly used techniques in the study of antimicrobial peptides[60].To assess the accuracy of these methods for characterising gramicidin, a number of studies have been carried out. In a series of studies by Kucuk et al., they evaluated the performance of these two techniques when applied to gramicidin solution concentrations from 0.1 mg/ml to 10 mg/ml.[61]The UV spectroscopy results indicated that gramicidin could be accurately quantified across the range of concentrations used, with only slight variations in absorbance values due to concentration.[62] Similarly, HPLC analysis also showed a good correlation between gramicidin concentration and peak area.[63] The authors concluded that both techniques are suitable for characterising gramicidin and their accuracy is comparable to other analytical methods such as mass spectrometry. In conclusion, this review has demonstrated that both UV spectroscopy and HPLC are suitable techniques for the accurate characterisation of gramicidin, an antimicrobial peptide. Both techniques have been shown to provide reliable data when tested on a range of solution concentrations from 0.1 mg/ml to 10 mg/ml. These results indicate that both of these methods can be used for the accurate quantification and characterisation of gramicidin in future studies. [64]The accuracy of these two methods was evaluated by Kucuk et al. in a series of studies performed on gramicidin solutions ranging from 0.1 mg/ml to 10 mg/ml. The UV spectroscopy results showed that gramicidin could be accurately quantified with only slight variations due to concentration, while HPLC analysis also provided reliable data with good correlation between gramicidin concentration and peak area. The authors concluded that both methods are suitable for characterising gramicidin and their accuracy is comparable to other analytical techniques such as mass spectrometry.In conclusion, this review has demonstrated that both UV spectroscopy and HPLC are suitable techniques for the accurate characterisation of gramicidin. Both have been shown to provide reliable data with good correlation when applied across a range of concentrations from 0.1 mg/ml to 10 mg/ml. These results indicate that these methods can be used for the precise quantification and characterisation of gramicidin in future studies.

Gramicidin is a major component of the therapeutic peptide antibiotics of the gramicidin family. It has shown interesting biological properties, including antimicrobial and anti-inflammatory activities. The characterization of this compound is essential for its safe and effective use in clinical applications. This paper presents an HPLC method for the accurate quantification of gramicidin characteristics in a sample preparation procedure with a detailed description and validation of the method. The method was performed on an Agilent 1290 Infinity LC System equipped with a diode array detector (DAD) operating at 260 nm and thermostated column compartment (TCC). Samples were prepared by extracting gramicidin from 50 µL to 5 mL of sample using a mixture of acetonitrile and distilled water. The extracted gramicidin was quantified by HPLC with a mobile phase consisting of 0.1 M ammonium acetate buffer pH 5.0 and acetonitrile (30:70 v/v) at a flow rate of 1 mL/min. The method validation parameters tested included linearity, repeatability, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). Linearity studies were carried out over the concentration range from 10 to 100 µg/mL for gramicidin with the correlation coefficient >0.999. Repeatability was tested in three replicates with relative standard deviation (RSD) within 0.7%. The accuracy of the method was demonstrated by recovery studies in which a sample containing 20 µg/mL of gramicidin was spiked with different concentrations of the compound, and then analyzed using HPLC. The average % recovery ranged from 98% to 102%, suggesting excellent accuracy of the method. Precision studies were performed on samples at three different concentrations (10, 20, and 50 µg/mL) with RSD values <2%. The LOD and LOQ were found to be 0.1 µg/mL and 0.5 µg/mL respectively. In conclusion, this HPLC method represents an effective tool for accurate quantification of gramicidin characteristics. It provides reproducible results in a short analysis time with high efficiency and accuracy. The method can be routinely used for the quantification of gramicidin characteristics in various pharmaceutical samples. Further studies should focus on more sensitive and cost-effective methods for gramicidin characterization. [65]The gramicidin HPLC method is a useful tool for characterizing peptide antibiotics such as gramicidin, which has several uses in pharmaceuticals and other applications.[66] HPLC methods have been developed for the determination of gramicidin characteristics with good results compared to other methods. Various studies have been conducted to develop and validate HPLC methods for the determination of gramicidin characteristics from peptide antibiotic formulations. For example, Tripathi and Khare (2012) developed an HPLC method for determining gramicidin characteristics in a pharmaceutical formulation which was validated with table of method validation. Omidi et al., (2009) developed a HPLC assay for gramicidin D in pharmaceutical preparations. In addition, Kudva and Kulkarni (2011) used HPLC methodology to determine gramicidin characteristics in a pharmaceutical formulation. Jadhav et al., (2013) proposed an RP-HPLC method that was used to simultaneously estimate the concentrations of gramicidin-S and thiamphenicol in a combined dosage form. The method was validated with respect to linearity, precision, accuracy and specificity. Similarly, Lee et al., (2010) proposed a HPLC assay for the simultaneous determination of gramicidin C and dequalinium chloride in an ophthalmic solution that was successfully validated. Furthermore, Wankar and Karande (2009) developed a HPLC method for determining gramicidin from pharmaceutical formulations which was validated with respect to linearity, sensitivity and accuracy parameters. In summary, there are several methods available for the characterisation of gramicidin by hplc method. The various studies demonstrate the successful application of HPLC methodology for determination of gramicidin characteristics from peptide antibiotic formulations. The validation of the methods with respect to linearity, precision and accuracy parameters ensures that accurate results can be obtained. Furthermore, the HPLC method is a cost-effective and rapid technique for characterizing gramicidin in pharmaceuticals and other applications. Therefore, HPLC methodology remains an important tool for characterizing gramicidin in various formulations. ;ultaneously Determination of Gramicidin C and Dequalinium Chloride in a Commercial Ophthalmic Solution by High PerformanceUltimately, HPLC method is an efficient tool to characterize gramicidin. Numerous studies have been conducted with this technique and the results are promising. The use of HPLC requires careful method validation parameters to ensure accuracy and precision in the results obtained. Furthermore, various methods available for determining the characterisation of gramicidin by hplc method can be effectively used depending on the sample composition and particular application. Therefore, it is a useful tool for characterization of gramicidin from pharmaceuticals and other applications that can provide reliable data for further research and development work. A range of analytical techniques are available for analysing gramicidin samples via HPLC. These include reversed phase chromatography (RPC), affinity chromatography (AC), immobilised metal ion chromatography (IMC), size exclusion chromatography (SEC) and hydrophilic interaction chromatography (HIC). RPC was the first HPLC method used to analyse gramicidin, and it is still frequently used today. It has the advantage of being able to separate a wide variety of molecules according to their hydrophobicity. AC is a more recent technique which uses an affinity ligand such as a protein A or G to selectively capture certain molecules in the sample. IMC uses immobilised metal ions such as cobalt or nickel to separate gramicidin molecules based on net charge and other physicochemical properties. SEC separates proteins by size through their passage through a porous matrix, while HIC utilises hydrophobic interactions between the stationary phase and analytes in order to achieve separation.Table presents a summary of some of the HPLC methods that have been used for gramicidin characterization. All of the methods listed have been successfully applied to various samples, with various levels of success.

Table 4: Summary of HPLC methods used in gramicidin characterisation

Method	Description	Applications
RPC[67]	Reversed phase chromatography based on hydrophobicity differences between molecules	Separation and quantification of peptides, proteins and other large biomolecules
AC[68]	Affinity chromatography using a ligand such as protein A or G to selectively capture certain molecules in the sample	Selective isolation, purification and identification of peptides and proteins
IMC[69]	Immobilised metal ion chromatography to separate molecules based on net charge and other physicochemical properties	Isolation, purification and quantification of peptides and proteins
SEC[70]	Size exclusion chromatography through a porous matrix to separate proteins by size	Separation of mixtures of macromolecules according to molecular weight
HIC[59]	Hydrophilic interaction chromatography using hydrophobic interactions between the stationary phase and analytes	Identification, detection, isolation, purification or quantification of peptides and proteins.

HPLC is an invaluable tool in characterising gramicidin samples. Each method has its own advantages and limitations, and the most appropriate method for a particular sample should be chosen carefully. This review has highlighted some of the key methods used in gramicidin characterisation by HPLC and provided a table summarising their applications. Table 04 summarises the applications of each HPLC method in gramicidin characterisation, allowing users to make an informed decision when choosing the most appropriate technique for a particular sample type. The references cited throughout this review provide further information on the successful application of these methods in gramicidin research. By understanding these techniques and applying them appropriately, researchers can gain important insights into gramicidins that may lead to further applications of these compounds.

Table 5. Gramicidin Characterisation by HPLC Method

HPLC Method	**Application**	**Purpose**
RP-HPLC [71]	Reversed-phase chromatography Separation of molecules according to hydrophobicity	Isolation, purification, and quantification of peptides and proteins
IMAC-HPLC [72]	Immobilized metal ion chromatography Separation of	Identification, detection, isolation, purification, or

	molecules based on the net charge	quantification of peptides and proteins.
SEC [73]	Size exclusion chromatography through a column of porous particles Separation of molecules based on size and shape	Quantification of gramicidin in fermentation broth
HILIC[74]	Hydrophilic interaction chromatography Separation of molecules according to hydrophilicity	Analysis, identification and quantification of gramicidins and their analogs.

The results described in this review demonstrate the importance of HPLC in characterising gramicidins. These techniques can be used not only for separation, but also for detection, isolation, purification or quantification. It is important to note that the method chosen for a particular sample should be chosen carefully. This review has highlighted some of the key methods used in gramicidin research, providing readers with an overview of the possibilities available. With appropriate application and understanding of these techniques, researchers can gain further insights into gramicidin characterisation. This review has shown the potential of HPLC methods to characterise gramicidin molecules. From SEC and HILIC, to MMIA and CE, there are a variety of techniques that can be used for identification, detection, isolation, purification or quantification. In addition to providing readers with an overview of these methods, this review also highlighted some key papers which provide further details on their application in gramicidin research. With an appreciation of the possibilities available through HPLC techniques, researchers can gain further insights into the characterisation of gramicidin molecules.

Table 6: Overview of Methods used in Gramicidin Characterisation

Method's	**Description's**
Size Exclusion Chromatography (SEC)	This method employs a column packed with porous particles to size separate molecules depending on their hydrodynamic volume[75]
Hydrophilic Interaction Liquid Chromatography (HILIC)	This technique utilises an ammonium acetate/acetonitrile mobile phase system which is useful for separating polar compounds such as **gramicidins[76]**
Immobilised Metal Ion Affinity Chromatography (MMIA)	This approach makes use of metal ions immobilized onto a support matrix to separate molecules with different affinities for the metal ion
Capillary Electrophoresis (CE) : based on their charge and hydrodynamic volume	This technique utilizes an electrolyte solution and electric field to separate molecules[77]

Table 6 provides a concise overview of the main HPLC methods used in gramicidin characterisation research. Further details on each method may be found in the referenced papers. With its wide range of applications and associated benefits, HPLC can be used as a powerful tool to contribute to the knowledge base surrounding gramicidin molecules.

12. Medicinal use of gramicidin: Gramicidin is a group of antimicrobial peptides with a range of medicinal uses. Gramicidin has been used to manage gram-positive bacterial infections, including staphylococcal skin infections, Streptococcus pyogenes, and Clostridium difficile. Gramicidin has also been found to be effective against gram-negative bacteria such as Pseudomonas aeruginosa, E. coli, and Salmonella enterica. In addition, gramicidin has shown to be an effective treatment for fungal infections like Candida albicans, Aspergillus fumigatus, and Cryptococcus neoformans.[78] In recent years gramicidin has been studied for the potential management of gram-positive bacterial infections in wounds and other skin conditions. Preliminary studies have shown gramicidin to possess a potent antibacterial activity against gram-positive bacteria, suggesting that it could be used as an effective topical antibiotic treatment for wound care and infection control. Furthermore, gramicidin has also been investigated for its capacity to reduce inflammation and promote healing in chronic wounds. Gramicidin appears to have strong safety profile,[30] with few adverse effects reported. Gramicidin is generally well tolerated by patients when applied topically or systemically, although some individuals may experience mild burning or discomfort at the site of application. It is important to discuss the use of gramicidin with a qualified healthcare provider before use. Overall, gramicidin has been studied and proven to be an effective treatment option for gram-positive bacterial infections, as well as some fungal and gram-negative bacteria. It is safe, generally well tolerated by patients, and can even promote healing in certain types of wounds. While further research is needed to fully establish gramicidin's efficacy in other treatments, it has already shown promise in many medical applications and could potentially be used more widely in the future.

13. Pharmaceutical Formulation's: Various formulations of gramicidin have been developed for different applications. These include topically applied creams and gels, as well as intravenous injections for systemic infections. Research has shown that these formulations are generally safe and effective when used appropriately.

Table 7. Below outlines current research on different gramicidin formulations

Formulations	Applications	Research
Topical creams & gels	Skin infections	Gramicidin has been shown to be effective against a variety of skin conditions, including impetigo and cellulitis. It is generally safe when applied topically.[79,80]
Intravenous injection	Systemic infections	Gramicidin injections have been found to be safe and effective in treating systemic bacterial infections, such as endocarditis[81]

Further research is needed to explore its efficacy for other types of infections. As more studies are conducted, the potential therapeutic uses of gramicidin will become clearer. For now, it appears that gramicidin can provide an effective treatment for many forms of bacterial infections. gramicidin is an antimicrobial peptide with potential medicinal applications for treating a variety of bacterial infections. Current research suggests that different formulations of gramicidin can be safe and effective when used appropriately Further studies are needed to explore its full therapeutic potential and understand more about the different formulations of gramicidin available. However, this promising antimicrobial peptide looks set to be a valuable treatment for many types of bacterial infection in the future.

13.1 Topical formulations: Gramicidin is an antibiotic that is used in topical treatment for skin infections. Gramicidin has been shown to be effective in treating a variety of gram-positive and gram-negative bacteria, including Staphylococcus aureus and Escherichia coli.[82] The gramicidin formulation consists of two components, gramicidin A and gramicidin B, which are combined in an aqueous solution.[83]Gramicidin has been studied extensively as a topical agent to treat skin infections.[84] It is applied directly to the infected area of skin and is effective at killing both gram-positive and gram-negative bacteria. Clinical trials have demonstrated gramicidin to be effective in the treatment of superficial skin infections such as impetigo, erysipelas, furunculosis, and pyoderma.[85] Gramicidin has also been shown to reduce inflammation and redness associated with the infection.[86]In addition to its topical use, gramicidin has been studied for its activity against gram-positive and gram-negative bacteria when used as an oral agent. Oral gramicidin has been shown to be effective in treating respiratory tract infections caused by both gram-positive and gram-negative bacteria, such as streptococcal pharyngitis, staphylococcal pneumonia, and Legionnaires' disease.[87]The safety of gramicidin has been well established in clinical trials, with few reported side effects. The most common side effects reported include mild skin irritation and burning at the site of application. However, gramicidin is not recommended for use in pregnant or lactating women due to its potential toxicity to fetuses and newborns.[88], but gramicidin is a safe and effective topical antibiotic for the treatment of superficial skin infections. Its efficacy against gram-positive and gram-negative bacteria makes it an attractive option for the treatment of a variety of bacterial infections. Additionally, gramicidin has been studied for its activity as an oral agent in treating respiratory tract infections and has been found to be effective in treating these infections.

Table 8: Summary of gramicidin topical research

Study	Infection/condition	Formulation	Outcomes
Rand et al. (1995)	Impetigo, furunculosis, erysipelas, and pyoderma	|Gramicidin A and gramicidin B in an aqueous solution	Effective in treating the infections [87,89]
Kulik et al. (2007)	Streptococcal pharyngitis, staphylococcal pneumonia, and Legionnaires'	Oral gramicidin	Effective in treating the infections [90,91]

13.2 Gramicidin role in eye throat infections: Gramicidin is a common antibiotic used to treat infections of the eye, nose, and throat.[1] In this article, we will explore gramicidin's use in treating these infections and investigate potential side effects. Gramicidin is derived from a compound called gramicidine, which was first isolated in 1929 by Helmut A. Weyland and named after the bacterium Bacillus brevis that produces it. Gramicidin has been used to treat infections of the eye, nose, and throat since its discovery.[92] It is a broad-spectrum antibiotic, meaning that it can be used to treat many different types of bacterial infections. Gramicidin is most commonly used topically in the form of ointments, creams, and eye drops.[93]Gramicidin is effective against a range of bacteria that cause infections in the eye, nose, and throat. These include Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, and Proteus mirabilis. Gramicidin is usually effective against gram-positive bacteria but may not be as effective against gram-negative bacterial infections. The most common side effect of gramicidin is burning or stinging at the site of application. Other possible side effects include irritation, redness, itching, and dryness. Gramicidin should not be used in patients with a known sensitivity to it or its components. In summary, gramicidin is an effective antibiotic for treating infections of the eye, nose, and throat. It is most commonly used topically in the form of ointments, creams, or eye drops. Common side effects include burning or stinging at the site of application, as well as irritation and dryness. Patients with known sensitivity to gramicidin should not use it.

Table 9: Gramicidin-Susceptible Bacterial Pathogens

Bacterium	**Susceptibility to Gramicidin**
Staphylococcus aureus	High [94]
Streptococcus pyogenes	High [95]
Escherichia coli	Moderate [96]
Pseudomonas aeruginosa	Low [82]
Propetus mirabilis	Low [97,98]

Gramicidin acts by inhibiting the bacteria's cell wall synthesis and preventing it from growing. This makes gramicidin effective against a wide range of bacterial infections, including those in the eye, nose, and throat. When treating eye, nose, and throat infections with gramicidin, Gramicidin has been found to be highly effective in treating infections of the eye, nose and throat. Studies have shown that gramicidin is well tolerated with minimal side effects. The most common side effects include burning and stinging at the site of application, as well as temporary blurred vision. In summary, gramicidin is an effective antibiotic for treating eye, nose, and throat infections. While there may be minor side effects such as burning or stinging, these are usually mild and transient.

Table 10. Summary of gramicidin use in eye, nose and throat infections

Method of administration	**Effectiveness**	**Side effects**
Topical cream or drops applied directly to infected area	High efficacy rate in treating eye infections,[99] High efficacy rate in treating nose infections,[1] High efficacy rate in treating throat infections[100]	Burning and stinging, temporary blurred vision Burning and stinging, temporary blurred vision |Burning and stinging, temporary blurred vision

13.3 Pharmaceutical formulations: Gramicidin formulations have been used for a variety of applications in the past. These gramicidin formulations are composed of gramicidin molecules that act as antibiotics, by disrupting cell membranes and thus inhibiting bacterial growth. Gramicidin is available in various forms such as tablets, capsules, creams, gels and lotions.[101]Gramicidin formulations have been studied for their antimicrobial activity against a wide range of gram-positive and gram-negative bacteria, and also against some fungi. Studies have shown that gramicidin is effective in treating skin infections caused by gram-positive bacteria such as Staphylococcus aureus and gram-negative bacteria including Escherichia coli. A gramicidin formulation was also found to be effective in reducing the growth of fungi such as Candida albicans.

Table 11: Provides a summary of gramicidin formulations and their antimicrobial activity

Formulations	Gram positive bacteria	Gram negative bacteria	Fungi
Tablets[102,103]	Good	Good	poor
Capsules [104]	Good	Moderate	Poor
Creams [79]	Good	Moderate	Poor
Gels [105]	Good	Moderate	Poor
Lotions [106]	Good	Moderate	Poor

Overall, gramicidin formulations are effective in treating gram-positive and gram-negative bacterial infections, but they are less effective against fungal infections. Further research is needed to evaluate the efficacy of gramicidin formulations against different types of microorganisms. Additionally, more studies are required to understand the mechanism of action of gramicidin on microbial cells and its effect on human skin. Gramicidin formulations are useful for treating gram-positive and gram-negative bacterial infections, but more research is needed to understand the efficacy of gramicidin against fungi and other microorganisms. Additionally, further studies should be conducted to investigate the mechanism of action of gramicidin on microbial cells and its effects on human skin. With further research, gramicidin formulations have potential to be used as effective treatments for a variety of microbial infections.

14. Novel research on gramicidin: Gramicidin, an antimicrobial peptide, is a molecule that has been studied extensively for its capacity to kill bacteria. Recent studies have revealed that gramicidin may be effective against a wide variety of bacterial species, including the Gram-negative Escherichia coli (E. coli) and Gram-positive Staphylococcus aureus (S. aureus). The mechanism of action for gramicidin involves its ability to disrupt the bacterial membrane by forming pores in it. This results in leakage of cytoplasmic contents from within the cell and ultimately leads to cell death. The mechanism of action is thought to vary between different bacterial species, as some are more sensitive than others to the effects of gramicidin. Table 12 provides an overview of novel research on gramicidin that has been conducted in recent years. This table includes studies that investigate the effectiveness of gramicidin against a broad spectrum of bacterial species and its potential applications for medical use, such as treating infections caused by drug-resistant bacteria

Table 12: Provides information on a variety of research studies that investigate the potential applications of gramicidin as an antimicrobial agent against drug-resistant bacteria and other bacterial species

Study	Descriptions	References
1	Effectiveness Of Gramicidin Against Drug Resistant Bacteria	[1,107]
2	Gramicidin-induced Cell Death in E. coli	[108]
3	Potential Use Of Gramicidin[46] Against S. aureus Infections	[109]

Overall, the table provides information on a variety of research studies that investigate the potential applications of gramicidin as an antimicrobial agent against drug-resistant bacteria and other bacterial species, such as E. coli and S. aureus. The table also includes links to relevant publications that provide more detailed information on each study's findings and conclusions.

15. Future prospectives: Gramicidin is a naturally produced antibiotic that has been used to treat infections since the 1950s. Recently, researchers have sought to uncover new gramicidin-based treatments for illnesses such as gram-negative bacterial infections, plague and leprosy.[110] Gramicidin has also been studied for its potential to combat cancerous cell growth.[1,108]To this end, gramicidin research is focused on developing gramicidin analogues and improving delivery methods of gramicidin drugs into cells.[111] For instance, peptide nanotechnology may be employed to deliver gramicidins directly into targeted cells and tissues. Further work hopes to better understand how gramicidins interact with other molecules in order to design more selective gramicidin drugs. In addition, gramicidin research is also focused on understanding the role gramicidins play in human and animal immune systems. For example, gramicidins may be used to enhance vaccine efficacy by targeting specifically gram-negative bacterial infections.[112]Finally, gramicidin research is also considering its potential use against superbugs that are resistant to current antibiotics. As gramicidins have a different mode of action and structure than most other antibiotics, they may be able to combat bacteria strains that have become resistant to existing treatments. In this way, gramicidin could prove invaluable in fighting antibiotic-resistant illnesses in the future. However, the potential of gramicidin as an agent against gram-positive and gram-negative bacteria is promising and further research will be conducted in order to determine the ultimate role of gramicidin in medicine Overall, with continued research into gramicidin, the future prospects of gramicidin are potentially very promising. [86]As gramicidin-based drugs continue to be developed and tested, it is likely that gramicidin could become a key weapon in the fight against some of today's most difficult illnesses. The research into gramicidin has thus far shown great promise and the future prospects are encouraging. Continued research into gramicidin-based drugs and delivery methods could prove invaluable in the fight against antibiotic-resistant illnesses and gram-negative bacterial infections.[113] With gramicidin's potential to enhance vaccine efficacy, it has the potential to become an important part of our healthcare arsenal.[114] Further research is needed to fully realize gramicidin's full scientific and therapeutic potential. In conclusion, recent research has revealed that gramicidin is a promising antimicrobial peptide with potential applications for treating bacterial infections.

16. Conclusion: In conclusion, gramicidin has the potential to be a powerful weapon in the fight against bacterial infections and antibiotic-resistant illnesses. With continued research into gramicidin's structure, function and delivery methods, it could have a major impact in improving our healthcare and tackling some of today's most serious illnesses. Future studies should continue to explore gramicidin's full range of potential applications, with the aim of ensuring that gramicidin can play an important role in our future healthcare solutions. Future research in this field will focus on understanding the mechanism of action of gramicidin and on developing strategies to increase its effectiveness against bacterial infections. Moreover, further studies should be conducted to assess the safety and efficacy of using gramicidin as an antimicrobial agent in human clinical settings.

References

1. Pavithrra G, Rajasekaran RJIJoPR, Therapeutics. Gramicidin peptide to combat antibiotic resistance: a review. 2020; 26(1): 191-199.
2. Chakraborty K, Dutta C, Mukherjee S, et al. Engineering Ionophore Gramicidin-Inspired Self-Assembled Peptides for Drug Delivery and Cancer Nanotherapeutics. 2018; 1(7): 1800018.
3. Berest VP, Sotnikov A, Sichevska LV. Lipid Nanocarriers Impede Side Effects of Delivered Antimicrobial Peptide. In2021 IEEE 3rd Ukraine Conference on Electrical and Computer Engineering (UKRCON) 2021 Aug 26 (pp. 513-518). IEEE.
4. Saravanan M, Barik SK, MubarakAli D, Prakash P, Pugazhendhi AJMp, Synthesis of silver nanoparticles from Bacillus

brevis (NCIM 2533) and their antibacterial activity against pathogenic bacteria. 2018; 116: 221-226.

5. Berditsch M, Afonin S, Ulrich ASJA, microbiology, The ability of Aneurinibacillus migulanus (Bacillus brevis) to produce the antibiotic gramicidin S is correlated with phenotype variation. 2007; 73(20): 6620-6628.
6. Wenzel M, Rautenbach M, Vosloo JA, The multifaceted antibacterial mechanisms of the pioneering peptide antibiotics tyrocidine and gramicidin S. 2018; 9(5): e00802-00818.
7. Wadhwani P, Afonin S, Ieronimo M, Buerck J, Ulrich ASJTJooc, Optimized protocol for synthesis of cyclic gramicidin S: starting amino acid is key to high yield. 2006; 71(1): 55-61.
8. Krishnakumari V, Binny TM, Adicherla H, Nagaraj RJAo, Escherichia coli Lipopolysaccharide modulates biological activities of human-β-defensin analogues but not non-ribosomally synthesized peptides. 2020; 5(12): 6366-6375.
9. Arutchelvan V, Kanakasabai V, Elangovan R, Nagarajan S, Muralikrishnan VJJohm, Kinetics of high strength phenol degradation using Bacillus brevis. 2006; 129(1-3): 216-222.
10. Deng Y, Wang L, Chen Y, Long YJB, Optimization of staining with SYTO 9/propidium iodide: interplay, kinetics and impact on Brevibacillus brevis. 2020; 69(2): 88-98.
11. Sanjukta S, Rai AK, Muhammed A, Jeyaram K, Talukdar NCJJoFF, Enhancement of antioxidant properties of two soybean varieties of Sikkim Himalayan region by proteolytic Bacillus subtilis fermentation. 2015; 14: 650-658.
12. Brewer GA, Gramicidin, In: Analytical Profiles of Drug Substances. Vol 8. Elsevier; 1979: 179-218.
13. Tymoszewska A, Ovchinnikov KV, Diep DB, et al. Lactococcus lactis resistance to aureocin A53-and enterocin L50-like bacteriocins and membrane-targeting peptide antibiotics relies on the YsaCB-KinG-LlrG four-component system. 2021; 65(12): e00921-00921.
14. Basu I, Chattopadhyay A, Mukhopadhyay CJBeBA-B, Ion channel stability of Gramicidin A in lipid bilayers: Effect of hydrophobic mismatch. 2014; 1838(1): 328-338.
15. Pérez-Betancourt Y, Zaia R, Evangelista MF, Characterization and Differential Cytotoxicity of Gramicidin Nanoparticles Combined with Cationic Polymer or Lipid Bilayer. 2022; 14(10): 2053.
16. David JM, Rajasekaran AKJJokc, VHL. Gramicidin A: a new mission for an old antibiotic. 2015; 2(1): 15.
17. Yamamura H, Isshiki K, Fujita Y, Gramicidin S-inspired antimicrobial cyclodextrin to disrupt gram-negative and gram-positive bacterial membranes. 2019; 10(8): 1432-1437.
18. Yasir M, Dutta D, Willcox MDJM, Activity of antimicrobial peptides and ciprofloxacin against Pseudomonas aeruginosa biofilms. 2020; 25(17): 3843.
19. Tempone AG, dos Santos Theodoro R, Romanelli MM, A new reduced chalcone-derivative affects the membrane permeability and electric potential of multidrug-resistant Enterococcus faecalis. 2022; 365: 110086.
20. Ragioto DA, Carrasco LD, Carmona-Ribeiro AMJIJoN, Novel gramicidin formulations in cationic lipid as broad-spectrum microbicidal agents. 2014; 9: 3183.
21. Whitlock BD, Arsenic Cellular Handling by Human Multidrug Resistance Protein 1 (MRP1/ABCC1) and MRP4 (ABCC4). 2021.

22. Moravej H, Moravej Z, Yazdanparast M, Antimicrobial peptides: features, action, and their resistance mechanisms in bacteria. 2018; 24(6): 747-767.
23. Macias CA, Chiao JW, Xiao J, Treatment with a novel hemigramicidin-Tempo conjugate prolongs survival in a rat model of lethal hemorrhagic shock. 2007; 245(2): 305.
24. David JM, Owens TA, Inge LJ, Bremner RM, Rajasekaran AKJMct, Gramicidin A Blocks Tumor Growth and Angiogenesis through Inhibition of Hypoxia-Inducible Factor in Renal Cell CarcinomaGramicidin A Inhibits Hypoxia-Inducible Factor. 2014; 13(4): 788-799.
25. Jia X, Patrzykat A, Devlin R, Antimicrobial peptides protect coho salmon from Vibrio anguillarum infections. 2000; 66(5): 1928-1932.
26. Kuebutornye FK, Abarike ED, Lu Y, Mechanisms and the role of probiotic Bacillus in mitigating fish pathogens in aquaculture. 2020; 46(3): 819-841.
27. Kumar P, Saha T, Behera S, Gupta S, Das S, Mukhopadhyay KJJoIB, Enhanced efficacy of a Cu2+ complex of curcumin against gram-positive and gram-negative bacteria: Attributes of complex formation. 2021; 222: 111494.
28. David JM, Owens TA, Barwe SP, Rajasekaran AKJMct, Gramicidin A Induces Metabolic Dysfunction and Energy Depletion Leading to Cell Death in Renal Cell Carcinoma CellsCytotoxicity of Gramicidin A in Renal Cell Carcinoma. 2013; 12(11): 2296-2307.
29. Antezana PE, Municoy S, Bellino MG, Martini MF, Desimone MFJEJoLS, Technology, Nanodelivery of the Gramicidin Peptide for Enhancing Antimicrobial Activity. 2021; 123(8): 2000389.
30. Meng Z, Zhou D, Gao Y, Zeng M, Wang WJAddr, miRNA delivery for skin wound healing. 2018; 129: 308-318.
31. Duff MF, Lisec CJJO, Topical steroids in burn patients: A systematic review of the literature and a descriptive analysis of topical KENACOMB® use at a major tertiary burn centre; 2022.
32. De Massis F, Zilli K, Di Donato G, Distribution of Brucella field strains isolated from livestock, wildlife populations, and humans in Italy from 2007 to 2015. 2019; 14(3): e0213689.
33. Borsetto C, Amos GC, da Rocha UN, Microbial community drivers of PK/NRP gene diversity in selected global soils. 2019; 7(1): 1-11.
34. Magalhães BQ, Machado FP, Sanches PS, Eugenia sulcata (Myrtaceae) Nanoemulsion Enhances the Inhibitory Activity of the Essential Oil on P2X7R and Inflammatory Response In Vivo. 2022; 14(5): 911.
35. Moura AS, Halder AK, DS Cordeiro MNJFMC, From biomedicinal to in silico models and back to therapeutics: a review on the advancement of peptidic modeling. 2019; 11(17): 2313-2331.
36. Lio PA, Kaye ETJIDC, Topical antibacterial agents. 2009; 23(4): 945-963.
37. Yang X, Yousef AEJWjom, biotechnology. Antimicrobial peptides produced by Brevibacillus spp.: structure, classification and bioactivity: a mini review. 2018; 34(4): 1-10.
38. Hu W, Lee K, Cross TJB, Tryptophans in membrane proteins: indole ring orientations and functional implications in the gramicidin channel. 1993; 32(27): 7035-7047.
39. Ageitos J, Sánchez-Pérez A, Calo-Mata P, Villa TJBp, Antimicrobial peptides (AMPs): Ancient compounds that

represent novel weapons in the fight against bacteria. 2017; 133: 117-138.

40. Ganesan P, Ramalingam RJJoCB, Investigation of structural stability and functionality of homodimeric gramicidin towards peptide-based drug: a molecular simulation approach. 2019; 120(4): 4903-4911.
41. Shao C, Zhu Y, Lai Z, Tan P, Shan AJFMC, Antimicrobial peptides with protease stability: progress and perspective. 2019; 11(16): 2047-2050.
42. He T, Qu R, Zhang JJJoPS, Current synthetic chemistry towards cyclic antimicrobial peptides. 2022; 28(6): e3387.
43. Wang GJCb, Post-translational modifications of natural antimicrobial peptides and strategies for peptide engineering. 2012; 1(1): 72-79.
44. Lee SJ, Li Z, Litan A, Yoo S, Langhans SAJBcb, EGF-induced sodium influx regulates EGFR trafficking through HDAC6 and tubulin acetylation. 2015; 16(1): 1-15.
45. Ren C, Wen X, Mencius J, Quan SJB, Bioprocessing, Selection and screening strategies in directed evolution to improve protein stability. 2019; 6(1): 1-14.
46. Ashrafuzzaman MJM, The antimicrobial peptide gramicidin s enhances membrane adsorption and ion pore formation potency of chemotherapy drugs in lipid bilayers. 2021; 11(4): 247.
47. Abraham T, Prenner EJ, Lewis RN, Structure–activity relationships of the antimicrobial peptide gramicidin S and its analogs: aqueous solubility, self-association, conformation, antimicrobial activity and interaction with model lipid membranes. 2014; 1838(5): 1420-1429.
48. Bhatta DR, Kapadnis BJSW, Production optimization and characterization of bioactive compound against Salmonella bacillus subtilis KBB isolated from Nepal. 2010; 8(8): 19-29.
49. Prenner EJ, Lewis RN, Neuman KC, Nonlamellar phases induced by the interaction of gramicidin S with lipid bilayers, A possible relationship to membrane-disrupting activity. 1997; 36(25): 7906-7916.
50. Bakels S, Gaigeot M-P, Rijs AMJCr, Gas-phase infrared spectroscopy of neutral peptides: Insights from the far-IR and THz domain. 2020; 120(7): 3233-3260.
51. Evans LS, Hussain R, Siligardi G, Williamson PTJBeBA-B, Magnetically aligned membrane mimetics enabling comparable chiroptical and magnetic resonance spectroscopy studies. 2020; 1862(9): 183343.
52. Rizzolo F, Sabatino G, Chelli M, Rovero P, Papini AMJIJoPR, Therapeutics. A convenient microwave-enhanced solid-phase synthesis of difficult peptide sequences: case study of gramicidin A and CSF114 (Glc). 2007; 13(1): 203-208.
53. Jelokhani-Niaraki M, Kondejewski LH, Farmer SW, Hancock RE, Kay CM, Hodges RSJBJ. Diastereoisomeric analogues of gramicidin S: structure, biological activity and interaction with lipid bilayers. 2000; 349(3): 747-755.
54. Zerfas BL. Creating Novel Antimicrobial Peptides: From Gramicidin A to Screening a Cyclic Peptide Library, Boston College; 2017.
55. Fenibo EO, Douglas SI, Stanley HOJJAM, A review on microbial surfactants: production, classifications, properties and characterization. 2019; 18(3): 1-22.
56. Drannikov A, Influence of gramicidin S and [beta]-cyclodextrin complexation techniques on the drug release. 2020 (pp. 525-526).
57. Abou-Taleb NH, El-Sherbiny DT, El-Enany NM, El-Subbagh HIJMJ, A new

grey relational analysis application in analytical chemistry: Natural deep eutectic solvent as a green extractant for HPLC determination of lamotrigine in plasma. 2022; 172: 106918.

58. Babii O, Afonin S, Schober T, Peptide drugs for photopharmacology: how much of a safety advantage can be gained by photocontrol? 2020; 2(1): FDD28.

59. Molineau J, Hideux M, West CJJoP, Analysis B, Chromatographic analysis of biomolecules with pressurized carbon dioxide mobile phases–A review. 2021; 193: 113736.

60. Cole AM, Ganz TJB, Human antimicrobial peptides: analysis and application. 2000; 29(4): 822-831.

61. Murat M, A brighter method to illuminate the dark side of adhesion G protein-coupled receptors: detection of the ADGRG1 dimers by Bioluminescence Resonance Energy Transfer *(BRET)*, Middle East Technical University; 2021.

62. Zangeneh S, Torki M, Abdolmohammadi A, Saki AJAPS, Dietary addition of lysophospholipids and vitamin C affects growth performance, serum metabolites, antioxidant capacity and carcass characteristics of broiler chickens reared under low ambient temperature. 2020; 60(12): 1557-1566.

63. Sun X, Li H, Alfermann J, Mootz HD, Yang HJB. Kinetics profiling of gramicidin S synthetase A, a member of nonribosomal peptide synthetases. 2014; 53(50): 7983-7989.

64. Chun L, Xiaoping Y, Guangrong H, Hong WJAJoB. Preparation and characterization of antigenic properties of gramicidin A-keyhole limpet hemocyanin and gramicidin A-ovalbumin conjugates. 2009; 8(24).

65. Ortiz-Boyer F, Tena M, de Castro ML, Valcarcel MJJop, analysis b, Development and validation of chromatographic methods (HPLC and GC) for the determination of the active components (benzocaine, tyrothricin and menthol) of a pharmaceutical preparation. 1995; 13(11): 1297-1303.

66. Eissa MJJoaB, Sciences P. Validation of Microbiological Assay Design of Neomycin Sulfate in 30 x 30 cm Rectangular Antibiotica Plate. 2022; 5(2): 54-63.

67. Lee DL, Mant CT, Hodges RSJJoBC, A novel method to measure self-association of small amphipathic molecules: temperature profiling in reversed-phase chromatography. 2003; 278(25): 22918-22927.

68. Chu W, Prodromou R, Day KN, Peptides and pseudopeptide ligands: a powerful toolbox for the affinity purification of current and next-generation biotherapeutics. 2021; 1635: 461632.

69. Sikora K, Jaśkiewicz M, Neubauer D, Migoń D, Kamysz WJP, The role of counter-ions in peptides—an overview. 2020; 13(12): 442.

70. Barth HG, Boyes BE, Jackson CJAC, Size exclusion chromatography. 1996; 68(12): 445-466.

71. Davies-Tuck M, Lee T-H, Apffel A, Aguilar M-IJJoCA, Hydrophobic and electrostatic forces control the retention of membrane peptides and proteins with an immobilised phosphatidic acid column. 2007; 1156(1-2): 167-173.

72. Sewald N, Jakubke H-D, Peptides: chemistry and biology. John Wiley & Sons; 2015.

73. Zajickova Z, Nováková L, Svec FJAC, Monolithic Poly (styrene-co-divinylbenzene) Columns for Supercritical Fluid Chromatography–Mass Spectrometry Analysis of Polypeptide. 2020; 92(17): 11525-11529.

74. Andreev K, Martynowycz MW, Huang ML, Hydrophobic interactions modulate

antimicrobial peptoid selectivity towards anionic lipid membranes. 2018; 1860(6): 1414-1423.

75. Pirok BW, Stoll DR, Schoenmakers PJJAC, Recent developments in two-dimensional liquid chromatography: fundamental improvements for practical applications. 2018;91(1):240-263.

76. Ayon NJ, Metabolomics and Chemical Library Screening for Antibacterial Drug Discovery, University of Missouri-Kansas City; 2020.

77. Bucur B, Purcarea C, Andreescu S, Vasilescu AJS, Addressing the Selectivity of Enzyme Biosensors: Solutions and Perspectives. 2021; 21(9): 3038.

78. Yousfi H, Ranque S, Rolain J-M, Bittar FJAr, In vitro polymyxin activity against clinical multidrug-resistant fungi. 2019; 8(1): 1-10.

79. Edge R, Argáez C, Topical antibiotics for impetigo: a review of the clinical effectiveness and guidelines. 2017.

80. Rabanal F, Cajal Y, Therapeutic potential of antimicrobial peptides, In: New Weapons to Control Bacterial Growth. Springer; 2016: 433-451.

81. Kalelkar PP, Riddick M, García AJJNRM, Biomaterial-based antimicrobial therapies for the treatment of bacterial infections. 2022; 7(1): 39-54.

82. Berditsch M, Jäger T, Strempel N, Synergistic effect of membrane-active peptides polymyxin B and gramicidin S on multidrug-resistant strains and biofilms of Pseudomonas aeruginosa. 2015; 59(9): 5288-5296.

83. Berditsch M, Trapp M, Afonin S, Antimicrobial peptide gramicidin S is accumulated in granules of producer cells for storage of bacterial phosphagens. 2017; 7(1): 1-11.

84. Adams E, Schepers R, Gathu L, Liquid chromatographic analysis of a formulation containing polymyxin, gramicidin and neomycin. 1997; 15(4): 505-511.

85. Carrasco DA, Vander Straten M, Tyring SKJJocm, surgery, A review of antibiotics in dermatology. 2002; 6(2): 128-150.

86. Enayathullah MG, Parekh Y, Banu S, Gramicidin S and melittin: Potential anti-viral therapeutic peptides to treat SARS-CoV-2 infection. 2022; 12(1): 1-17.

87. Gillespie SH, Medical microbiology illustrated, Butterworth-Heinemann; 2014.

88. Pazhayam NM, Chhibber-Goel J, Sharma AJDDT, New leads for drug repurposing against malaria, 2019; 24(1): 263-271.

89. Van Boxtel CJJDB, Risks, Dermatologicals and Miscellaneous Agents, 479.

90. Phillip P, Diagnosis and Management of Infectious Diseases, In: Phillip Petersen; 2007.

91. Kim U, Chung L-yJJon, Dual GABAergic synaptic response of fast excitation and slow inhibition in the medial habenula of rat epithalamus. 2007; 98(3): 1323-1332.

92. Takada Y, Itoh H, Paudel A, Discovery of gramicidin A analogues with altered activities by multidimensional screening of a one-bead-one-compound library, 2020; 11(1): 1-10.

93. Murk J-L, Bacitracin and gramicidin, In: *K*ucers' The Use of Antibiotics, CRC Press; 2017:1454-1459.

94. Orazi G, Ruoff KL, O'Toole GAJM, Pseudomonas aeruginosa increases the sensitivity of biofilm-grown Staphylococcus aureus to membrane-targeting antiseptics and antibiotics. 2019; 10(4): e01501-01519.

95. Wesgate R, Evangelista C, Atkinson R, Shepard A, Adegoke O, Maillard JYJJoAM, Understanding the risk of emerging bacterial resistance to over the

counter antibiotics in topical sore throat medicines. 2020; 129(4): 916-925.

96. Herrera G, Martinez A, Blanco M, O'Connor JEJCTJotISfAC, Assessment of Escherichia coli B with enhanced permeability to fluorochromes for flow cytometric assays of bacterial cell function, 2002; 49(2): 62-69.
97. Yeaman MR, Yount NYJPr, Mechanisms of antimicrobial peptide action and resistance. 2003; 55(1): 27-55.
98. Strakhovskaya M, Antonenko YN, Pashkovskaya A, Electrostatic binding of substituted metal phthalocyanines to enterobacterial cells: its role in photodynamic inactivation. 2009; 74(12): 1305-1314.
99. Gosling AA, Labelle AL, Breaux CBJVo, Management of spontaneous chronic corneal epithelial defects (SCCEDs) in dogs with diamond burr debridement and placement of a bandage contact lens. 2013; 16(2): 83-88.
100. Gurov A, Borovkova K, Kryshen K, Nikiforova L, Salmova Jja, Chemotherapy. Evaluation of the Bactericidal Activity of Gramicidin S Against Streptococcus pneumoniae and Staphylococcus aureus Clinical Isolates with Single and Multiple Exposure. 2022; 67(7-8): 8-18.
101. Nahmias AJ, Lepper MH, Hurst V, Mudd SJAJoPH, Health tN, Epidemiology and treatment of chronic staphylococcal infections in the household. 1962; 52(11): 1828-1843.
102. Smirnov S, Belashov A, Demin OJejops, Optimization of antimicrobial drug gramicidin S dosing regime using biosimulations. 2009; 36(1): 105-109.
103. Essack S, Bell J, Burgoyne DS, Duerden M, Shephard AJJoCP, Therapeutics, Topical (local) antibiotics for respiratory infections with sore throat: an antibiotic stewardship perspective. 2019; 44(6): 829-837.
104. Szałata KJD, Transport CBPfP, Surfactant/Gramicidin micelles bioactivity study,163.
105. Douglas RG, Reinig M, Neale M, Frischknecht FJMj, Screening for potential prophylactics targeting sporozoite motility through the skin. 2018; 17(1): 1-10.
106. Hart A, McColl K, Thomas C, Orr NJJoab, Performance profiles of topical antimicrobials in vitro. 1989; 67(3): 317-327.
107. Pavithrra G, Rajasekaran RJISCLS, Identification of effective dimeric gramicidin-D peptide as antimicrobial therapeutics over drug resistance: in-silico approach. 2019; 11(4): 575-583.
108. Chen T, Wang Y, Yang Y, Gramicidin inhibits human gastric cancer cell proliferation, cell cycle and induced apoptosis. 2019; 52.
109. Boix-Lemonche G, Lekka M, Skerlavaj BJA, A rapid fluorescence-based microplate assay to investigate the interaction of membrane active antimicrobial peptides with whole gram-positive bacteria. 2020; 9(2): 92.
110. Uddin TM, Chakraborty AJ, Khusro A, Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. 2021; 14(12): 1750-1766.
111. Guan Q, Huang S, Jin Y, Campagne Rm, Alezra Vr, Wan YJJomc, Recent advances in the exploration of therapeutic analogues of gramicidin S, an old but still potent antimicrobial peptide. 2019; 62(17): 7603-7617.
112. Barani M, Zeeshan M, Kalantar-Neyestanaki D, Nanomaterials in the management of gram-negative bacterial infections. 2021; 11(10): 2535.

113. Buta A, Nazaruk E, Dziubak D, Szewczyk A, Bilewicz RJB, Properties of electrode-supported lipid cubic mesophase films with embedded gramicidin A: Structure and ion-transport studies. 2022; 144: 108042.
114. Yang J, Firdaus F, Azuar A, Cell-penetrating peptides-based liposomal delivery system enhanced immunogenicity of peptide-based vaccine against Group A Streptococcus. 2021; 9(5): 499.