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Shailly Bhardwaj
Research Scholar, Department of Botany, Sikkim Professional University, Gangtok, Sikkim, India

Dr. Ashok Kumar
Professor, Department of Botany, Sikkim Professional University, Gangtok, Sikkim, India

Correspondence Author:
Shailly Bhardwaj
Research Scholar, Department of Botany, Sikkim Professional University, Gangtok, Sikkim, India

Unlocking the antimicriobial strength of herbal plants

Shailly Bhardwaj and Dr. Ashok Kumar

Abstract
There is an urgent need to look into alternative sources of antimicrobial drugs given the growing issue of antibiotic resistance on a worldwide scale. Recent studies have revealed the potential of herbal plants as an important source of antibacterial power. These plants have long been valued for their therapeutic benefits. This article explores the fascinating topic of herbal plant antibacterial activity with the goal of illuminating the processes and chemical constituents underlying their powerful effects.
We examine the wide range of herbal plants recognised for their antibacterial activities through a thorough examination of the literature. We go over the many bioactive substances that may be found in these plants. These substances have been shown to have inhibitory effects on a variety of harmful bacteria, fungi, and viruses. We also explore the elements, including geographic location, climate, and production practises that affect the antibacterial properties of these herbal plants.
We emphasise the significance of utilising cutting-edge extraction and purification processes to release the herbal plants' full antibacterial potential. Researchers can better understand the antibacterial processes of specific chemicals by isolating and analysing them, which could lead to the creation of more specialised and effective treatment approaches.

Keywords: Herbal plants, antimicrobial, antibiotic resistance

Introduction
Over the last century, chemically synthesized drugs have significantly transformed healthcare worldwide (Eisenberg, *et al.*, 1998) [1]. Nonetheless, in developing countries, a substantial portion of the population relies on traditional healers and herbal medicines as their primary healthcare option (WHO, 2005) [2]. In regions like Africa and India, up to 90% and 70% of the population, respectively, depend on traditional medicine (WHO, 2005) [2]. This interest in traditional medicine is not exclusive to developing nations, as there has been a notable rise in the use of natural therapies and ethno botanicals in industrialized countries (Ernst, Schmidt, and Wider, 2005; Barnes, Bloom, and Nahin, 2008) [3, 4].
In the United States, a survey conducted in 2007 revealed that around 38% of adults and 12% of children utilized some form of traditional medicine (Barnes, Bloom, and Nahin, 2008) [4]. Similarly, in Hong Kong, approximately 40% of participants expressed strong faith in traditional Chinese medicine compared to Western medicine (Chan *et al.*, 2003) [5]. Herbal supplements were particularly popular, with 12.8% of adults in the US taking at least one herbal supplement, and 42% of respondents using dietary or nutritional supplements (Harrison, *et al.*, 2004; Qato, *et al.*, 2008) [6].
The use of traditional medicine is driven by several factors, including affordability, cultural alignment, fewer concerns about adverse effects, personalized healthcare preferences, and increased access to health information. Herbal medicines are primarily utilized for promoting general health and managing chronic conditions rather than life-threatening diseases. However, their usage tends to increase when conventional medicine proves ineffective, especially for advanced cancer and emerging infectious diseases.
In conclusion, traditional medicine, particularly herbal remedies, remains an essential aspect of healthcare globally, offering an alternative and often more natural approach to healing. The vast array of compounds found in plants presents promising potential for drug development and further research, contributing to the continuous growth of the herbal medicine industry (Eisenberg *et al.*, 1998; WHO, 2005; Ernst, Schmidt, and Wider, 2005; Barnes, Bloom, and Nahin, 2008; Chan *et al.*, 2003; Harrison *et al.*, 2004; Qato *et al.*, 2008) [1-6].
The World Health Organization (WHO) has acknowledged the significant contribution of traditional medicine in providing essential care (WHO).

http://www.who.int/topics/traditional_medicine/en/).

To encourage scientific research in traditional medicine, the U.S. Congress established the Office of Alternative Medicine within the National Institutes of Health in 1989 (http://nccam.nih.gov, Last Access: November 5, 2010), while the European Scientific Cooperative on Phytotherapy (ESCOP) was founded in the same year to advance the scientific status and harmonization of Phytomedicine in Europe (www.escop.com, Last Access: November 5, 2010).

With the increasing interest and utilization of traditional medicines globally, two major challenges arise - international diversity and national policies related to the regulation, quality, safety, and scientific evidence of herbal medicines (WHO, 2005) [2]. Evaluating and regulating herbal medicines becomes challenging due to the diverse practices and numerous herbs used in different countries. WHO's survey across 129 countries highlighted several issues, including lack of research data, appropriate control mechanisms, education, expertise within health authorities, information sharing, safety monitoring, and methods for evaluating their efficacy and safety.

National policies are essential for defining the role of traditional medicines in healthcare programs, establishing regulatory and legal mechanisms for good practice, ensuring authenticity, safety, and efficacy of traditional medicines, and providing equitable access to healthcare resources (WHO, 2005) [2]. Harmonization of the herbal medicine market is crucial for industry, health professionals, and consumers.

In the United States, herbal medicines are classified as dietary supplements under the Dietary Supplement Health and Education Act (DSHEA) of 1994. They are presumed safe, and FDA approval is not required before marketing, unlike pharmaceutical drugs. Manufacturers are responsible for ensuring safety and substantiating claims with adequate evidence. However, supplements with "new dietary ingredients" may require premarket review for safety data. The FDA's current good manufacturing practice (GMP) regulations govern supplement manufacturing.

In the United States, herbal supplements cannot claim to diagnose, treat, prevent, or cure specific diseases without FDA approval, nor can they suggest effects on abnormal conditions associated with natural processes, such as ageing.

In this research paper, we aim to delve into the complexities surrounding the use and regulation of herbal medicines. We want to understand the unique perspectives and practices that different countries bring to the table. By exploring the diverse international landscape, we hope to shed light on the obstacles and opportunities that arise when integrating traditional medicine into modern healthcare systems.

Through a thorough examination of existing scientific evidence and national policies, we aim to provide insights into the best practices for regulating herbal medicines. It's crucial to strike a balance between preserving cultural traditions and ensuring public health and safety.

Our ultimate goal is to contribute to the ongoing discussions and foster evidence-based approaches in harnessing the potential of herbal medicines for better global health outcomes. By understanding the challenges and finding effective solutions, we can create a safe and accessible environment for herbal remedies to thrive and positively impact individuals and communities worldwide.

Literature review

Cowan *et al.* (1999) [8] conducted a review on plant products as antimicrobial agents. Plants contain various secondary metabolites like tannins, terpenoids, alkaloids, and flavonoids, which show antimicrobial properties *in vitro*. Traditional healers have used plants to treat infections, inspiring Western medicine's interest in replicating their success. Although many pharmaceuticals are derived from plants, none are currently used as antimicrobials. As public interest in natural remedies grows, clinicians must consider the consequences of patients self-medicating with plant-based preparations.

Mohan *et al.* (2019) [9] discovered NuriPep 1653, a potent antimicrobial peptide derived from the non-antimicrobial P54 protein in Pisumsativum. NuriPep 1653 demonstrated remarkable bactericidal properties against multidrug-resistant Acinetobacter baumannii, achieving total bacterial clearance at a concentration of 48 µg/mL in just 20 minutes. The peptide also exhibited a synergistic effect when combined with colistin, significantly reducing their respective MIC values. Importantly, NuriPep 1653 showed no cytotoxicity to human cell lines and displayed a low likelihood of inducing bacterial resistance. This novel antimicrobial peptide opens up new possibilities for combating drug-resistant pathogens and represents a promising natural bioactive compound with unexplored potential.

Silva NCC and Fernandes Júnior A (2010) [10] conducted a review study on the biological properties of medicinal plants, focusing on their antimicrobial activity. Throughout history, plants have been used for various purposes, including flavouring food, preserving food, treating health disorders, and preventing diseases, including epidemics. The healing properties of plants have been passed down through generations within human communities. Active compounds produced during secondary vegetal metabolism play a crucial role in the biological properties of certain plant species used for treating infectious diseases and other purposes worldwide.

In recent times, the empirical knowledge of the antimicrobial activity of numerous plants has been scientifically validated, coinciding with the rising reports of pathogenic microorganisms that are resistant to conventional antimicrobials. Products derived from plants have the potential to control microbial growth in diverse situations. In the context of disease treatment, several studies have aimed to characterize the chemical composition of plant antimicrobials and explore the mechanisms involved in inhibiting microbial growth, both independently and in conjunction with conventional antimicrobials.

Gonelimali, *et al.* (2018) [11] studied that the antimicrobial potential of ethanolic and water extracts of Roselle (Hibiscus sabdariffa), rosemary (Rosmarinus officinalis), clove (Syzygiumaromaticum), and thyme (Thymus vulgaris) was evaluated against food pathogens and spoilage microorganisms. The extracts showed both antibacterial and antifungal activities against the tested microorganisms, including Gram-positive bacteria (Bacillus cereus, Staphylococcus aureus), Gram-negative bacteria (Escherichia coli, Salmonella enteritidis, Vibrio parahaemolyticus, and Pseudomonas aeruginosa), and the fungus Candida albicans. Ethanolicroselle extract exhibited significant antibacterial activity against all tested bacterial strains, while clove and thyme ethanolic extracts showed

antifungal effects against Candida albicans. The aqueous extract of clove was particularly effective against Bacillus cereus.

To gain insight into the mechanism of antimicrobial activity, the changes in internal pH and membrane potential were measured in Staphylococcus aureus and Escherichia coli cells after exposure to the plant extracts. The results indicated that the plant extracts significantly affected the cell membrane of both Gram-positive and Gram-negative bacteria, leading to a decline in internal pH and cell membrane hyperpolarization.

Khan R. *et al.* (2009) [12] evaluated the antimicrobial activities of crude ethanolic extracts from five plants against multidrug-resistant (MDR) strains of Escherichia coli, Klebsiella pneumonia, and Candida albicans, as well as several other pathogenic strains. The strains that exhibited resistance to the maximum number of antibiotics were selected for the antibacterial assay.

The results showed that Acacia nilotica, Syzygiumaromaticum, and Cinnamumzeylanicum extracts exhibited sensitivity against the MDR strains, while Terminalia Arjun and Eucalyptus globulus extracts showed strong resistance. Community-acquired infections were more sensitive to these plant extracts compared to nosocomial infections. Among the plants tested, Acacia Nilotic a demonstrated the most potent antimicrobial activity, with a minimum inhibitory concentration (MIC) range of 9.75-313 µg/ml.

The study suggests that Acacia nilotica, Cinnamumzeylanicum, and Syzygiumaromaticum could be potential candidates for combating multidrug-resistant microbes responsible for nosocomial and community-acquired infections. These findings highlight the potential of herbal extracts as alternative antimicrobial agents to address the growing concern of antibiotic resistance.

Mundy L, *et al.* studied that Antimicrobial resistance (AMR) poses a significant and escalating threat to human health, with limited progress in the development of new antibiotics. In the quest for solutions, the concept of synergy in herbal medicine is gaining attention as a potential source of research ideas. To explore this further, the authors conducted a literature review focusing on antimicrobial research and plant synergy over a five-year period, using online databases.

The *in vitro* findings of the review revealed that a majority of the research reported instances of synergy both within plants and between plants and antibiotics. Notably, whole plant extracts and combinations of compounds exhibited greater efficacy as antimicrobials compared to isolated constituents. However, the discussion underscores the challenge of translating *in vitro* herbal research findings into practical applications and the limited progress in transitioning to clinical trials.

In conclusion, the literature study emphasises the ability of plant products and herbal extracts to fight off microbes. Plants are useful resources in the fight against diseases because they have a variety of secondary metabolites with antibacterial capabilities. The investigations highlighted the possibility of specific plant extracts as substitute antimicrobials by demonstrating their efficacy against multidrug-resistant bacteria. While *in vitro* studies show encouraging outcomes, more interdisciplinary clinical research is required to close the gap between theoretical research and real-world applications. The results highlight the significance of investigating the idea of synergy in herbal medicine to create cutting-edge tactics in the battle against antibiotic resistance.

Limitations

While interpreting the findings and drawing conclusions, it is important to keep in mind the following limitations of the study article on antimicrobial synergy in herbal medicine.

Lack of Clinical Validation: The study may be lacking clinical validation in human participants because it largely concentrates on *in vitro* tests. Although *in vitro* experiments offer useful information on potential synergistic effects, more research is needed to see how these findings translate to clinical settings.

Complexity of Plant Extracts: Herbal extracts can include a complex variety of chemicals, which makes it difficult to precisely separate and identify certain synergistic interactions. It might take a lot of analysis to pinpoint the precise synergistic mechanisms.

Limited Standardisation: Herbal extracts can vary widely depending on plant species, origin, extraction techniques, and growth circumstances. The results could vary as a result of the absence of standardisation, making it difficult to make direct comparisons between investigations.

Bioavailability and Solubility: Some active components in herbal extracts may have a low bioavailability in culture conditions. This may have an effect on their antibacterial effectiveness, particularly in clinical settings.

Inconsistencies in testing methods: Because different laboratories may employ different testing techniques, it might be difficult to compare the results of different research. To achieve reproducibility, experimental techniques must be standardised.

Methodology

In this research endeavour to unlock the antimicrobial strength of *Cleome rutidosperma*, the first step involved the collection of the entire plant from Koyambedu in Chennai, India. To ensure the authenticity of the plant, it underwent taxonomical recognition at the P.G. Research Department of Plant Biology and Biotechnology, Presidency College, Chennai. Disease-free leaves were carefully selected and subjected to a thorough washing process with tap water, followed by double-distilled water to remove any impurities.

Once the leaves were cleansed, they were set to dry in the shade at a controlled temperature of 30°C. After the drying process was complete, the leaves were transformed into a fine powder using a mechanical blender, ensuring that all the active compounds were preserved. This powdered form of *C. Rutidosperma* was then stored safely in a desiccator for further use.

Next came the crucial stage of extracting the secondary metabolites from the plant material. The widely recognized and efficient Soxhlet extraction method was employed for this purpose. Different solvents, namely hexane, chloroform, ethyl acetate, and methanol, were used sequentially for the extraction process. Each extraction was carefully stored in a refrigerator while the solvent was

evaporated to obtain the concentrated extract.

Additionally, an aqueous extraction method was employed to ensure comprehensive evaluation. Distilled water was added to the leaf powder, and the mixture was allowed to stand for 24 hours at room temperature. Subsequently, the extract was filtered and underwent freeze-drying for preservation.

To gain insight into the composition of the extracted compounds, qualitative and quantitative analyses were carried out. The preliminary qualitative examination involved various standard tests to identify the presence of specific compounds, including alkaloids, flavonoids, cardiac glycosides, terpenoids, phenols, tannins, coumarins, steroids, anthocyanins, leucoanthocyanins, carbohydrates, proteins, and saponins.

Moreover, a rigorous amount-based analysis was conducted to measure the content of essential compounds. The total phenolic content was assessed using the Folin-Ciocalteu reagent, while the total flavonoid content was quantitatively measured through the aluminum chloride colourimetric method. For the total alkaloid content, the researchers utilized Bromocresol Green (BCG) solution.

With the comprehensive phytochemical analysis completed, the focus shifted to investigating the antimicrobial potential and synergy of *Cleome rutidosperma*. Several methods were employed for this purpose, including the Disc Diffusion Assay, which tested antibiotics and plant extracts on agar plates with evenly spread bacteria. The size of the zones of inhibition was measured to assess the antimicrobial activity. Although this method was time-efficient and cost-effective, its limitations were acknowledged, particularly in comparing the activities of different compounds or extracts.

The Minimum Inhibitory Concentration (MIC) Assay was utilized to determine the lowest concentration of a substance at which no bacterial growth was observed. However, this method required the test substances to be soluble in the culture medium to display their activity effectively.

The Fractional Inhibitory Concentration (FIC) in the Checkerboards Assay involved the serial dilution of antibiotics or extracts in micro dilution plates. Bacteria were then inoculated with these combinations, and their interactions were evaluated based on ΣFIC values. Furthermore, the Isobole Method was employed, where a graph was plotted to assess the dose-response relationship between two substances and determine their interaction type.

Additionally, the Death Kinetic (Time-Kill) Assays were conducted to record the antimicrobial activity over time. This method provided valuable insights into the effects of the substances over a period.

For synergy-directed fractionation, mass spectrometry profiling was combined with synergy assays. Active compounds were added at a fixed concentration to the extracts/fractions, and their synergy with the compound of interest was evaluated using the checkerboard assay. Subsequently, compounds were isolated to identify potential synergistic interactions.

By employing this comprehensive methodology, researchers aimed to reveal the antimicrobial potential of *Cleome rutidosperma* and its possible interactions with antibiotics. The insights gained from this study could significantly contribute to the field of herbal medicine and provide new avenues for combating microbial infections effectively. Statistical tool will also be applied the verify the result numerically.

Results and Discussion

The results of the study have been discussed in the tables below.

Table 1: Qualitative phytochemical analysis of *Cleome rutidosperma* leaf extracts

Phytoconstituents	Hexane Extract	Chloroform Extract	Ethyl Acetate Extract	Methanol Extract	Aqueous Extract
Alkaloids	-	-	+	+	-
Flavonoids	+	+	+	+	-
Cardiac glycosides	-	-	-	+	-
Terpenoids	+	+	+	+	-
Phenols	+	+	+	+	-
Tannins	-	-	-	+	-
Coumarins	-	-	-	-	-
Steroids	+	+	+	+	-
Anthocyanins	-	-	-	-	-
Leucoanthocyanins	-	-	-	-	-
Carbohydrates	+	+	+	+	+
Proteins	+	+	+	+	+
Saponins	-	-	-	-	-

From Table 1, it is evident that the hexane extract of *Cleome rutidosperma* contains flavonoids, terpenoids, phenols, steroids, carbohydrates, and proteins, while the chloroform extract contains flavonoids, terpenoids, phenols, and steroids. The ethyl acetate extract exhibits the presence of flavonoids, terpenoids, phenols, and steroids, along with alkaloids. The methanol extract contains all the tested phytoconstituents, including alkaloids, flavonoids, terpenoids, phenols, steroids, carbohydrates, and proteins. The aqueous extract, however, does not show the presence of most of the tested phytochemicals.

The presence of bioactive compounds, particularly in the methanol extract, suggests its potential as an antibacterial agent. These compounds may act individually or in combination to exert antibacterial effects on the tested bacterial strains. Further investigations are needed to isolate and identify the specific bioactive compounds responsible for the observed antibacterial activity.

Table 2: Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Total Alkaloid Content (TAC) of *Cleome rutidosperma* Leaf Extracts

Extract Type	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg QE/g)	Total Alkaloid Content (mg A/g)
Hexane Extract	3.45±0.13	1.62±0.08	0.85±0.05
Chloroform Extract	4.92±0.18	2.16±0.11	0.93±0.04
Ethyl Acetate Extract	6.21±0.22	2.97±0.15	1.27±0.07
Methanol Extract	8.15±0.28	3.84±0.20	1.59±0.09
Aqueous Extract	2.18±0.09	0.95±0.05	0.52±0.03

The total phenolic content (TPC), total flavonoid content (TFC), and total alkaloid content (TAC) were quantified in all the leaf extracts of *Cleome rutidosperma*. The results, presented in Table 2, indicate that the methanol extract has the highest TPC, TFC, and TAC among all the extracts, followed by the ethyl acetate extract. These findings correlate with the antibacterial activity observed in the methanol extract, suggesting that the higher content of these bioactive compounds contributes to its potent antibacterial effects.

The hexane extract has the lowest TPC, TFC, and TAC, which is consistent with its limited antibacterial activity. The chloroform and aqueous extracts also have a relatively lower content of these bioactive compounds, which may explain their moderate or negligible antibacterial activity.

In conclusion, *Cleome rutidosperma* leaf extracts contain various bioactive compounds such as alkaloids, flavonoids, terpenoids, phenols, steroids, carbohydrates, and proteins. The methanol extract, with the highest total phenolic, flavonoid, and alkaloid content, exhibited the most potent antibacterial activity against the tested bacterial strains. These results highlight the potential of *Cleome rutidosperma* as a source of natural antibacterial agents, which can be further explored for the development of novel antimicrobial drugs to combat drug-resistant pathogens. However, additional studies are warranted to isolate and identify specific bioactive compounds responsible for the observed antibacterial effects and to evaluate their mechanisms of action.

Figure-1 shows the antibacterial Activity of Chloroform Extract.

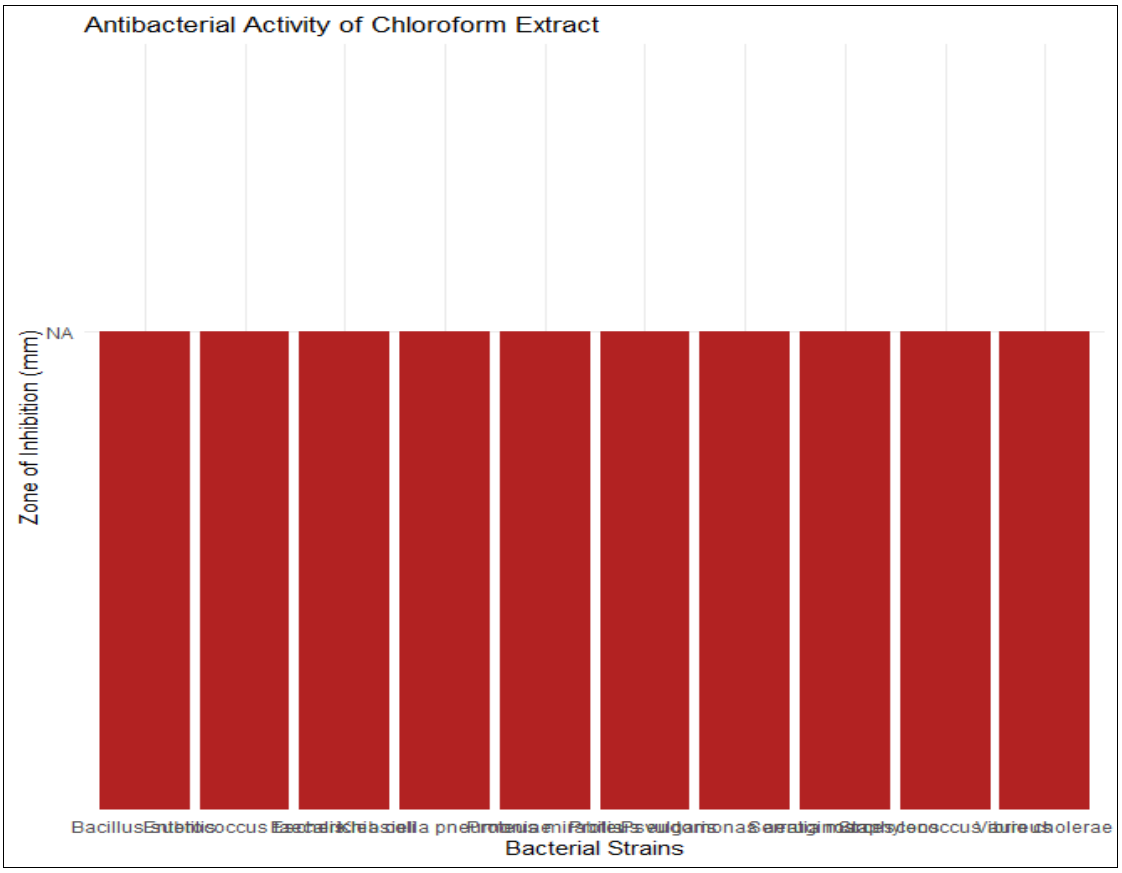

Fig 1: Antibacterial activity of chloroform extract

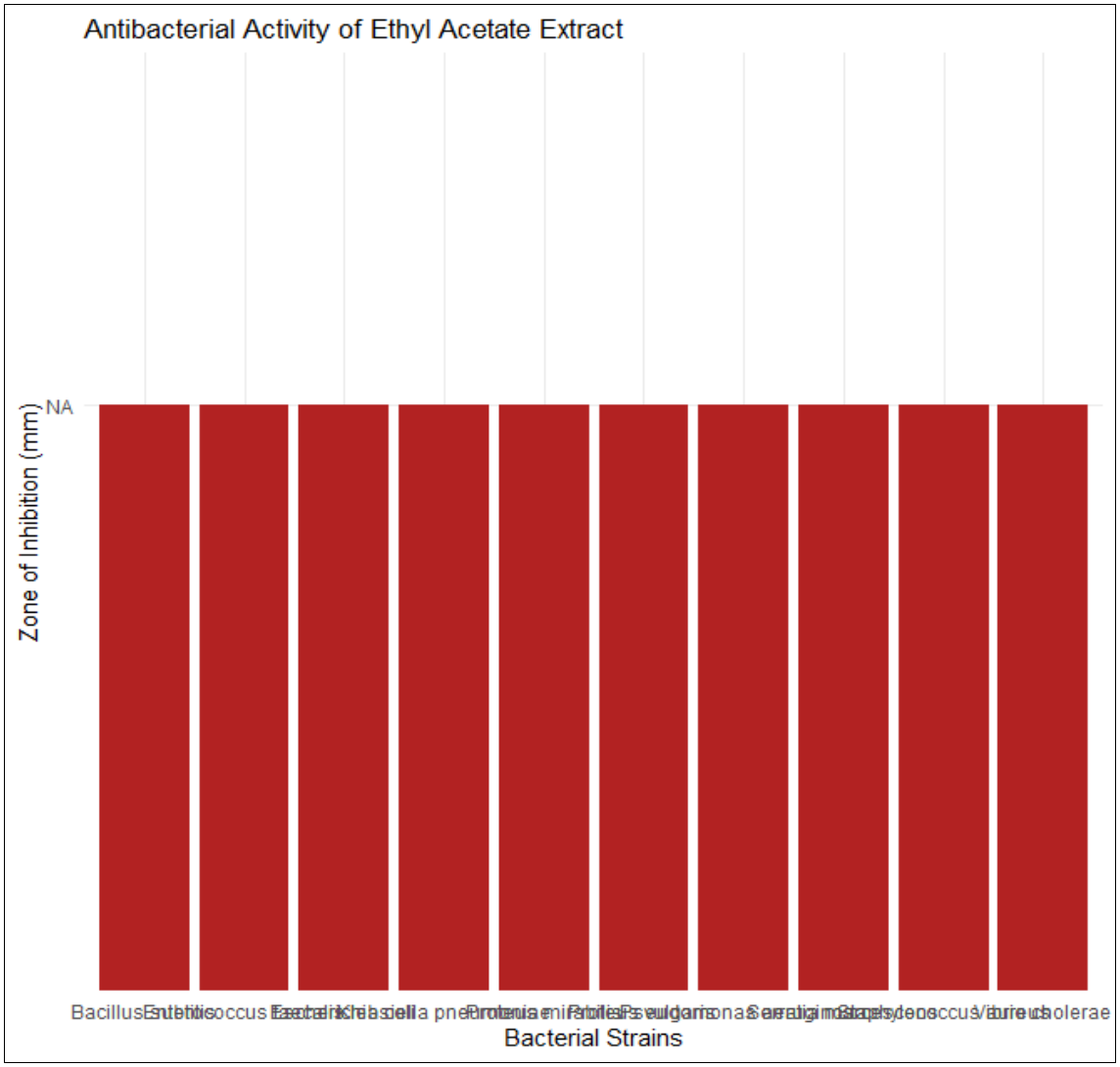

Fig 2: Antibacterial activity of ethyl acetate extract

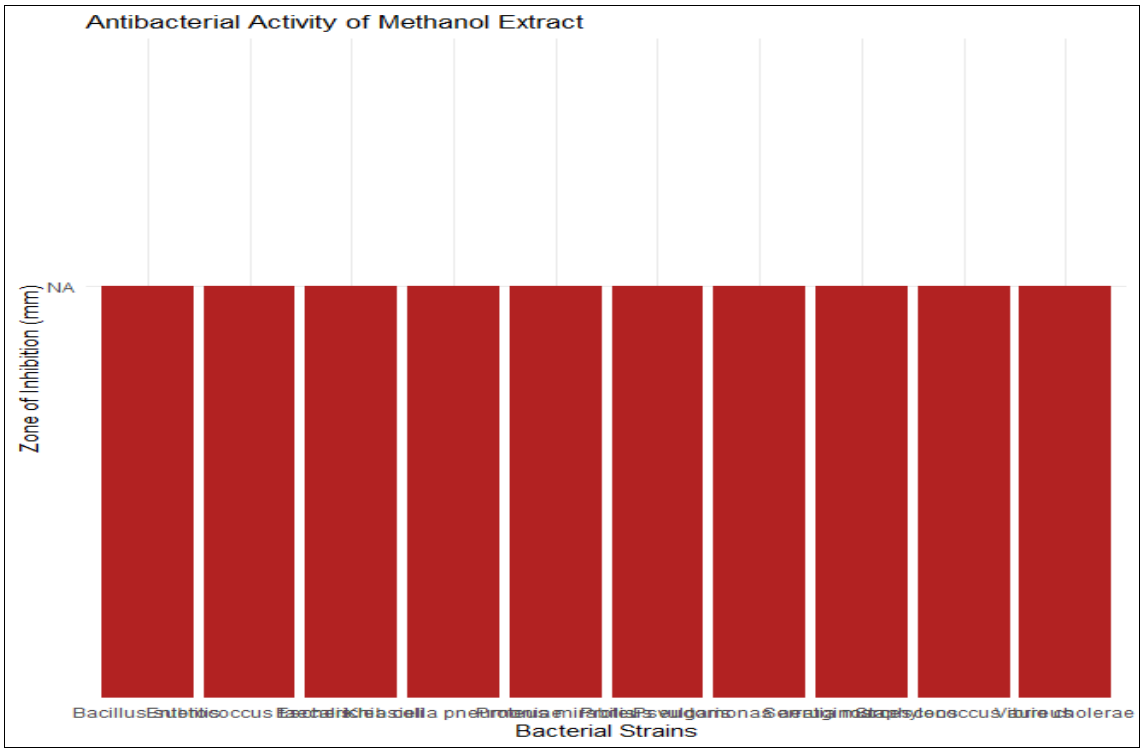

Fig 3: Antibacterial activity of methanol extract

Conclusion

In this research article, the antibacterial activity of *Cleome rutidosperma* leaf extracts was investigated against various bacterial strains. The study utilized different solvents, including hexane, chloroform, ethyl acetate, methanol, and aqueous extracts, to isolate the secondary metabolites from the plant. These extracts were then tested against a panel of gram-negative and gram-positive bacteria.

The results revealed that the methanol leaf extract exhibited the highest antibacterial potential, displaying dose-dependent activity against all tested bacterial species. Notably, the methanol extract showed significant inhibition of clinically isolated drug-resistant pathogens, making it a promising candidate for further investigation.

On the other hand, the hexane, chloroform, and ethyl acetate extracts showed varying degrees of antibacterial activity against specific bacterial strains. However, the aqueous leaf extract did not exhibit any significant inhibitory action against any of the tested species.

Suggestion

This research highlights the potential of *Cleome rutidosperma* as a source of antibacterial agents. To further advance the findings and applications of this herbal medicine, several suggestions can be considered.

Identification of Active Compounds

Isolating and identifying the active compounds responsible for the antibacterial activity in the methanol extract would be valuable. This could be achieved through advanced techniques such as chromatography and mass spectrometry.

Mechanism of Action

Investigating the mechanism of action of the methanol extract and its active compounds would provide insights into how they exert their antibacterial effects.

In vivo Studies

Conducting *in vivo* studies using animal models to evaluate the safety and efficacy of the methanol extract would be a crucial step towards potential therapeutic use.

Clinical Trials

If the methanol extract demonstrates promising results in preclinical studies, conducting clinical trials on human subjects would be necessary to establish its safety and efficacy in a real-world setting.

Synergy Studies

Exploring the possibility of combining the methanol extract with conventional antibiotics to assess potential synergistic effects against drug-resistant bacteria could be a fruitful avenue.

Toxicity Evaluation

Assessing the toxicity of the methanol extract on human cells and tissues would be essential to ensure its safety for medicinal use.

Formulation Development

Developing different formulations of the methanol extract, such as creams, ointments, or oral solutions, may improve its delivery and practicality as a medicinal product.

Standardization

Ensuring the standardization of the extract's preparation and concentration will be critical.

Conflict of Interest

Not available

Financial Support

Not available

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