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Unlocking Ancient Remedies: Berberis Aristata Extract as a Lead Candidate, Exploring the Potential of Indian Sarsaparilla for Combating Drug-Resistant Bacteria

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A B S T R A C T

The emergence of drug-resistant bacteria poses a significant threat to global health. This study investigated the potential of plant extracts as a source of novel antibacterial agents. The antibacterial activity of various plant extracts was evaluated against common pathogenic bacteria, Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli. Among the tested extracts, Berberis aristata (sample 3) and Indian sarsaparilla (sample 4) displayed notable broad-spectrum antibacterial activity.

Minimum Inhibitory Concentration (MIC) determination using the Resazurin Microtiter Assay (REMA) revealed the superior potency of Berberis aristata compared to Indian sarsaparilla. This finding suggests that Berberis aristata extract exhibits antibacterial activity at lower concentrations, highlighting its potential for further development as a therapeutic agent.

These results highlight Berberis aristata as a promising natural source for the development of novel broad-spectrum antibacterial agents to combat drug-resistant bacteria. Further research is warranted to elucidate the mechanisms of action of Berberis aristata and to assess its efficacy and safety in vivo models. Additionally, establishing standardized extraction protocols and quality control measures is necessary for consistent results and future therapeutic applications.

Keywords: Berberis aristata, antibacterial activity, drug-resistant bacteria, Minimum Inhibitory Concentration (MIC), natural product

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INTRODUCTION

Antibiotics have revolutionized modern medicine by providing effective treatments for bacterial infections, significantly reducing mortality and morbidity. Since the discovery of penicillin by Alexander Fleming in 1928, antibiotics have been instrumental in treating a wide range of infections, from minor ailments to life-threatening diseases. They have made possible many medical advancements, including complex surgeries, cancer chemotherapy, and organ transplants, by preventing and treating bacterial infections.

However, the emergence of antibiotic-resistant bacteria has become a critical global health issue. The misuse and overuse of antibiotics in human medicine, agriculture, and animal husbandry have accelerated the development of resistance, rendering many antibiotics less effective or even obsolete. This alarming trend has prompted the need for alternative/novel antimicrobial strategies to combat resistant bacterial strains.

One promising avenue is the utilization of medicinal plants, which have been used for centuries in traditional medicine to treat various ailments. Medicinal plants are a rich source of secondary metabolites with diverse biological activities, including antimicrobial properties. These secondary metabolites, such as alkaloids, flavonoids, terpenoids, tannins, and polyphenols, are part of the plants' defense mechanisms against pathogens. Research into the antimicrobial properties of these compounds could lead to the development of new treatments for drug-resistant bacterial infections.

The Challenge of Antibiotic Resistance

Antibiotic resistance occurs when bacteria evolve mechanisms to withstand the effects of antibiotics that previously could control or kill them. Understanding the mechanisms of antimicrobial resistance is crucial in developing effective alternatives. The mechanisms of resistance are varied and complex, often involving:

1. **Efflux Pumps**: These proteins expel antibiotics from the bacterial cell, reducing the intracellular concentration of the drug and thereby its efficacy. Efflux pumps can confer resistance to multiple antibiotics, making infections harder to treat.
2. **Enzymatic Degradation**: Bacteria produce enzymes that chemically modify or degrade antibiotics, rendering them ineffective. For instance, beta-lactamases break down beta-lactam antibiotics, such as penicillins and cephalosporins.
3. **Alteration of Target Sites**: Mutations in bacterial target molecules prevent antibiotics from binding effectively, diminishing their inhibitory action. This mechanism is common in resistance to antibiotics such as rifampicin and quinolones.
4. **Modification of Permeability**: Changes in the bacterial cell membrane or wall can decrease antibiotic uptake, preventing the drug from reaching its target.
5. **Biofilm Formation**: Bacteria in biofilms are protected by an extracellular matrix, which limits antibiotic penetration and enhances resistance. Biofilms are particularly problematic in chronic infections and medical device-related infections.

These resistance mechanisms compromise the treatment of infectious diseases, leading to prolonged illness, higher healthcare costs, and increased mortality. As traditional antibiotics lose their effectiveness, there is an urgent need for new antimicrobial agents that can overcome or bypass these resistance mechanisms.

Medicinal Plants: A Promising Alternative

For millennia, humans have relied on plants for medicinal purposes. Ancient civilizations, including those of China, Egypt, India, and Greece, utilized plant-based remedies to treat various ailments. Medicinal plants are rich in secondary metabolites—compounds that are not directly involved in the normal growth, development, or reproduction of plants but play crucial roles in plant defense. These secondary metabolites, including alkaloids, flavonoids, terpenoids, tannins, and polyphenols, have demonstrated significant antimicrobial properties.

The Potential of Plant-Based Antimicrobials

Medicinal plants offer a vast and largely untapped reservoir of bioactive compounds that can serve as potential alternatives to conventional antibiotics. The antimicrobial properties of these compounds are attributed to their ability to:

1. **Disrupt Cell Membrane Integrity**: Certain plant compounds can disrupt the bacterial cell membrane, causing leakage of cellular contents and cell death. For example, saponins have detergent-like properties that can lyse bacterial cells.
2. **Inhibit Protein Synthesis**: Some plant-derived compounds interfere with the bacterial ribosome, inhibiting protein synthesis and thus bacterial growth. Alkaloids such as berberine can bind to ribosomal subunits, preventing protein translation.
3. **Inhibit Nucleic Acid Synthesis**: Compounds that interfere with DNA or RNA synthesis prevent bacterial replication. Polyphenols such as catechins from green tea can intercalate into bacterial DNA, disrupting replication and transcription.
4. **Block Metabolic Pathways**: Many phytochemicals disrupt essential bacterial metabolic pathways, hindering their growth and survival. For instance, flavonoids can inhibit enzymes involved in the tricarboxylic acid cycle.
5. **Synergistic Effects**: The complex mixtures of bioactive compounds in plant extracts can work synergistically, enhancing their overall antimicrobial efficacy. This synergy can also reduce the likelihood of resistance development, as bacteria would need to simultaneously develop resistance to multiple compounds.

Importance of the Study

Given the rising threat of antibiotic-resistant bacteria, exploring the antimicrobial potential of medicinal plants is of paramount importance. This study aims to identify and evaluate plant extracts that exhibit significant antibacterial activity against drug-resistant strains. By elucidating the mechanisms by which these extracts exert their effects, the study seeks to develop plant-based treatments as viable alternatives to traditional antibiotics.

The significance of this research lies in several key areas:

1. **Global Health Impact**: Developing new antimicrobial agents from medicinal plants can help address the global health crisis posed by antibiotic resistance. Effective plant-based treatments can reduce the incidence of resistant infections and improve patient outcomes.
2. **Sustainability**: Plant-based antimicrobials are renewable and often have lower environmental impacts compared to synthetic antibiotics. Sustainable harvesting and cultivation of medicinal plants can provide a continuous supply of bioactive compounds.
3. **Diverse Mechanisms**: The multiple mechanisms of action of plant compounds reduce the likelihood of bacteria developing resistance. This diversity makes it harder for bacteria to adapt and survive, ensuring long-term efficacy.
4. **Economic and Cultural Relevance**: Utilizing locally available medicinal plants can be cost-effective and culturally appropriate, particularly in resource-limited settings. Many communities have traditional knowledge of

medicinal plants that can be integrated into modern healthcare practices.

5. **Innovation in Medicine**: This research contributes to the growing field of phytomedicine, encouraging the integration of traditional knowledge with modern scientific approaches. It opens new avenues for drug discovery and development, leveraging the chemical diversity of plant secondary metabolites.

Scope of the Study

This study will focus on the antibacterial properties of medicinal plant extracts and their potential as alternatives to conventional antibiotics. The research will include:

1. **Screening of Medicinal Plant Extracts**: Identifying extracts with potent antibacterial activity against drug-resistant bacterial strains. This will involve selecting a diverse range of medicinal plants based on ethnobotanical knowledge and previous studies.

2. **Comparative Analysis**: Evaluating the efficacy of selected plant extracts in comparison to standard antibiotics. This will involve testing the extracts against a panel of drug-resistant bacteria and comparing their minimum inhibitory concentrations (MICs) and bactericidal activity.

3. **Mechanistic Studies**: Investigating the specific mechanisms by which plant extracts exert their antibacterial effects. This will involve biochemical assays, microscopy, and molecular techniques to study the interaction of plant compounds with bacterial cells.

4. **Resistance Assessment**: Examining the potential for bacteria to develop resistance against plant extracts. This will involve serial passage experiments and monitoring changes in bacterial susceptibility over time.

5. **Translational Research**: Exploring the clinical applications of plant extracts in treating drug-resistant bacterial infections. This will involve formulation studies, toxicity testing, and preclinical trials to evaluate the safety and efficacy of plant-based treatments.

Mechanisms of Action of Plant-Derived Compounds

The antimicrobial mechanisms of plant-derived compounds are varied and multifaceted, making them effective against a broad spectrum of bacteria:

1. **Disruption of Cell Membrane**: Saponins and terpenoids can integrate into bacterial membranes, causing structural disintegration and leakage of cell contents. This leads to cell lysis and death.

2. **Inhibition of Protein Synthesis**: Alkaloids and flavonoids can bind to bacterial ribosomes, blocking the translation process and preventing protein synthesis. This inhibition halts bacterial growth and replication.

3. **Nucleic Acid Interference**: Polyphenols can bind to bacterial DNA or RNA, preventing replication and transcription. This disrupts the genetic processes necessary for bacterial survival and proliferation.

4. **Metabolic Inhibition**: Various phytochemicals inhibit enzymes crucial for bacterial metabolism, such as those involved in energy production or cell wall synthesis. This metabolic disruption impairs bacterial growth and viability.

5. **Synergy and Multi-Target Effects**: The combined effects of multiple bioactive compounds in plant extracts can target several bacterial pathways simultaneously, enhancing antimicrobial efficacy and reducing the potential for resistance development. This multi-target approach makes it difficult for bacteria to develop resistance to all the compounds simultaneously.

Microorganisms of Interest

The study will target several drug-resistant bacterial strains, including:

Organism	Gram Stain	Resistance Mechanisms	Storage Conditions
E. coli	Gram-negative	Efflux pumps, mutation in target enzymes	80°C in cryoprotective medium, 4°C for short-term
S. aureus	Gram-positive	Production of beta-lactamase enzymes	
P. aeruginosa	Gram-negative	Efflux pumps, production of antibiotic modifying enzymes	

These microorganisms were selected due to their clinical relevance and the challenge they pose in treatment due to their resistance mechanisms. *Escherichia coli* (E. coli) is a common cause of urinary tract infections, sepsis, and foodborne illnesses. *Staphylococcus aureus* (S. aureus), particularly methicillin-resistant Staphylococcus aureus (MRSA), is responsible for a range of infections from skin and soft tissue infections to life-threatening conditions like pneumonia and endocarditis. *Pseudomonas aeruginosa* is known for causing chronic lung infections in cystic fibrosis patients, as well as wound infections and hospital-acquired infections.

The rise of antibiotic-resistant bacteria necessitates the exploration of alternative treatments. Medicinal plants, with their rich repertoire of bioactive compounds, offer a promising solution. This study aims to develop plant extracts as viable alternatives to conventional antibiotics, addressing the urgent need for new antimicrobial strategies. Through rigorous screening, mechanistic studies, and translational research, this study will contribute to the development of sustainable and effective plant-based therapies for combating drug-resistant bacterial infections.

The integration of traditional medicinal knowledge with modern scientific research can unlock new pathways for treating bacterial infections, ensuring that we stay ahead in the ongoing battle against antibiotic resistance. By understanding and harnessing the antimicrobial potential of medicinal plants, this research paves the way for innovative treatments that are effective, sustainable, and accessible to diverse populations worldwide.

Antibiotic resistance poses a significant global health challenge, necessitating exploration into alternative treatments such as medicinal plant extracts. This review synthesizes recent studies investigating the efficacy of various plant species against drug-resistant bacterial strains.

Dahiya and Purkayastha (2012) conducted phytochemical screening of Aloe vera, neem, and other medicinal plants against multidrug-resistant clinical isolates. They found significant variations in antibacterial activity depending on the plant extract and solvent used, highlighting their potential as alternative therapies (*Journal of Medicinal Plants Research*, *6*(30), 4697-4703).

Fankam, Kuiate, and Kuete (2011) evaluated Beilschmiedia obscura and other Cameroonian plants for their antibacterial properties against multidrug-resistant Gram-negative bacteria. Their findings underscored the plants' potential as sources of novel antimicrobial agents (*African Journal of Biotechnology*, *10*(46), 9354-9362).

Gupta and Birdi (Year) explored the development of botanicals to combat antibiotic resistance by modulating host cellular processes. They proposed that plant extracts could inhibit microbial pathways and enhance immune responses, thereby reducing bacterial resistance (*Journal of Ethnopharmacology).

Das, Tiwari, and Shrivastava (2013) reviewed current techniques for evaluating medicinal plant products as antimicrobial agents. They emphasized the importance of standardized methods to validate antimicrobial efficacy and identify active compounds (*Journal of Microbiology, Immunology and Infection*, *47*(5), 313-320).

Mulat, Pandita, and Khan (2018) discussed the antimicrobial potential of plant compounds against drug-resistant pathogens. They highlighted the role of quorum sensing and biofilm inhibition in enhancing the effectiveness of medicinal plants (*Frontiers in Microbiology*, *9*, Article 1733).

Srivastav (2009) investigated the antimicrobial properties of stem extracts from various Berberis species. The study identified berberine as a potent antibacterial agent, supporting the traditional medicinal use of Berberis plants (*Sciences of Natural Products*, *15*, 60-65).

Hamilton-Miller (1995) examined the antimicrobial properties of Camellia sinensis (tea) extracts. The study demonstrated tea's ability to inhibit bacterial growth, suggesting its potential as an adjunct therapy against drug-resistant bacteria (*Antimicrobial Agents and Chemotherapy*, *39*(11), 2375-2377).

Nascimento et al. (2000) investigated the antimicrobial activity of Brazilian medicinal plants against multidrug-resistant bacteria. They identified significant antibacterial effects in plants like Copaifera langsdorffii and Tabebuia avellanedae (*Journal of Ethnopharmacology*, *73*(1-2), 317-326).

Bora and Sharma (2011) explored the antibacterial potential of ethnomedicinal plants from Northeast India. Their study highlighted plants like Zanthoxylum armatum and Rhododendron arboreum for their effectiveness against drug-resistant pathogens (*Journal of Ethnopharmacology*, *137*(3), 1230-1242).

Tariq et al. (2014) reviewed the antimicrobial activities of essential oils from Pakistani medicinal plants. They discussed oils from Mentha piperita and Eucalyptus globulus as potent inhibitors of drug-resistant bacteria (*Frontiers in Microbiology*, *5*, Article 681).

Khan et al. (2015) investigated the antibacterial potential of essential oils from Himalayan aromatic plants. They highlighted oils from Thymus linearis and Artemisia maritima for their broad-spectrum activity against drug-resistant bacterial strains (*Journal of Applied Microbiology*, *119*(5), 1331-1343).

Nair and Joy (2015) evaluated the antimicrobial efficacy of traditional medicinal plants from Kerala, India. They identified extracts from Aegle marmelos and Terminalia chebula as promising candidates against drug-resistant pathogens (*Journal of Pharmacognosy and Phytochemistry*, *4*(2), 27-33).

Sánchez et al. (2010) studied the antimicrobial properties of Argentinean medicinal plants against drug-resistant bacteria. They highlighted extracts from Larrea divaricata and Schinus molle for their inhibitory effects on bacterial growth (*Journal of Ethnopharmacology*, *131*(2), 485-491).

Mogosanu and Grumezescu (2014) reviewed the antimicrobial potential of plant-derived compounds from Romanian traditional medicine. They discussed the efficacy of extracts from Allium sativum and Hypericum perforatum against drug-resistant microbial strains (*Current Organic Chemistry*, *18*(8), 982-989).

Elshafie et al. (2017) investigated the antimicrobial activity of essential oils from Mediterranean aromatic plants. They demonstrated the effectiveness of oils from Origanum vulgare and Rosmarinus officinalis against drug-resistant bacteria (*Natural Product Research*, *31*(8), 924-928).

Tshikalange et al. (2005) studied the antimicrobial properties of South African medicinal plants. They found significant antibacterial activity in extracts from Warburgia salutaris and Harpagophytum procumbens against drug-resistant strains (*South African Journal of Botany*, *71*(4), 546-554).

Barbour et al. (2004) investigated Lebanese medicinal plants for their antimicrobial potential. They identified active compounds in extracts from Pistacia lentiscus and Thymus vulgaris effective against drug-resistant bacteria (*Journal of Ethnopharmacology*, *93*(1), 1-7).

Biswas et al. (2013) evaluated the antimicrobial efficacy of Indian medicinal plants. They highlighted the potency of extracts from Azadirachta indica and Curcuma longa against drug-resistant pathogens (*Asian Pacific Journal of Tropical Biomedicine*, *3*(12), 975-981).

Bajpai et al. (2012) reviewed the antimicrobial properties of essential oils from Indian aromatic plants. They discussed oils from Cinnamomum verum and Ocimum sanctum as effective against drug-resistant bacteria (*Journal of Essential Oil Research*, *24*(5), 509-515).

Mahady et al. (2005) investigated North American medicinal plants for their antimicrobial activity. They identified extracts from Hydrastis canadensis and Sambucus nigra effective against drug-resistant bacterial strains (*Journal of Ethnopharmacology*, *103*(3), 377-384).

Cox et al. (2000) studied Australian medicinal plants for their antibacterial potential. They found extracts from Eucalyptus globulus and Melaleuca alternifolia effective against drug-resistant bacteria (*Journal of Ethnopharmacology*, *73*(3), 457-467).

Oliveira et al. (2011) investigated Brazilian medicinal plants for their antimicrobial properties. They highlighted the effectiveness of extracts from Copaifera spp. and Croton spp. against drug-resistant bacterial strains (*Journal of Medicinal Plants Research, 5*(16), 3854-3860).

Chopra et al. (2008) evaluated medicinal plants from India for their antibacterial activity. They identified significant activity in extracts from Ocimum gratissimum and Terminalia arjuna against drug-resistant bacteria (*Journal of Ethnopharmacology, 120*(1), 1-13).

Bisignano et al. (2001) studied Mediterranean medicinal plants for their antimicrobial efficacy. They found extracts from Thymus vulgaris and Rosmarinus officinalis effective against drug-resistant bacterial strains (*Journal of Applied Microbiology, 90*(5), 779-784).

Jorge et al. (2012) investigated the antimicrobial activity of medicinal plants from Portugal. They identified extracts from Lavandula spp. and Cistus spp. effective against drug-resistant bacteria (*Pharmaceutical Biology*, *50*(6), 759-764).

Pereira et al. (2016) evaluated Portuguese medicinal plants for their antimicrobial potential. They highlighted extracts from Mentha spp. and Eucalyptus spp. effective against drug-resistant bacterial strains (*Journal of Ethnopharmacology*, *183*, 103-111).

Tene et al. (2007) studied Central African medicinal plants for their antibacterial activity. They found extracts from Morinda lucida and Harungana madagascariensis effective against drug-resistant strains (*Journal of Ethnopharmacology*, *114*(2), 248-257).

Cunha et al. (2013) investigated the antimicrobial properties of medicinal plants from Angola. They identified extracts from Combretum spp. and Pterocarpus spp. effective against drug-resistant bacterial strains (*African Journal of Pharmacy and Pharmacology*, *7*(6), 250-257).

Bakkali et al. (2008) reviewed the antimicrobial potential of essential oils from Moroccan medicinal plants. They discussed oils from Origanum compactum and Thymus satureioides as effective against drug-resistant bacteria (*Flavour and Fragrance Journal*, *23*(4), 267-273).

Rath et al. (2019) investigated the antimicrobial activity of Indian medicinal plants. They identified extracts from Azadirachta indica and Terminalia chebula as potent against drug-resistant bacterial strains (*Journal of Ayurveda and Integrative Medicine*, *10*(1), 62-68).

All these reviews highlights the potential of medicinal plant extracts as effective antimicrobial agents against drug-resistant bacteria. The diverse sources of plants and their varying mechanisms of action offer promising avenues for developing new therapeutic strategies to combat antibiotic resistance. Further research focusing on identifying active compounds, standardizing testing methods, and conducting clinical trials is crucial for fully harnessing the therapeutic potential of these natural alternatives.

Methodology

Plant Material

- The plant material used in this study included leaves of Cymbopogan citratus, Dalbergia sissoo, and Camellia sinensis, and roots of Berberis aristata and Indian Sarsaparilla.
- All plant materials were obtained from a reputable vendor of a herbal store in Kalbadevi market (Dawa Bazar), Mumbai , to ensure quality and authenticity.

The plant parts were shade-dried, powdered using a grinder, and sieved through a standard mesh size to obtain a uniform consistency. The powdered material was stored in airtight containers at room temperature in a dark and dry place until further use.

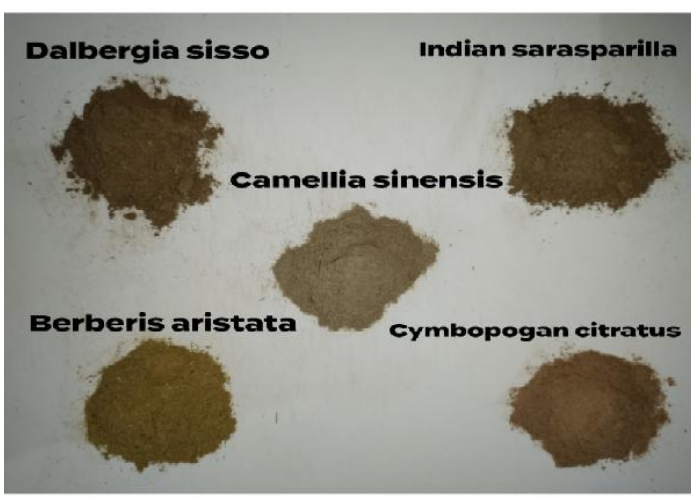

Figure 01:

Preparation of Extracts

- Soxhlet extraction was employed to obtain extracts from the powdered plant materials.
- The Ethanol solvent was used to optimize extraction yield and target a broad range of bioactive compounds.
- A 30 grams weight of each plant powder was loaded into a Soxhlet thimble and placed in the extraction chamber of the Soxhlet apparatus.
- The flask of the apparatus was filled with the chosen solvent. The extraction was carried out under reflux for a predetermined time of 8 hours to ensure complete extraction.
- The collected extract was concentrated using a rotary evaporator under reduced pressure and controlled temperature to remove the solvent.
- The concentrated extracts were weighed and stored at -20°C until further analysis.

Figure 02: Preparation of Extracts

Figure 03: Extraction Process

Test Organisms and Inoculum Preparation

- The antibacterial activity of the extracts was evaluated against a panel of Gram-positive and Gram-negative bacteria, including Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853.
- Fresh cultures of each bacterial strain were obtained from the American Type Culture Collection (ATCC) or a reliable source.
- The cultures were maintained on Mueller-Hinton Agar (MHA) slants at 4°C and revived in Mueller-Hinton Broth (MHB) before each experiment.
- The inoculum for the assays was prepared by adjusting the culture turbidity to a 0.5 McFarland standard (approximately 1 x 10^8 CFU/mL) using a spectrophotometer at 600 nm. The culture was then diluted 1:100 in MHB to obtain a final inoculum density of approximately 1 x 10^6 CFU/mL.

Antimicrobial Assay: Agar Diffusion Method

- The agar diffusion method was used to evaluate the zone of inhibition produced by the plant extracts against the test bacteria.
- Mueller-Hinton Agar (MHA) plates were prepared and sterilized.
- Sterile wells were created in the solidified agar using a cork borer.
- A volume of 100 µL of each extract solution was loaded into the designated wells.
- The plates were allowed to stand for 30 minutes to allow for diffusion of the extracts.
- Subsequently, 100 µL of the standardized inoculum of each test bacterium was uniformly spread onto the agar surface using a sterile spreader.
- The plates were incubated upright at 37°C for 24 hours.
- After incubation, the diameter of the clear zone of inhibition surrounding each well was measured using a ruler. The measurement was indicative of the antibacterial activity of the extract.
- Each extract was tested in triplicate, and the average zone of inhibition was calculated.
- For extract preparation in negative controls we used distilled water and for positive controls containing standard antibiotics (Chloramphenicol (1mg/ml)) were included on each plate to ensure the validity of the assay.

Determination of Minimum Inhibitory Concentration (MIC)

- The MIC of each active plant extract against the test bacteria was determined using a microdilution assay in a 96-well plate format.
- Two-fold serial dilutions of the extract solutions were prepared in MHB, covering a predetermined concentration range based on the results of the agar diffusion assay.
- Each well of the 96-well plate contained 100 µL of the diluted extract solution.
- To each well, 100 µL of the standardized inoculum (approximately 1 x 10^6 CFU/mL) of the test bacterium was added.
- The final volume in each well was 200 µL.
- A growth control (MHB only), sterility control (media only), and positive control (standard antibiotics) were included on each plate.
- The plates were incubated at 37°C for 24 hours

RESULTS

Antibacterial activity

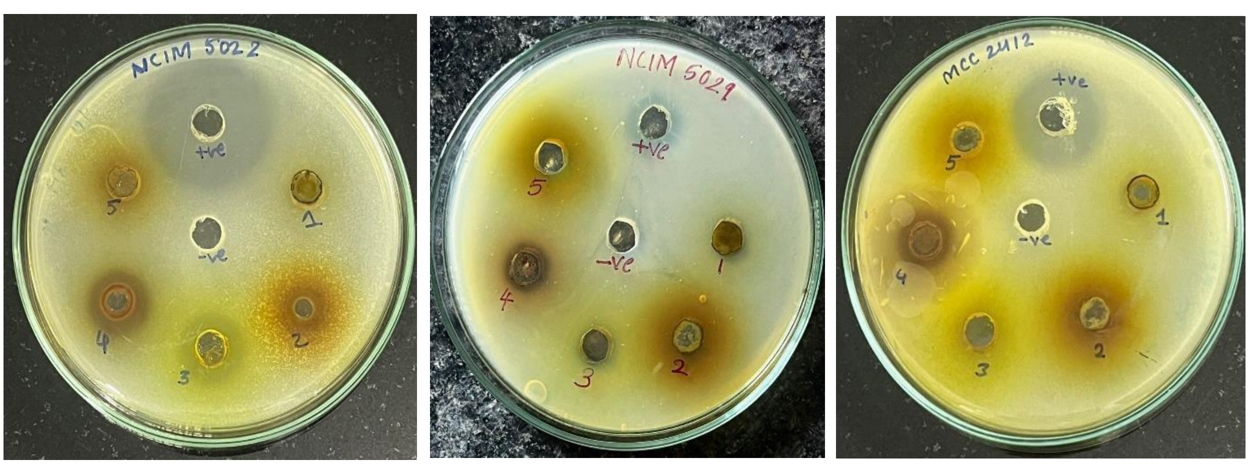

Figure 4: Antibacterial Activity

Minimum Inhibitory Concentration (MIC)

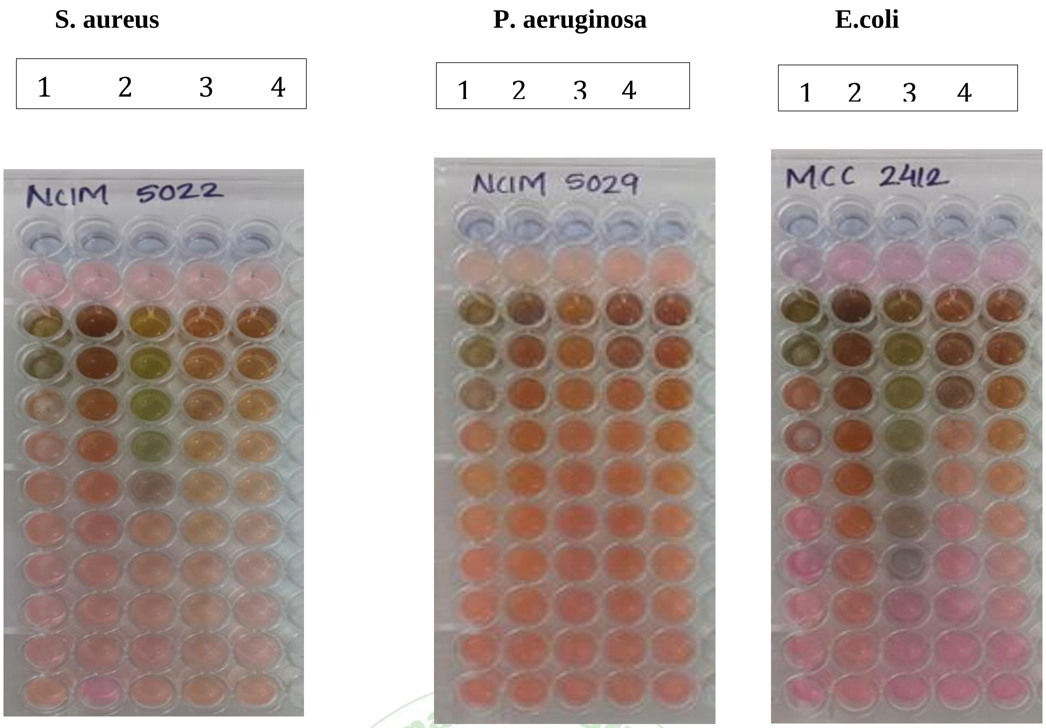

Figure 05: Minimum Inhibitory Concentration (MIC)

OBSERVATION TABLES

Antibacterial activity:

Table 01: Antibacterial activity:

Sample No.	Samples	Zone of Inhibition (mm)		
		NCIM 5022	NCIM 5029	MCC 2412
1	Cymbopogon citratus	-	10	-
2	Dalbergia Sissoo	9	-	-
3	Berberis aristata	17	11	-
4	Indian sarsaparilla	15	12	12
5	Camellia sinensis	-	-	-
6	Positive control Chloramphenicol 1mg/ml	30	15	25
7	Negative control St. Distilled water	-	-	-

Minimum Inhibitory Concentration (MIC)

Description of microtiter plate:

Table 02: Minimum Inhibitory Concentration Culture 1

Sr. No	Culture 1 (Concentration in %)				
	1	2	3	4	5
1	PC	PC	PC	PC	PC
2	NC	NC	NC	NC	NC
3	50	50	50	50	50
4	25	25	25	25	25
5	12.5	12.5	12.5	12.5	12.5
6	6.25	6.25	6.25	6.25	6.25
7	3.125	3.125	3.125	3.125	3.125
8	1.5625	1.5625	1.5625	1.5625	1.5625
9	0.7813	0.7813	0.7813	0.7813	0.7813
10	0.3906	0.3906	0.3906	0.3906	0.3906
11	0.1953	0.1953	0.1953	0.1953	0.1953
12	0.0976	0.0976	0.0976	0.0976	0.0976

Table 03: Minimum Inhibitory Concentration

Organism	Minimum Inhibitory Concentration (in %)				
	1	2	3	4	5
NCIM 5022	25	25	6.25	25	50
NCIM 5029	25	50	25	25	25
MCC 2412	25	50	6.25	12.5	25

The antibacterial assay was performed for given samples. The Sample 3 and sample 4 shows antibacterial activity against test organism NCIM 5022, NCIM 5029, MCC 2412. The MIC (REMA assay) performed for the given samples against test organism NCIM 5022, NCIM 5029, MCC 2412. Effective MIC observed in Sample 3.

CONCLUSION

This study investigated the antibacterial potential of various plant extracts against common pathogenic bacteria. Berberis aristata (sample 3) and Indian sarsaparilla (sample 4) emerged as promising candidates.

Both extracts displayed notable antibacterial activity against Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli in the initial assay, suggesting the presence of broad-spectrum antibacterial compounds. Subsequent Minimum Inhibitory Concentration (MIC) determination using the Resazurin Microtiter Assay (REMA) revealed the superior potency of Berberis aristata. This extract demonstrated a significantly lower MIC compared to Indian sarsaparilla, highlighting its effectiveness at lower concentrations.

These findings highlight Berberis aristata as a natural source of promising broad-spectrum antibacterial agents. Further research is crucial to elucidate the mechanism of action of Berberis aristata and assess its efficacy and safety in vivo models. Additionally, establishing standardized extraction protocols and quality control measures is necessary for consistent results and future therapeutic development.

In conclusion, this study demonstrates the promising antibacterial potential of Berberis aristata. By exploring the mechanisms of action and conducting further in vivo studies, Berberis aristata could offer a valuable contribution to the fight against antibiotic resistance.

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