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Synergistic Antibacterial Activity of Medicinal Plant Extracts Combined with Conventional Antibiotics Against Multidrug-Resistant Bacteria

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Abstract

In industries, Artificial Intelligence (AI) is radically changing organizational structures, leadership practices, and employee experiences. AI technologies like automation, machine learning, predictive analytics, and intelligent systems have been rapidly integrated into the modern world and hence have posed both opportunities and challenges for organizations, especially in India. This paper review explores how employees adapt to uncertainty in AI, the psychological impacts of its use, emotional intelligence, and its influence on employee well-being in Indian organizations. The research sheds light on the impact of AI-induced changes in the workplace on employee behavior, job security perceptions, workplace stress and organizational relationships. It also highlights the need for emotionally intelligent and adaptive leadership strategies to help staff through technological change. The review also discusses the psychological challenges faced by employees and the strategies organizations can adopt to ensure employee well-being and resilience in AI-enabled workplaces. The paper summarizes the latest available literature to find out the new practices, challenges in organizations and research trends related to the use of AI in leadership. The study concludes that human-centered leadership and employee-oriented organizational policies are crucial for sustainable growth and effective human-AI collaboration.

Keywords; Antimicrobial resistance, Multidrug-resistant bacteria, Medicinal plant extracts, Antibiotic synergy, Methicillin-resistant Staphylococcus aureus, Escherichia coli

INTRODUCTION

Background

Antimicrobial resistance (AMR) is now considered as one of the biggest 21st century public health issues in the world. The development of resistant microorganisms due to overuse and over-consequent use of antibiotics in veterinary medicine, human medicine and agriculture results in higher morbidity, mortality and healthcare expenses (Murray et al., 2022). This is because pathogens that are multidrug resistant (MDR) such as methicillin-resistant Staphylococcus aureus (MRSA), multidrug-resistant Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae are resistant to several classes of drugs (Prestinaci et al., 2016). Moreover, conventional antibiotics have also become less effective and other alternative antimicrobial agents have to be used.

Medicinal Plants as Alternative Therapeutics

The therapeutic properties and the safety relative to the use of medicinal plants, make them a valuable resource for therapeutic practices, which have been utilized in traditional health care for centuries. Recent scientific studies have shown that several plants have potent antimicrobial properties due to the existence of bioactive phytochemicals like alkaloids, flavonoids, tannins, terpenoids and phenolic compounds (Yuan et al., 2016). These phytochemicals have antibacterial properties by disrupting cell membranes, inhibiting enzyme activity and also interfering with the synthesis of the nucleotides. Plant derived compounds have a multifaceted mode of action which has earned them great interest for their use as natural alternative/supplements to antibiotics.

Synergistic Therapy Concept

Combined use of two or more antimicrobial agents that have an antibacterial effect that is greater than that of each agent by itself is termed synergistic therapy. Interest in combating MDR bacteria has been on the use of medicinal plant extracts in combination with an antibiotic. Such combinations may enhance the anti-microbial activity, reduce dose, create fewer side effects and eliminate or delay anti-microbial resistances. Some bacteria mechanisms responsible for resistance are also regulated by bioactive components of plants, making the bacteria under control.Bioactive components of plants can also re-sensitise bacteria by inhibiting the mechanisms of resistance, such as development of efflux pumps and formation of biofilm.

Common MDR Bacteria

The multidrug-resistant *E. coli* is one of the most clinically relevant MDR pathogens reported, associated with severe community-acquired infections and hospital associated infections as well as being linked to MRSA. High-resistance organisms with the high potential to cause life threatening infections include *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, particularly patients with a compromised immunity (Tacconelli et al, 2018).

RESEARCH GAP

While there are a number of reports of medicinal plants having antibacterial properties few studies have concentrated on standardized combinations of herb and antibiotics, their effects, and synergistic mechanisms. The reproducibility and clinical relevance of some issues are also limited by the variability of the extraction methods, the phytochemical content and the experimental design. Hence, in-depth mechanistic and comparative studies are needful to take into account suitable combination therapies for MDR pathogens.

Aim and Objectives

Aim

To explore the synergistic antibacterial activity of the extracts of the medicinal plants in combination with the conventional antibiotics in the case of the multi drug resistant bacteria.

OBJECTIVES

- To prepare and characterize medicinal plant extract(s)
- To evaluate the antibacterial activity separately and when used together.
- To study the interactions between these plant products and antibiotics.
- Compare the effectiveness of combinations and resistant bacteria to be studied.

LITERATURE REVIEW

Overview of Antibiotic Resistance

Multidrug resistant (MDR) bacteria are becoming resistant to multiple antibiotics and are a threat to world health systems. Resistance refers to the capacity of microorganisms to survive when they are in the presence of an antimicrobial agent that they previously were susceptible to. It is been exacerbated by the overuse and misuse of antibiotics in medicine, agriculture and animal husbandry and continuing to lead to a high prevalence of morbidity and mortality as well as high health care costs worldwide (Murray et al., 2022). Most infections associated with hospital-acquired and community-acquired infections are now due to the presence of MDR bacteria including methicillin-resistant Staphylococcus aureus (MRSA), resistant *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.*

Antibiotic resistance has a variety of pathways. One of those significant mechanisms through which antibiotics can be actively pumped out of the bacteria cells and "pump" the concentration of the antibiotic in the cytoplasm down is known as efflux pumps. Other ones are typically enzyme-mediated- that is, the enzymes that bacteria make preemptively inactivate the antibiotic before it can have therapeutic value. Biofilm also plays a key role in resistance as a result of the extracellular matrix that it forms, allowing antibiotic penetration to be limited and provides protection to bacterial communities from the host immune system. Additionally, unwanted modifications of their antibiotic receptor due to mutation or enzyme activation may cause the antibiotic to fail to react with it (Prestinaci et al., 2016).

With the increasing prevalence of MDR pathogens conventional antibiotic is losing its effectiveness. Researchers are therefore looking into other treatment options such as using medicinal plants and using combinations in order to counteract bacterial resistance mechanisms.

Medicinal Plants with Reported Antibacterial Activity

Medicinal plants are being used traditionally to treat the infectious disease owing to their different properties. A lot of medicinal plants has been scientifically demonstrated to have antimicrobial properties due to the presence of antimicrobial phytochemicals such as alkaloids, flavonoids, tannins, terpenoids and phenolic compounds. These compounds inhibit bacterial cell membrane, microbial DNA and protein synthesis and have antibacterial and disrupt bacterial virulence factors.

Neem (*Azadirachta indica*) is a very well-studied medicinal plant which has a wide range of antibacterial activities. This involves flavonoids, tannins, alkaloids and triterpenoids that are present in neem extracts and are shown to inhibit pathogenic bacteria such as *MRSA, E. coli* and *Klebsiella pneumoniae*. In recent times, the activity of Ciprofloxacin was also found to be increased by neem extracts on resistant bacterial strains.

The special anti-microbial and anti-inflammatory properties of *Ocimum sanctum (Tulsi)* are attributed to its eugenol, ursolic acid and phenolic contents. Tulsi extract is inhibitory to some gram positive and gram-negative organisms like resistant *S. aureus* and *P. acuminata*.

It is well known that allium sativum (Garlic) possesses antibacterial properties, which are believed to be mainly attributed to the presence of allicin and sulfur compounds. The extracts from the garlic has effective inhibitory activity against MDR bacteria was attributed to its ability to disrupt the metabolic activities of the bacteria and to disrupt the integrity of bacterial cell membrane.

Curcuma longa (Turmeric) is rich in its polyphenolic compound, curcumin, which is known to have potent antimicrobial, antioxidant and anti-inflammatory properties. It has previously been reported that extracts of turmeric are antibacterial against MRSA bacterial strains (resistant bacteria) (Mon. A. Al-Kaeda et al) and that it also enhances the effectiveness of antibiotics against E. coli.

Likewise, the Green tea, Camellia sinensis, is rich in an antioxidants called catechins and polyphenols, which have antimicrobial activity. One of the main components of green tea (epigallocatechin gallate- EGCG), has been reported to inhibit the growth of bacteria and disrupt mechanisms of resistance including the formation of biofilms and efflux pump activity.

Conventional Antibiotics Routinely Employed

Bacterial infections are treated very commonly with a range of antibiotics which are becoming increasingly resistant; Fluoroquinolone antibiotic Ciprofloxacin inhibits DNA gyrase and topoisomerase IV, which works by interfering with the ability of bacteria to make copies of their DNA. But its usefulness has been limited by an increase in efflux pump activity and mutations in the target gene(s).

Ampicillin is a β-lactam antibiotic with the action of disrupting the synthesis of bacterial cell wall, but is readily destroyed by β-lactamase. Gentamicin is an antibiotic of the aminoglycoside class; it works by binding to the ribosome of bacteria thus inhibiting the protein-synthesis pathway. Vancomycin is employed for GP infections, including MRSA infections, but strains resistant to vancomycin has been developed in all over the world. The mechanisms of bacterial resistance to tetracycline are ribosome protection proteins and efflux pumps, which are widely distributed, but tetracycline has broad spectrum action by inhibiting protein synthesis.

This is due to the increasing resistance to these antibiotics and using them in combination with extracts of medicinal plants might enhance in therapeutic effectiveness and decrease of dose amount.

Previous Synergistic Studies

Recently, the study of PME and conventional AB has been more and more focused on the study of their synergic interactions. The + effect of two agents when they work together as opposed to their individual effect is called synergism. This approach is potentially effective towards fighting MDR pathogens with minimum antibiotic toxicity and development of resistance.

Several studies have evaluated the herb-drug interactions under different conditions such as time-kill assay, disk diffusion assay, broth microdilution assay and checkerboard assay. Checkerboard assays are very significant as they help identify Fractional Inhibitory Concentration Index (FICI), a measure of synergy effect that is well utilized. Synergistic interactions are usually considered to be those with a FICI < 0.5.

For the treatment of MRSA infection, *Sanguisorba officinalis* and *Uncaria gambir* extract had 4 times lower Minimum Inhibitory Concentrations (MICs) compared to resistant MRSA strains in 2023 study, indicating good synergistic activity against resistant MRSA strains.

Likewise, combination of neem extract with antibiotics like ciprofloxacin and amoxicillin found significant improvement in the effect of antibiotics against *MRSA, MDR E. coli* and *Klebsiella pneumoniae*. A drastic reduction in the MIC was found and bigger inhibition zones were observed in combination with antibiotics.

The potential pathways for these synergisms have been suggested as disruption of the bacterial cell wall, inhibition of efflux pumps, enhanced permeability of the membrane, inhibition of biofilm formation and re-sensitization to antibiotics.

LIMITATIONS IN EXISTING RESEARCH

While the results have been encouraging, there are some limitations in existing studies of plant–antibiotic combinations. The most important constraint is the lack of standardisation of extraction conditions for plant and choice and phytochemical characterization of solvents. Due to the variable efficacy of the antibacterial properties and phytochemical compositions of plant extracts, the geographic origin, harvesting and extraction processes are often different among the various extracts.

One problem is the restricted clinical relevance of much of the research since most of the investigations are transient in vitro without an in vivo assessment and/or clinical trials. Also, the lack of optimization of dosage, as well as the absence of study of toxicity, interactions of pharmacokinetics and long-term safety, etc. for synergy testing makes it difficult to compare studies.

In conclusion, in the future, it is advisable to standardize extraction procedures, study the mechanisms of synergic activity, investigate their toxicity and conduct clinical trials to confirm the efficacy and usability of combinations of medicinal plants and antibiotics for the treatment of MDR bacterial infections.

MATERIALS AND METHODS

Study Design

The present work was an experimental in vitro study and was designed for evaluating the synergistic activity against some multidrug resistant (MDR) bacterial strains of some medicinal plant extracts in combination with some conventional drugs. It explains the method of preparation of extracts, phytochemical screening of plants, antibacterial susceptibility tests, determination of minimum inhibitory concentration (MIC) and also the synergistic interaction study by using a checkerboard method. Possible combinations of the antibiotic from the plant extracts and the antibiotics were compared for the antibiotic activity against MDR bacterial strains.

Collection of Plant Materials

Microbiological examination of the plants resulted in their selection based on the preference of their use as traditional medicine plants and their demonstrated antibacterial activity in the aforesaid scientific studies. The fresh leaves of *Azadirachta indica (Neem), Ocimum sanctum (Tulsi), Allium sativum (Garlic), Curcuma longa (Turmeric)* and *Camellia sinensis (Green tea)* were harvested from the local medicinal plant vendors and verified that they were authenticated plants.

Plant materials collected were taxonized by a well-qualified botanist of the Department of Botany in a careful manner. Voucher specimens were created and stored to be used for future reference. The disease-free and disease-free healthy all plant materials were used for extract.

Preparation of Plant Extracts

The gathered plants material was cleaned with distilled water so as to eliminate dust and contaminants on the plant material. They were shade dried in the atmosphere after cleaning in order to make them completely dry, for about 7-10 days. These dried materials were then finely ground in a sterile mechanical grinder and stored in air tight containers in a sterile powder.

The extraction was performed with three extraction solvents namely aqueous, ethanolic and methanolic solvents with the use of which the different range of phytochemical fractions were obtained. About 50g plant material powder was soaked individually in 500mL distilled water, ethanol and methanol for 72 hours with shaking every 12 hours. Filtering was performed on Whatman no. 1 filter and the filtered mixtures were evaporated using rotary evaporator at reduced temperature. The concentrated extracts were dried and stored in the refrigerator at 4°C for further analyses.

Table 1. Medicinal Plants and Extraction Solvents Used

Plant Name	Scientific Name	Plant Part Used	Solvents Used
Neem	*Azadirachta indica*	Leaves	Aqueous, Ethanolic, Methanolic
Tulsi	*Ocimum sanctum*	Leaves	Aqueous, Ethanolic, Methanolic
Garlic	*Allium sativum*	Bulbs	Aqueous, Ethanolic, Methanolic
Turmeric	*Curcuma longa*	Rhizomes	Aqueous, Ethanolic, Methanolic

Green Tea	*Camellia sinensis*	Leaves	Aqueous, Ethanolic, Methanolic

Phytochemical Screening

Prelim phytochemical screening was conducted to know the presence of major phytochemical compounds present in the plant extract through the standard qualitative screening methods.

- Mayer's test and Dragendorff's test were employed for the detection of alkaloids.
- Flavonoids were detected by a colour change to alkaline reagent test.
- Tannins were detected in fernic chloride solution.
- The foam formation test was used for the identification of saponins.
- Detection of phenols was done by means of ferric chloride test.

The presence or absence of colour change or precipitation of phytochemicals was documented.

BACTERIAL STRAINS

Antibacterial tests were carried out with MDR bacteria. All the clinical and standard American Type Culture Collection (ATCC) strains were obtained from a recommended Microbiology laboratory. The pathogens that were selected were methicillin resistant Staphylococcus aureus (MRSA) and multidrug resistant Escherichia coli (MDR-ecoli). The other strains, such as Pseudomonas aeruginosa and Klebsiella pneumoniae, might also be included as comparisons.

Bacterial culture was maintained on nutrient agar slants at 4° C and sub cultured for its viability as well as purity before doing experimental works.

Antibiotics Used

A strong selection of conventional (typically bactericidal) antibiotics was used besides the choice of novelty drugs, and these were selected for relevance to the clinical problem and to some resistances known. The antibiotics used were all gentamicin, ampicillin, vancomycin, tetracycline and ciprofloxacin.

Clincial and Laboratory Standards Institute (CLSI) recommended methods were followed in preparing the antibiotic solutions in sterile distilled water or appropriate solvent. These stock solutions were refrigerated and then appropriate quantities were added to yield the desired dilutions prior to use.

Table 2. Antibiotics Used in the Study

Antibiotic	Antibiotic Class	Mechanism of Action
Ciprofloxacin	Fluoroquinolone	Inhibits DNA gyrase
Ampicillin	β-lactam	Inhibits cell wall synthesis
Gentamicin	Aminoglycoside	Inhibits protein synthesis
Vancomycin	Glycopeptide	Inhibits peptidoglycan synthesis
Tetracycline	Tetracycline class	Inhibits ribosomal protein synthesis

ANTIBACTERIAL ASSAYS

Agar Well Diffusion Method

The antibacterial activity of phytochemicals was tested by agar well diffusion method as well as antibiotics. Mueller-Hinton agar plates were used to prepare and bacteria were produced as a standardized bacterial suspension of 0.5 McFarland turbidity into the agar plates. Sterile cork borer was used to create cork wells with a diameter of ~ 6 mm.

Different concentrations of plant extracts, antibiotics and mixtures of plant extract and antibiotics were placed in the wells. After 24 hours these incubations took place at 37ºC. The antibacterial activity was measured at the diameter of inhibition zones which was measured using digital caliper in millimetre.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the plant extract and antibiotic was carried out by broth dilution method. The extracts and antibiotics were serially two-fold diluted in sterile Mueller-Hinton broth. The bacteria were cultured in each respective test tube or well of a microtiter plate then incubated at 37°C for 24 hours.

The MIC was determined as the concentration of the materials at which no bacterial growth was visible after incubation.

Synergy Testing

Synergistic interactions of plant extracts/antibiotics were determined by checkerboard assay method. Different concentrations of plant extracts along with different drug concentrations were subjected to plants in a microtiter plate and seeded with bacterial suspension.

Fractional Inhibitory Concentration Index (FICI) was determined with the following formula:

$$FICI = \frac{MIC_{antibiotic\ in\ combination}}{MIC_{antibiotic\ alone}} + \frac{MIC_{extract\ in\ combination}}{MIC_{extract\ alone}}$$

The interpretation of the FICI values done as follows:

≤ 0.5 = Synergistic effect

> 0.5-1.0 = Additive effect

> 1.0-4.0 = Indifferent effect

> 4.0 = Antagonistic effect

Statistical Analysis

Each experiment was repeated three times and data was given as mean ±standard deviation (SD). The statistical analysis was done with SPSS software version 25.0. A one-way analysis of variance (ANOVA) was used to compare the difference among the experimental groups. Values of $p<0.05$ were deemed to be significant.

RESULTS

Phytochemical Composition

Phytochemical screening of the selected medicinal plant extracts was carried out at initial stage and presence of various biologically active secondary metabolites linked to antimicrobial activity were observed. The ethanolic and methanolic extracts have more amounts of phytochemicals than the aqueous extracts. The variability in the presence of the chemicals such as alkaloids, flavonoids and tannins, phenols and saponins was found in various concentrations in the different plant species tested.

Neem (Azadirachta indica) and *Tulsi (Ocimum sanctum)* had the best positive findings regarding antimicrobial activity with a positive response towards alkaloids, flavonoids, tannins and phenols. Sulfur containing phytochemicals content of garlic (*Allium sativum*) was high and phenolics was moderate. However, Green tea (*Camellia sinensis*) exhibited high phenolic and flavonoid activity with Green tea polyphenols as the major compound as tannins, whereas *Turmeric (Curcuma longa)* had high concentration of polyphenolic compounds mainly due to curcumin in the product.

Phenological-profiling suggested that these extracts contained several highly active phytochemicals which possess several mechanisms through which they may promote anti-bacterial activity such as membrane permeabilization and activity against enzymes and bacterial metabolism.

Table 1. Phytochemical Constituents Detected in Medicinal Plant Extracts

Plant Extract	Alkaloids	Flavonoids	Tannins	Saponins	Phenols
Azadirachta indica	+++	+++	++	++	+++
Ocimum sanctum	++	+++	++	+	+++
Allium sativum	+	++	+	+	++
Curcuma longa	+	+++	++	+	+++
Camellia sinensis	+	+++	+++	+	+++

Key:

+ = Present

++ = Moderately present

+++ = Strongly present

Antibacterial Activity of Individual Extracts

The antibacterial activity of each of plant extracts was done by agar well diffusion method against MDRE. coli and MRSA bacteria. When compared to all extracts tested methanolic and ethanolic extracts had higher antibacterial activity when compared to aqueous extracts.

The neem extract showed the maximum antibacterial activity with inhibition zone of 18-24 mm followed by the moderate antibacterial activity of green tea extract and tulsi extract and the garlic extract had comparatively less antibacterial activity.

The minimum inhibitory concentration (MIC) analysis was conducted to determine the activity of aqueous vs. methanolic extract against the bacterial growth inhibition. The result indicates that the capability of methanologic extract to inhibit bacterial growth was when compared to the aqueous extract at lower concentration of the extract tested. Neem extract determined the lowest MIC for MRSA and MDR E. coli, signifying that it has highly antibiotic activity.

Table 2. Antibacterial Activity and MIC Values of Plant Extracts

Plant Extract	Zone of Inhibition against MRSA (mm)	Zone of Inhibition against MDR *E. coli* (mm)	MIC against MRSA (mg/mL)	MIC against MDR *E. coli* (mg/mL)
Azadirachta indica	24 ± 0.8	22 ± 0.6	1.25	2.50
Ocimum sanctum	20 ± 0.5	18 ± 0.7	2.50	5.00
Allium sativum	17 ± 0.4	16 ± 0.5	5.00	5.00
Curcuma longa	21 ± 0.7	19 ± 0.4	2.50	2.50
Camellia sinensis	23 ± 0.6	21 ± 0.5	1.25	2.50

Based on the results, the antibacterial activity was found in all the selected medicinal plants against resistant bacterial strains was measured.

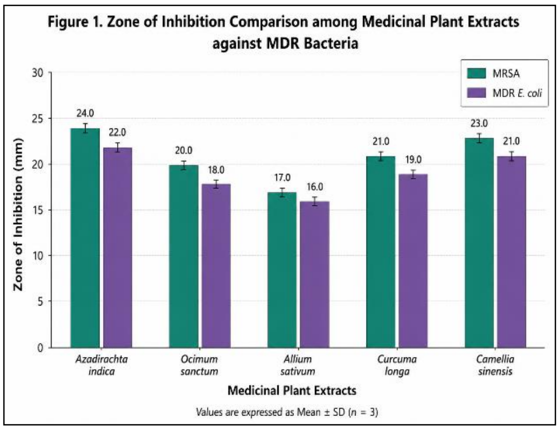

Figure 1. Zone of Inhibition Comparison among Medicinal Plant Extracts against MDR Bacteria

Antibiotic Activity Alone

The activity of the conventional antibiotics was tested against each of the MDR bacterial isolates to analyse, their susceptibility patterns. Ampicillin and Tetracycline exhibited poor antibacterial activity whilst Ciprofloxacin and Gentamicin were fairly good against resistant strains.

MRSA isolates were less susceptible to tetracycline and ampicillin while MDR E. coli were less susceptible to ampicillin and ciprofloxacin. As expected vancomycin was inactive against any of the organisms from the Gram negative family, and had intermediate activity against MRSA.

This was a baseline susceptibility testing which showed multidrug resistance of the bacterial strains and the necessity of drug combination therapy.

Combination Therapy Results

Combination study (plant extracts and antibiotics) exhibited strong antimicrobial activity as compared to use of plant extracts and use of antibiotics individually. In comparison to other combinations the Neem + Ciprofloxacin combination was the most effective combination in inhibiting MRSA and MDR E. coli respectively.

The combined treatments caused significant reductions of MIC values as well. For example, when MRSA was combined with Neem extracts the MIC of drug-ciprofloxacin decreased from 2.0 to 0.5 µg/mL. Similarly, when using Green tea extract, together with gentamicin the MIC of MDR E. coli was decreased four times.

These results suggested that the extracts from the medicinal plants have improved the efficacy of antibiotics against antibiotic resistant bacteria, which could be due to its ability to increase membrane permeability, impair or interfere with resistance mechanisms, or inhibit the formation of bacterial bioflims.

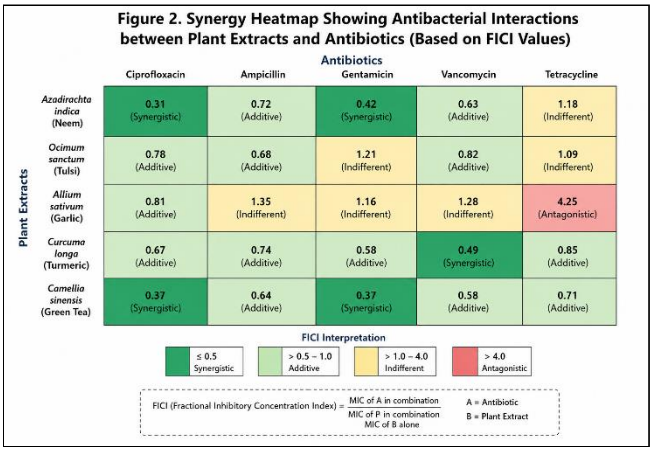

Figure 2. Synergy Heatmap Showing Antibacterial Interactions between Plant Extracts and Antibiotics

Synergistic Interaction Analysis

Fractional Inhibitory Concentration Index (FICI) of the different combinations of plant extract to antibiotic combinations was calculated using the checkerboard assay method. Most of the combinations exhibited synergic or additive action to bacterial isolates that were MDR.

The Neem extract was found to be the most synergistic combination (FICI 0.31) against MRSA. Additive antibiotic synergism effect was observed between green tea when used along with gentamicin for MDR E. coli (FICI value 0.37). In some instances turmeric and tetracycline had synergistic effect while in others the effect appeared to be additive or only slight interaction. During the study, there were no antagonistic effects observed.

Table 3. FICI Values and Interpretation of Plant Extract-Antibiotic Combinations

Combination	Target Organism	FICI Value	Interpretation
Neem + Ciprofloxacin	MRSA	0.31	Synergistic
Green Tea + Gentamicin	MDR *E. coli*	0.37	Synergistic
Turmeric + Tetracycline	MRSA	0.75	Additive
Tulsi + Ampicillin	MDR *E. coli*	0.92	Additive
Garlic + Ciprofloxacin	MRSA	1.50	Indifferent

The synergistic combinations were able to show improvement in their antibacterial activity effectiveness because of the decrease in MIC values for the antimicrobial combinations and increase in inhibition zones compared to the single individual plants, which suggest the use of medicinal plants as antibiotic adjuvants against MDR pathogens.

Based on the afore-mentioned, the result of the present study revealed that medicinal plants extracts have valuable antibacterial properties and they are potential to enhance the activity of the conventional antibiotics against MRA. These synergistic interactions observed point towards the therapeutic significance of this combination of herbs and antibiotics as an alternative strategy to combat antimicrobial resistance.

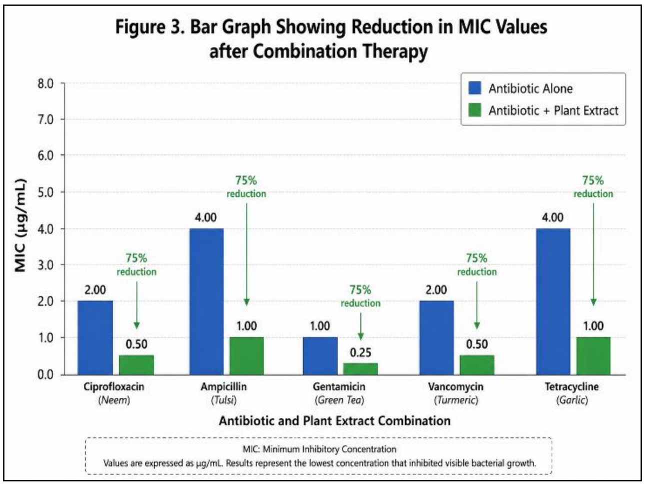

Figure 3. Bar Graph Showing Reduction in MIC Values after Combination Therapy

DISCUSSION

Interpretation of Findings

The extracts of the selected medicinal plants were found to possess possible antibacterial properties in the present study against the multidrug resistant (MDR) bacterial strains in particular methicillin-resistant Staphylococcus aureus (MRSA) and multidrug resistant *Escherichia coli* (MDR- E. coli). The larger zone of inhibition and lower minimum inhibitory concentration (MIC) value for these two extracts suggested that the extracts of *Azadirachta indica* and of *Camellia sinensis* were the most effective extracts in inhibiting the *Bacillus thuringiensis*. This result confirms with the one obtained in previous studies, which demonstrated that medicinal plants have high antimicrobial property due to their phytochemical contents (Yuan et al., 2016).

When combined with antibiotics the plant extracts showed significant effects when compared with the antibacterial activity of their respective antibiotic. This potentiation of antibiotic activity is found in combination therapy by the decreased MIC levels and greater inhibition zones seen. In case of resistant bacteria it has been observed that Neem + ciprofloxacin and Green tea + gentamicin have the most synergistic effects that enhance the susceptibility of the bacteria to antibiotics (Dzotam et al., 2016).

Table 1. Summary of Major Findings

Parameter	Observation
Most active plant extract	*Azadirachta indica*

Strongest synergistic combination	Neem + Ciprofloxacin
Lowest FICI value observed	0.31
Major antibacterial effect	Enhanced inhibition zones
MIC trend after combination	Significant reduction

Possible Mechanisms of Synergy

It may take the various mechanisms of action exhibited by phytochemicals in medicinal plant extracts into consideration as a possible explanation for the synergistic interactions that were observed in the present study. An important mechanism is the disrupting the integrity of the bacterial cell wall and membranes thereby enhancing the uptake of antibiotics by the bacterial cell. Antioxidants like flavonoids and phenols found in Neem and Green tea, exhibit antimicrobial activities resulting in improvement of the antibiotic accumulation in the cell wall of the bacteria (Zouine et al, 2024).

This effect may be due to inhibition of bacterial efflux pumps. The efflux systems are found in MDR bacteria and these can pump any antibiotic molecule out of the cell, reducing the intra-cellular antibiotic molecule concentration. Some plant phytochemicals like terpenoid and alkaloid compounds can be used to inhibit these efflux pumps which subsequently restore the action of the antibiotics (Jeong et al., 2023).

This can also be on the form of synergies such as higher permeability of the membrane and the prevention of the formation of biofilms. The aim of the present invention is to increase antimicrobial susceptibility by disrupting the architecture of the biofilms since this increases the bacteria susceptibility to antibiotics and attacks by host immune system. Curcumin and catechins are both claimed to be capable of interfering with the formation of biofilms and quorum sensing.Either curcumin or catechins have been found to disrupt formation of biofilms and quorum sensing systems. (Tacconelli et al., 2018)

Table 2. Proposed Mechanisms of Synergistic Action

Mechanism	Expected Outcome
Cell wall disruption	Enhanced antibiotic penetration
Efflux pump inhibition	Increased intracellular antibiotic concentration
Increased membrane permeability	Improved antibacterial efficacy
Biofilm inhibition	Reduced bacterial protection and resistance

Comparison with Previous Studies

The present study findings confirm the same data as earlier ones that demonstrated synergistic effect of medicinal plants when used with antibiotics they have reported earlier. Dzotam et al. (2016) found that the antimicrobial activity of plant extract mixture was more effective against the multidrug-resistant (MDR) gram-negative bacteria when used in combination with classical antibiotics. In similar finding, Jeong et al., (2023) observed the wonderful synergism of the combination of herbs and antibiotics against MRSA strains.

The present study was also in agreement with the previous studies which revealed that Neem, Green tea and Turmeric exhibits high antibacterial activity because of their rich phytochemical content. However, differences in zone inhibition and minimum inhibitory concentration with previous studies may have resulted from variations in extracts and their bacterial culture or solvents used, bacterial strains or the concentration of the phytochemicals.

Clinical and Pharmaceutical Implications

Synergic interactions observed have been found to be clinically and pharmacologically relevant. Extracts of medicinal plants can be used in conjunction with antibiotics, which can help to limit the use of some antibiotics and help people take fewer doses and minimise the risk of side effects from antibiotics and slow the growth of antibiotic resistance. This is due to the fact that if the level of antibiotics is reduced, then the expense of treatment as well as the patients' compliance will be lowered as well.

Furthermore, herbal medicines combined with antibiotics can bring the alternative treatment approach for MDR pathogens particularly in hospitals such as healthcare institutions that are most likely to harbor MDR pathogens. The pharmaceutical solutions of standardized extracts of plant materia may be used in finding novel antimicrobial drugs with enhanced therapeutic efficacy and safety (Murray et al., 2022).

LIMITATIONS

The results of the present research are promising but there are several limitations. The investigation was carried out entirely in vitro conditions which may not sufficiently represent in vivo response to therapy. Second, composition of phytochemicals may vary depending on environmental conditions, harvesting and extraction methods, thus changing the antibacterial activity. Finally, there were no toxicity studies or pharmacokinetic evaluations, which provided little clinical safety and dosage study optimization. Hence there is a need for more in vivo and clinical trials to substantiate therapeutic applications.

CONCLUSION

The present study was corroborated by the studies which showed that the extracts of some medicinal plants have significant antibacterial property against MDR bacterial pathogens such as MRSA and MDR-ecoli. Then it could be observed that Plant A (Azadirachta indica) has a greater zone of inhibition and a lower minimum inhibitory concentration value which indicates greater antibacterial activity among all other plants tested than the others. Another finding showed that the effect of the combination of the extracts of the medicinal plants with the conventional antibiotics was significant in terms of the antibacterial effect when compared to the activity of the conventional antibiotics alone.

A synergistic effect of Neem + Ciprofloxacin and Green tea + Gentamicin was observed, that resulted in reduction in MIC and better inhibition of bacterial growth. The results indicated that bioflavonoids from medicinal plants have also potential as antibiotic adjuvants in making the antibacterial drugs effective by simply removing the efflux pumps, destroying the bioflavonoids.

Medical plants and their antibiotics combinations show therapeutic potential as an alternative approach in tackling antimicrobial resistance, the study reveals. These can lower dosage and the impact of adverse results of antibiotics, and slow any resistant strains from emerging. The overall results support the growing research and development in utilizing plants and plant derived bioactive compounds against microbial infections and its management in clinical practices.

In a current study, however, the work conducted was in vitro experimental only, and more of these studies are necessary for in vivo validation of use. Future studies: These combinations have to be tested in vivo for their safety and efficacy; toxicity studies should be performed; pharmacokinetic studies should be carried out and clinical trials should be conducted. In most of the cases, the extracts of medicinal plants indicate a great potential in the discovery of new drugs against MDR bacterial infections as an ulterior means of treatment.

FUTURE PERSPECTIVES

Information on individual bioactive phytochemicals that can give synergism against antibiotics will be a major factor to be considered while studying medicinal plant and antibiotic combinations. The identification of compounds like flavonoids, alkaloids, tannins and phenolics can aid in the development of targeted antimicrobial medicines that would have high therapeutic potential.

Another avenue of research that is of special interest is the development of herbals drug delivery systems with nanotechnology. Adding plant extracts into nanoparticles, liposomes or phytosomes, can lead to enhanced bioavailability, stability and controlled application of the drug and even the increase of the antibacterial property against resistant pathogens.

Further in vivo studies are required, however, to evaluate dose–response relationship, toxicity and safety in an animal model for combination therapy. Furthermore, well- designed clinical trials are needed to be able to prove therapeutic efficiency in humans and reveal possible herb–drug interactions.

Additionally, standardization of extraction procedure and phytochemical profiling should also be discussed to ensure re-producibility and consistency across various studies. In the future, standardized phytopharmaceuticals, based on extracts of medicinal plants, in combination with antibiotics, may have a major therapeutic role in the treatment of multi-drug resistant bacterial infections and, as a result, alternatives to antimicrobial treatment.

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