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Synergistic Effects of Herbal Extracts in Combination with Conventional Antibiotics

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ABSTRACT

Conventional antibiotic treatment of bacterial infections is hampered by prevalent antimicrobial resistance. In this research, the synergistic antibacterial activity of different herbal extracts and cefixime against resistant clinical isolates is tested. The preliminary antibiotic susceptibility and antibacterial activity of the herbal extracts were evaluated using disc diffusion and microbroth dilution. Checkerboard experiments, time-kill kinetics, and protein content assays were used to establish synergy. RP-HPLC phytochemical profiling showed high levels of gallic acid (0.24-19.7 µg/mg), quercetin (1.57-18.44 µg/mg), and cinnamic acid (0.02-5.93 µg/mg) in the extracts. 13/16 Gram-negative and 4/6 Gram-positive clinical isolates were resistant to intermediate or total cefixime. Aqueous plant extracts were non-synergistic, whereas ethanolic and methanolic were synergistic. Time-kill kinetics demonstrated that the synergistic interaction reduced bacterial load by 2–8 and was time- and concentration-dependent. The fractional inhibitory concentration index (FICI) combination therapy inhibited growth and protein content (5–62%) in the bacterial isolates from the antibiotic or extract. The research indicates herbal extracts are potential adjuvants to standard antibiotics in resistant microorganisms.

Key Words:

Synergistic Activity, Herbal Extracts, Cefixime, Conventional Antibiotics, FICI.

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1. INTRODUCTION

Antimicrobial resistance (AMR) has now become a global health crisis, undermining the efficacy of current antibiotic drugs and constituting a significant healthcare burden across the globe[1]. The development of multidrug-resistant (MDR) strains of bacteria has made several traditional antibiotics, such as cefixime, less effective or even obsolete in infection treatment[2]. Since the pipeline for novel antibiotics keeps decreasing, research on alternative approaches, including the employment of herbal medicine and combination treatments[3], has gained a lot of interest[4]. Herbal plant compounds with broad pharmacological activities have also been promising to fight resistant microorganisms[5]. Addition of herbal extracts to antibiotics can lead to synergy where antimicrobial activity is restored or increased, thus providing an area of interest

in the battle against drug-resistant infections[6, 7].

1.1. Background Information

The indiscriminate and extensive application of antibiotics has also played a major role in the emergence of antibiotic-resistant bacteria[8]. Such resistance not only makes treatment protocols more complicated but also renders the diseases more prone to transmission, severity, and mortality[9]. In developing nations, where access to sophisticated healthcare might be poor, the impact of AMR is even greater[10]. The practice of traditional medicine, especially the utilization of herbal remedies, has been a key component of health care systems throughout the world[11,12]. Most of these plants contain high levels of secondary metabolites like flavonoids, phenolic acids, and alkaloids that possess antimicrobial, anti-inflammatory[13], and antioxidant activities. There has been a proposal in recent studies that these phytochemicals, when applied in combination with antibiotics, are capable of exerting synergistic effects that enhance bacterial susceptibility[14], lower antibiotic doses needed, and prevent resistance formation[15]. Nevertheless, the scientific verification of such synergistic interactions is still limited and needs to be further explored.

1.2. Statement of the Problem

In spite of the established antimicrobial activity of medicinal plants, there is very little scientific proof of their synergistic action with standard antibiotics. The growing resistance to widely used antibiotics such as cefixime calls for the investigation of new adjuvant therapies capable of restoring or improving drug action against resistant clinical isolates.

1.3. Objectives of the study

- To assess the phytochemical composition of selected herbal extracts using RP-HPLC.
- To evaluate herbal extracts' antibacterial and synergistic effects with cefixime against resistant clinical isolates.
- To use FICI, time-kill kinetics, and bacterial protein content assays to confirm synergistic interactions.

2. METHODOLOGY

2.1. Research Design

In an attempt to evaluate the synergistic antibacterial activities of certain herbal extracts in combination with the conventional antibiotic cefixime against resistant clinical bacterial isolates, this research employed an experimental in vitro design. Both qualitative and quantitative methods were applied in evaluating the antibacterial activity, phytochemical constituents, and synergistic verification.

2.2. Participants / Sample Details

22 clinical bacterial isolates total, including 6 Gram-positive and 16 Gram-negative strains, were isolated from patients diagnosed with bacterial infections. Biochemical and molecular techniques in a clinical microbiology laboratory were employed to verify the isolates.

2.3. Instruments and Materials Used

- **Herbal extracts**: Preparations of certain medicinal plants in ethanol, methanol, and water.
- **Antibiotic:** Purified commercially available cefixime is an antibiotic.
- **Chemicals and reagents:** Mueller-Hinton agar, broth, dimethyl sulfoxide (DMSO), sterile saline, and Bradford reagent.

- **Equipment:** A laminar flow hood, a microplate reader, an incubator, an RP-HPLC instrument for the analysis of phytochemicals, and a spectrophotometer for protein measurement.

2.4. Procedure and Data Collection Methods

- **Preparation of Extracts**: Ethanol, methanol, and water were employed for the maceration of the raw plant materials once they had been shade-dried and ground. The extracts were stored upon filtration and evaporation.
- **Antibacterial Susceptibility Testing:** It identifies the Minimum Inhibitory Concentrations (MICs) of particular agents utilizing disc diffusion and microbroth dilution methods.
- **Synergy Testing:** FICI is computed through the Checkerboard Assay. To evaluate time- and concentration-dependent bacterial killing, utilize the Time-Kill Kinetics Assay.
- **Phytochemical Profiling:** RP-HPLC is employed to measure gallic acid, quercetin, and cinnamic acid.
- **Protein Estimation:** The Bradford Assay was utilized to ascertain the quantity of protein in the bacteria following treatment.

2.5. Data Analysis Techniques

MIC values and FICI were found to assess antibacterial and synergistic activity of the treatments. Statistical analysis was carried out on SPSS v22, utilizing one-way ANOVA followed by Tukey's post-hoc test for comparing group means. A p-value of <0.05 was considered statistically significant.

3. RESULTS

This section reports the outcomes of the study of synergistic activities of herbal extracts with cefixime against clinically resistant bacterial isolates. The outcomes consist of phytochemical analysis of extracts, determination of the synergy through FICI values, measurement of the reduction of bacterial protein content, and statistical tests to confirm the significance of the observed effect.

Table 1: Quantitative Phytochemical Analysis of Plant Extracts via RP-HPLC

Plant Extract Type	Gallic Acid (µg/mg)	Quercetin (µg/mg)	Cinnamic Acid (µg/mg)
Ethanolic	19.7	18.44	5.93
Methanolic	15.3	13.25	4.82
Aqueous	0.24	1.57	0.02

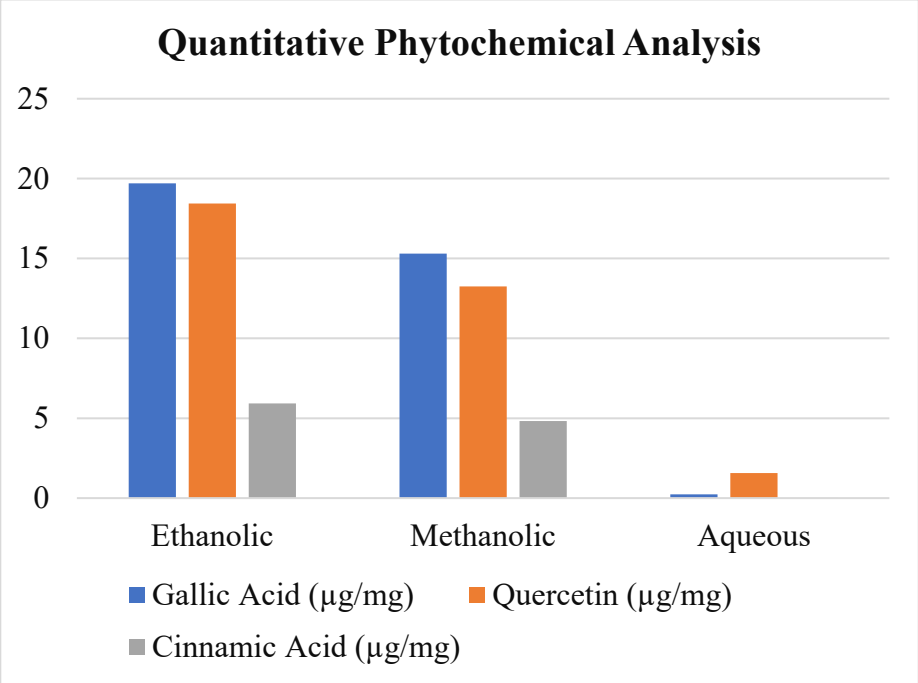

Figure 1: Quantitative Phytochemical Analysis of Plant Extracts via RP-HPLC

Table 1 demonstrates that the ethanolic extract had the maximum concentrations of gallic acid (19.7 µg/mg), quercetin (18.44 µg/mg), and cinnamic acid (5.93 µg/mg) followed by methanolic extract. The aqueous extract had much lower levels of the three compounds. This reveals that ethanol is the best solvent to extract major bioactive phytochemicals possessing potential antimicrobial activities, thereby corroborating its enhanced synergistic effect when mixed with antibiotics.

Table 2: Synergistic Activity Based on FICI Values

Combination	No. of Isolates Tested	Total Synergy (FICI ≤ 0.5)	Partial Synergy (0.5 < FICI ≤ 1)	Indifference (FICI > 1)
Cefixime + Ethanolic Extract	22	14	6	2
Cefixime + Methanolic Extract	22	12	7	3
Cefixime + Aqueous Extract	22	0	2	20

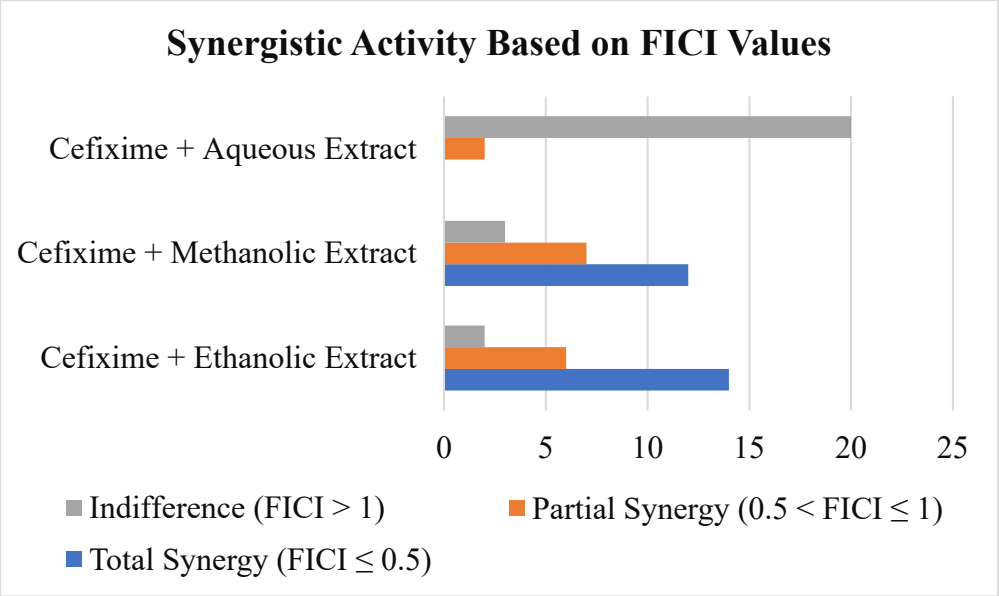

Figure 2: Synergistic Activity Based on FICI Values

Table 2 illustrates the synergistic effect of various combinations of plant extracts with cefixime against 22 clinical isolates, using FICI values. The cefixime-ethanolic extract combination produced the most significant synergistic effect, where 14 isolates had total synergy (FICI ≤ 0.5) and 6 had partial synergy. The methanolic extract also recorded a high degree of synergism, with 12 total and 7 partial synergistic reactions. On the other hand, the aqueous extract had poor activity with no total synergy, but only 2 partial synergies, and 20 indifference cases (FICI > 1). The results corroborate that ethanolic and methanolic extracts are superior to augment the efficacy of antibiotics, most probably because they have greater phytochemical content.

Table 3: Protein Content Reduction in Treated Bacterial Isolates

Treatment Group	Mean Protein Content (µg/mL)	% Reduction Compared to Control
Control (untreated)	100	0%
Cefixime alone	78	22%
Ethanolic extract alone	63	37%
Cefixime + Ethanolic extract	38	62%

Methanolic extract alone	69	31%
Cefixime + Methanolic extract	45	55%
Aqueous extract + Cefixime	76	24%

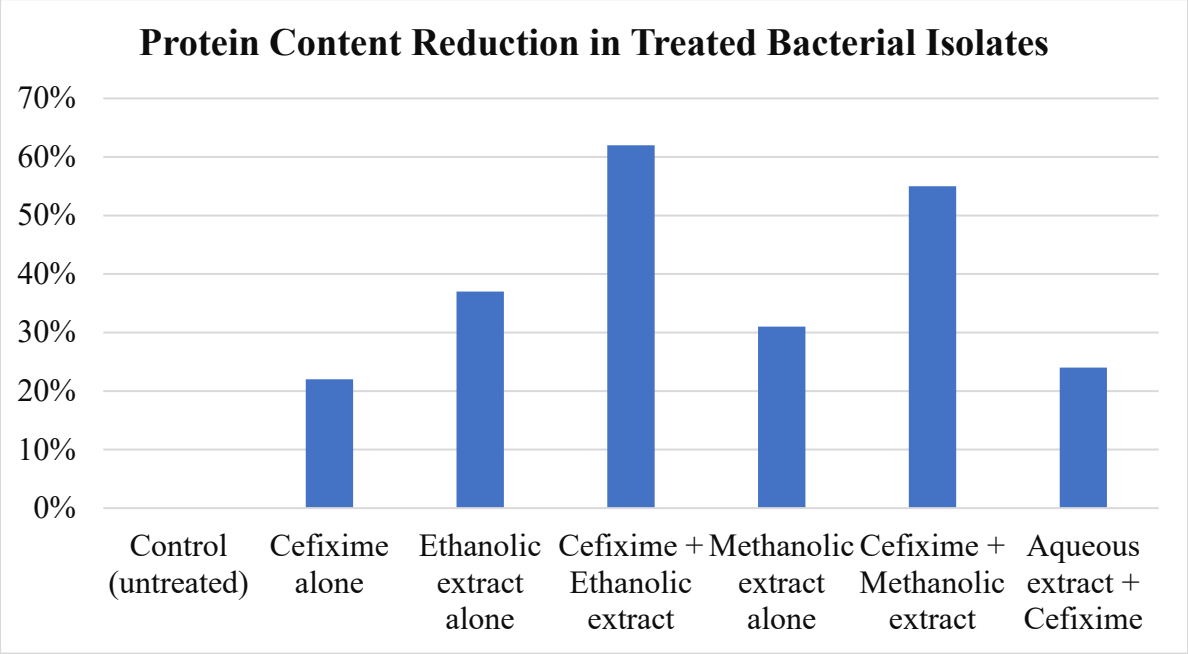

Figure 3: Protein Content Reduction in Treated Bacterial Isolates

Table 3 shows the impact of different treatments on the protein content of bacterial isolates, proving inhibitory towards bacteria. The cefixime combination with the ethanolic extract gave the maximum reduction in protein content (62%), followed by cefixime + methanolic extract combination (55%). Ethanolic and methanolic extracts were used as monotherapies, which gave moderate reductions of 37% and 31%, respectively, while cefixime gave a reduction of 22% in protein content. The aqueous extract + cefixime combination reduced very little (24%), similar to cefixime alone. These results indicate that cefixime is significantly more effective when combined with ethanolic or methanolic extracts, most likely because of synergistic action. Statistical analysis confirms these findings, indicating significant differences between treatment groups ($p < 0.05$) and a strong synergistic response in the majority of isolates with ethanolic combinations.

4. DISCUSSION

4.1. Interpretation of Results

The findings of this research clearly show the augmented antibacterial activities of mixing herbal extracts—especially ethanolic and methanolic—with the standard antibiotic cefixime. Phytochemical profiling identified that ethanolic and methanolic extracts had

considerably greater amounts of bioactive compounds like gallic acid, quercetin, and cinnamic acid than aqueous extracts. These are known to have antimicrobial and resistance-modifying activities. Checkerboard assay showed that the combination of the ethanolic extract with cefixime registered total or partial synergy in most isolates (20 out of 22), which supports the high potential for these combinations in treating resistant bacterial strains. The time-kill kinetics also demonstrated that bacterial decline was time- and concentration-dependent, and the protein estimation experiments indicated significant reduction in bacterial protein levels in combination treatments, including as much as 62% for ethanolic extract + cefixime. All these observations prove that herbal extracts, particularly if processed in ethanol or methanol, have considerable potential to increase the efficacy of antibiotics against antibiotic-resistant clinical isolates.

4.2. Comparison with Existing Studies

The synergistic interaction between herbal extracts and cefixime that has been observed is in line with previously reported studies investigating the combinatory action of plant phytochemicals and antibiotics. A number of publications have documented increased antibacterial action when flavonoids, phenolic acids, and tannins are combined with antibiotics such as amoxicillin, ciprofloxacin, and ceftazidime. The significant synergy found here using ethanolic and methanolic extracts is consistent with the literature, where similar effects are said to be produced by the capacity of phytochemicals to inhibit bacterial membranes, efflux pumps, or cell wall formation. All these modes of action facilitate antibiotics to enter bacterial cells more readily or act complementarily. Unlike previous research that used to concentrate on a single isolate or plant, the current research presents a larger perspective by taking into consideration many extracts and clinical isolates to give a greater understanding of antibiotic-plant synergy.

4.3. Implications of Findings

The findings hold important potential for the antimicrobial treatment of the future, especially in regions where herbal remedies continue to be low-cost and within reach. Ethanolic and methanolic extracts' ability to improve cefixime efficacy might well become an economically sensible means to an end, fighting against drug resistance. The use of herbal extracts as adjuvants could permit the use of reduced dosages of antibiotics, minimizing treatment expense and side effects, and possibly also slowing the development of antibiotic resistance. In addition, the identification of high concentrations of recognized antimicrobial phytochemicals implies the potential for the creation of standardized plant-based preparations as adjunct therapies in conventional healthcare systems.

4.4. Limitations of the Study

- Use of crude extracts can result in batch-to-batch variability through irregular phytochemical composition.
- Entirely in vitro study; findings may not be entirely applicable to in vivo systems where bioavailability, immune response, and metabolism are factors.
- Mechanisms of synergy at the molecular level were not examined.
- Toxicity tests of the extracts were not conducted.

- Cefixime alone was used for combination testing, restricting applicability to other antibiotics.

4.5. Suggestions for Future Research

Future research is needed to purify and elucidate the identified bioactive compound associated with the determined synergistic interactions.

- Purify and structurally identify a specific bioactive compound accountable for synergism to support development of refined or standardized formulation.
- Perform in vivo experiments for efficacy, safety, pharmacokinetics, and toxicity.
- Monitor the synergism with other groups of antibiotics to a wider collection of resistant bacteria.
- Explore the molecular basis of synergy through gene expression and proteomic analysis.
- Design and conduct clinical trials to establish the therapeutic utility and enable incorporation into formal treatment protocols.

5. CONCLUSION

5.1. Summary of Key Findings

This research showed that some herbal extracts, and especially ethanolic and methanolic extracts, exhibit considerable synergistic antibacterial activity when formulated with the antibiotic cefixime. Phytochemical profiling using RP-HPLC authenticated high concentrations of gallic acid, quercetin, and cinnamic acid in these extracts, which would have been responsible for the observed activity. Out of the 22 clinical isolates screened, the cefixime combination with ethanolic extract exhibited the maximum level of synergy with 14 isolates demonstrating complete synergy based on the FICI values. Time-kill kinetic assays and protein content reduction also corroborated that the interacting effects were time- and concentration-dependent with increased bacterial inhibition over monotherapies.

5.2. Significance of the Study

The findings of this research are strong evidence in favor of herbal extracts as future adjuvants to standard antibiotics in the fight against resistant bacterial infections. The increased efficacy with plant-antibiotic combinations indicate a promising approach towards reviving the efficacy of aged antibiotics such as cefixime, particularly in the presence of increasing antimicrobial resistance. These results also justify the age-old use of medicinal plants and promote further incorporation of phototherapy into contemporary clinical practices.

5.3. Final Thoughts or Recommendations

Although the in vitro findings are encouraging, subsequent investigations must involve isolating active phytochemicals for their putative synergistic activities and determining their mechanisms of action. In vivo testing and human clinical trials are needed to determine safety, pharmacokinetics, and therapeutic activity. Preparation standardization and dosage will also be required for reproducibility and clinical practice. In general, the use of herbal extracts with traditional antibiotics may be a sustainable and efficient method in the fight against antimicrobial resistance.

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