RESEARCH PAPER

Synergistic Antibacterial Activity Of A Polyherbal Formulation Against Streptococcus Pyogenes: An In Vitro Evaluation

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Abstract

The increasing prevalence of antimicrobial resistance in streptococcus pyogenes necessitates the exploration of effective plant based antibacterial alternatives. This study investigated the in vitro antibacterial efficacy of a polyherbal formulation comprising curcuma longa (turmeric), zingiber officinale (ginger), and crocus sativus (saffron) against s. Pyogenes. Antibacterial activity was evaluated using agar well diffusion assays to assess zones of inhibition and minimum bactericidal concentration (mbc) assays to confirm bactericidal effects, with comparisons made against standard antibiotics azithromycin and ciprofloxacin. While individual herbal extracts exhibited limited inhibitory activity, the polyherbal formulation demonstrated significantly enhanced, concentration dependent antibacterial effects, producing inhibition zones superior to azithromycin and comparable to or exceeding ciprofloxacin at higher concentrations. Serial dilution studies revealed a threshold driven synergistic response, and concordant mbc findings confirmed true bactericidal action. The enhanced activity is attributed to the synergistic, multi target effects of curcuminoids, gingerols, and saffron phytochemicals. These findings highlight the potential of the polyherbal formulation as a promising natural antibacterial alternative against s. Pyogenes, warranting further mechanistic and in vivo investigations.

Keywords: Streptococcus Pyogenes, Zone Of Inhibition, Polyherbal Extracts, Turmeric, Ginger, Saffron, Ciprofloxacin.

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1. Introduction

St*reptococcus pyogenes*, a Gram-positive Group A β-haemolytic bacterium, is a major causative agent of acute pharyngitis and a range of invasive and post-infectious complications, including rheumatic fever and glomerulonephritis. [1] Although antibiotic therapy remains highly effective for the management of streptococcal infections, the growing concerns of antimicrobial resistance, treatment failure, and recurrent infections highlight the need for complementary or alternative therapeutic strategies. [2]

Plant-based antimicrobials have gained renewed scientific interest due to their broad spectrum of biological activities and lower propensity for resistance development. Polyherbal formulations are believed to offer enhanced antibacterial efficacy through synergistic interactions among multiple phytochemicals targeting diverse microbial pathways. Such combinations may exert antibacterial effects by disrupting cell membrane integrity, inhibiting essential enzymes, impairing nucleic acid synthesis, and reducing biofilm formation. [3]

Curcumin, the principal bioactive constituent of turmeric (Curcuma longa), is widely acknowledged for its pronounced antimicrobial activity. It demonstrates antibacterial effects against a wide range of Gram-positive and Gram-negative microorganisms. The antibacterial action of curcumin is attributed to multiple mechanisms, including disruption of bacterial cell wall integrity, suppression of virulence factor production, and inhibition of biofilm development. In addition, curcumin promotes intracellular oxidative stress within bacterial cells, further contributing to its antimicrobial efficacy. Previous studies have reported notable activity of curcumin against pathogens such as *Streptococcus pyogenes* and methicillin-sensitive Staphylococcus aureus. When used alongside conventional antibiotics, curcumin has been shown to produce synergistic antibacterial effects, thereby enhancing therapeutic outcomes. Nevertheless, limitations related to its poor bioavailability and chemical stability must be addressed to maximize its clinical applicability. [4]

Zingiber officinale (ginger) is well recognized for its strong antibacterial potential, primarily owing to its diverse bioactive constituents, including gingerols, shogaols, paradols, and zingerone. These compounds collectively exert antimicrobial effects by compromising bacterial membrane integrity, inhibiting protein synthesis, and inducing oxidative damage within microbial cells. Ginger extracts have demonstrated effectiveness against a broad spectrum of bacterial species, encompassing both Gram-positive and Gram-negative organisms. Its reported activity against antibiotic-resistant strains further underscores its value as a natural adjunct or alternative to synthetic antimicrobials. Despite its promising antibacterial performance, challenges associated with improving bioavailability and formulation stability remain active areas of investigation. [5]

Saffron, obtained from the stigmas of Crocus sativus, is valued not only for its culinary and medicinal significance but also for its notable antibacterial activity. Key phytochemicals present in saffron, such as crocin, crocetin, and safranal, exhibit considerable antimicrobial effects against various pathogenic microorganisms. These bioactive compounds exert their antibacterial action by penetrating bacterial cell walls, inhibiting protein biosynthesis, and impairing biofilm formation. Research indicates that saffron is effective against both Gram-positive and Gram-negative bacteria, highlighting its broad-spectrum antimicrobial capability. Furthermore, incorporation of saffron into advanced delivery systems, such as nanoemulsions, has been reported to enhance its stability and bioavailability. However, additional studies are required to better elucidate its mechanisms of action and optimize its clinical application. [6]

Despite increasing evidence supporting the antibacterial properties of these individual herbs, limited data are available on their combined activity against *Streptococcus pyogenes*. Therefore, the present study aims to evaluate the in vitro antibacterial efficacy of a polyherbal combination of turmeric, ginger, and saffron using zone of inhibition, minimum inhibitory concentration, and minimum bactericidal concentration assays, with comparative reference to the standard antibiotic Ciprofloxacin. The findings of this study may provide scientific insight into the potential application of this polyherbal formulation as an adjunct or alternative approach in the management of streptococcal infections. [7]

2. Materials and Methods

2.1 Herbal Extracts

2.1 Herbal Extracts and Polyherbal Formulation

Standardized commercial extracts of Curcuma longa (turmeric; ≥95% curcuminoids), Zingiber officinale (ginger; ≥10% gingerols), and Crocus sativus (saffron; ≥30% safranal) were used in this study. Individual extracts were stored according to manufacturer specifications and freshly prepared prior to experimentation. [8]

The polyherbal formulation was prepared by combining the three extracts in predetermined proportions to obtain a final composite preparation. Serial dilutions of the polyherbal formulation were prepared to yield six graded concentrations (11.0%, 14.1%, 17.2%, 20.3%, 23.5%, and 36.0%) for antibacterial evaluation. Individual herbal extracts were also tested at their respective working concentrations to assess comparative antibacterial activity. [9]

2.2 Microbial Strain and Inoculum Preparation

Streptococcus pyogenes was used as the test organism. The bacterial strain was cultured in Mueller–Hinton broth and incubated at 37 °C for 18–24 hours. The inoculum was standardized to match 0.5 McFarland turbidity standard (≈1.5×10^8 CFU/mL) to ensure uniform bacterial density across all assays.

2.3 Agar Well Diffusion Assay (Zone of Inhibition)

The antibacterial activity of individual herbal extracts, the polyherbal formulation, and standard antibiotics was assessed using the agar well diffusion method. Mueller–Hinton agar plates were uniformly inoculated with the standardized *S. pyogenes* suspension using sterile cotton swabs. [10]

Wells of uniform diameter were aseptically punched into the agar and filled with specified concentrations of turmeric, ginger, saffron, polyherbal formulation, azithromycin (0.5%), and ciprofloxacin (0.5%). Plates were incubated at 37 °C for 24 hours. Following incubation, the diameter of the zone of inhibition (IZD) around each well was measured in millimeters. All assays were performed in triplicate, and results were expressed as mean ± standard deviation. [11]

\2.4 Evaluation of Concentration-Dependent Antibacterial Activity

To assess the synergistic and dose-dependent antibacterial effect of the polyherbal formulation, six serial dilutions (D1–D6) were evaluated using the agar diffusion method. Considering Azithromycin and Ciprofloxacin as negative control. The variation in inhibition zone diameter across concentrations was used to determine the threshold concentration required for maximal antibacterial activity.

2.5 Determination of Minimum Bactericidal Concentration (MBC)

Broth dilution assays were initially performed to determine the minimum inhibitory concentration (MIC); however, accurate visual interpretation was limited due to the inherent turbidity of the herbal extracts. Therefore, bactericidal activity was confirmed using minimum bactericidal concentration (MBC) determination. [12]

Following exposure of *S. pyogenes* to different concentrations of the polyherbal formulation, aliquots from tubes showing no visible growth were subcultured onto blood agar plates and incubated at 37 °C for 24 hours. The absence of bacterial colonies on subculture plates was interpreted as confirmation of bactericidal activity.

2.6 Statistical Analysis

All experiments were conducted in triplicate. Results were expressed as mean ± standard deviation. Comparative evaluation of antibacterial activity was based on zone diameter measurements and bactericidal outcomes.

3. Results

Agar diffusion results demonstrated clear, concentration-dependent zones of inhibition against *Streptococcus pyogenes.* Higher polyherbal concentrations produced wider zones, indicating stronger antibacterial action.

MBC testing revealed complete bacterial eradication at the highest polyherbal concentration tested, while lower concentrations exhibited partial inhibition.

Table 1. Antibacterial activity of different concentrations of polyherbal extracts against *S. pyogenes.*

Plant Name	**D1**	**D2**	**D3**	**D4**	**D5**	**D6 (Positive Control)**
Turmeric	6.0 %	7.5 %	9.0 %	10.5 %	12.0 %	18.0 %
Ginger	4.0 %	5.5 %	7.0 %	8.5 %	10.0 %	15.0 %
Saffron	1.00 %	1.10 %	1.20 %	1.30 %	1.50 %	3.0%
Total	11.00%	14.10%	17.20%	20.30%	23.50%	36.00 %

The study was carried out using five different concentrations of the combined herbal extracts along with two control groups. The positive control was prepared by inoculating 1 mL of Mueller–Hinton broth with a few colonies of *Streptococcus pyogenes* using a sterile micro-streaker (Figure 1). The negative control consisted of a mixture of turmeric, ginger, and saffron extracts containing

18% curcuminoids, 15% gingerols, and 3% safranal, yielding a total extract concentration of 36% in 1 mL of Mueller–Hinton broth.

Serial dilutions of the combined herbal extracts were prepared in Mueller–Hinton broth and designated as D1, D2, D3, D4, and D5, as shown in Table 3. These dilutions corresponded to final concentrations of approximately 11.00%, 14.10%, 17.20%, 20.30%, and 23.50%, respectively. For each dilution, 1 mL of the extract mixture was combined with 1 mL of Mueller–Hinton broth in sequential tubes. After preparation of all test concentrations, 1 mL of the bacterial suspension was added to each tube. The inoculated tubes were incubated at 37°C for a minimum of 24 hours. To ensure reproducibility and accuracy of results, all experiments were performed in quadruplicate.

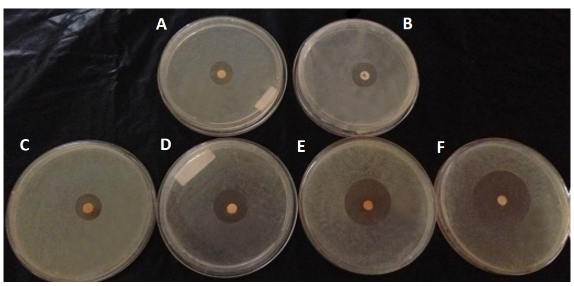

Figure: 1 Agar well diffusion plates illustrating antibacterial activity against Streptococcus pyogenes, where A: Turmeric (12.0%), B: Ginger (10.0%), C: Saffron (1.5%), D: Azithromycin (0.5%), E: Ciprofloxacin (0.5%), and F: Polyherbal formulation (23.5%). The polyherbal formulation produced the largest zone of inhibition, indicating superior antibacterial activity.

Table 2. Antibacterial activity of polyherbal vs Turmeric, Ginger, Saffron, Azithromycin & Ciprofloxacin against *S. pyogenes.*

Antibacterials	**Concentration**	**IZD Diameter (mm)**
Turmeric (A)	12.00 %	8.20 ± 0.12
Ginger (B)	10.00 %	8.30 ± 0.24
Saffron (C)	1.50 %	8.10 ± 0.32
Azithromycin (D)	0.50 %	14.50 ± 0.68
Ciprofloxacin (E)	0.50 %	24.50 ± 0.78
Polyherbal (F)	23.50 %	32.6 ± 1.34

Synergistic effect of the Polyherbal combination shows superior IZD against individual contribution in IZD Turmeric, Ginger, Saffron, Azithromycin & Ciprofloxacin by Agar diffusion method on *S. pyogenes.*

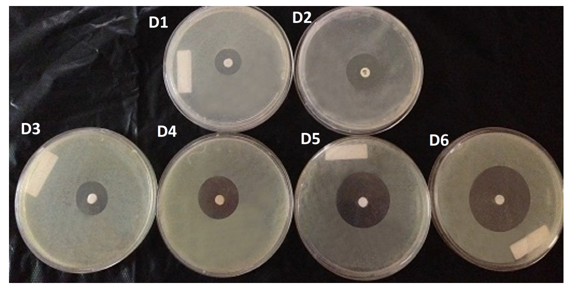

Figure: 2 Agar well diffusion assay showing concentration-dependent antibacterial activity of the polyherbal formulation against *Streptococcus pyogenes*. Plates D1–D6 represent increasing polyherbal concentrations (11.0%–36.0%), demonstrating progressively larger zones of inhibition and a pronounced synergistic effect at higher concentrations.

Table 3. Antibacterial activity of series of polyherbal dilutions against *S. pyogenes.* Synergistic effect of the Polyherbal combination dilutions shows superior IZD by Agar diffusion method on *S. pyogenes*

Polyherbal	**Concentration**	**IZD Diameter (mm)**
D1	11.00%	9.90 ± 0.22
D2	14.10%	10.80 ± 0.64
D3	17.20%	12.40 ± 0.72
D4	20.30%	14.50 ± 0.98
D5	23.50%	30.24 ± 1.18
D6	36.00%	34.60 ± 1.62

4. Discussion

The zone of inhibition (IZD) assay provides a preliminary yet robust indication of antibacterial potency by reflecting the ability of antimicrobial agents to diffuse through agar and inhibit bacterial growth. In the present study, the IZD findings strongly corroborate the bactericidal activity observed in minimum bactericidal concentration (MBC) assays, together confirming the enhanced antibacterial efficacy of the polyherbal formulation against *Streptococcus pyogenes*. The substantially larger inhibition zones produced by the polyherbal combination, compared with individual plant extracts and standard antibiotics, indicate a pronounced synergistic interaction among its phytochemical constituents.

Individually, turmeric, ginger, and saffron exhibited modest inhibitory activity, with IZD values ranging between 8.10 and 8.30 mm. These findings align with previous observations that single-plant extracts often display limited antibacterial activity due to restricted target specificity and sub-optimal concentration of active compounds. Turmeric's curcuminoids, ginger's gingerols, and saffron's bioactive molecules such as crocin and safranal are each known to exert antibacterial effects; however, when used alone, their action may be insufficient to cause extensive bacterial growth suppression. The relatively small IZD values obtained for these individual extracts support this notion and emphasize the limitations of monotherapeutic phytochemicals against resilient pathogens such as *S. pyogenes*.

In contrast, the polyherbal formulation demonstrated a markedly superior antibacterial response, producing an inhibition zone of 32.6 ± 1.34 mm at 23.5% concentration. This value not only exceeded those of the individual herbal components but also surpassed that of azithromycin (14.5 ± 0.68 mm) and approached or exceeded ciprofloxacin (24.5 ± 0.78 mm), a potent fluoroquinolone antibiotic. Such enhancement strongly suggests a synergistic rather than additive interaction among the constituent phytochemicals. The synergy likely arises from the simultaneous targeting of multiple cellular sites, thereby overwhelming the bacterial defense mechanisms.

Mechanistically, the antibacterial synergy of the polyherbal formulation can be attributed to the complementary actions of curcuminoids, gingerols, and saffron phytochemicals. Curcuminoids are known to disrupt bacterial cell membranes by increasing membrane permeability, leading to leakage of essential intracellular components. Gingerols further potentiate this effect by causing lipid bilayer destabilization and denaturation of membrane-bound proteins. Concurrently, saffron constituents interfere with enzymatic activity and metabolic pathways, impairing energy production and nucleic acid synthesis. The combined effect of these mechanisms results in profound membrane damage, protein denaturation, and metabolic disruption, ultimately leading to bacterial cell death.

The concentration-dependent study of polyherbal dilutions provides further insight into this synergistic interaction. As shown in the serial dilution assay, a gradual increase in IZD was observed from D1 (11.0%) to D4 (20.3%), indicating a dose-responsive antibacterial effect. Notably, a sharp escalation in inhibition zone diameter occurred at D5 (23.5%), where the IZD increased dramatically to 30.24 ± 1.18 mm, followed by a further increase at D6 (36.0%). This sudden rise suggests the existence of a critical concentration threshold, beyond which the synergistic interactions among phytochemicals become maximally effective. Such behaviour is characteristic of multi-component systems where cooperative effects are triggered once adequate concentrations of all active constituents are present.

The strong correlation between large IZDs and bactericidal endpoints observed in MBC assays further validates the lethal nature of the polyherbal formulation's action. While IZD assays primarily indicate growth inhibition, the concordance with MBC results confirms that the observed zones are not merely bacteriostatic but reflective of true bacterial killing. This is particularly significant in the context of *S. pyogenes*, a pathogen associated with both mild and invasive infections, where bactericidal activity is clinically desirable.

Overall, these findings demonstrate that the polyherbal formulation exhibits superior antibacterial efficacy through a multi-target mode of action. The synergistic interplay between curcuminoids, gingerols, and saffron phytochemicals enhances membrane disruption, protein inactivation, and metabolic inhibition, resulting in amplified antibacterial activity compared with individual components and some conventional antibiotics. This study highlights the therapeutic potential of rationally designed polyherbal formulations as effective alternatives or adjuncts to standard antibacterial agents, particularly in the face of increasing antimicrobial resistance.

5. Conclusion

The polyherbal formulation comprising *Curcuma longa*, *Zingiber officinale*, and *Crocus sativus* demonstrated significant antibacterial activity against *Streptococcus pyogenes*, as confirmed by zone of inhibition and bactericidal assays. While individual herbal extracts exhibited limited inhibitory effects, their combined formulation produced markedly enhanced antibacterial efficacy, indicating a strong synergistic interaction among the constituent phytochemicals.

The polyherbal formulation showed superior inhibition compared to standard antibiotics such as azithromycin and comparable or enhanced activity relative to ciprofloxacin. A clear concentration-dependent increase in antibacterial activity was observed, with a pronounced threshold effect, highlighting the importance of optimal phytochemical synergy. The concordance between IZD and MBC findings confirms that the formulation exerts true bactericidal activity.

Overall, the enhanced antibacterial effect is attributed to the multi-target action of curcuminoids, gingerols, and saffron phytochemicals, leading to membrane disruption, protein denaturation, and metabolic interference in *S. pyogenes*. These findings support the potential of this polyherbal formulation as a promising natural antibacterial alternative and warrant further mechanistic and in vivo investigations.

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