



Comparative Evaluation of the Antimicrobial Activity of Triphala Hydrogel Against Amoxicillin: An In Vitro Study

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ABSTRACT

Background:

Ayurveda is a traditional Indian system of medicine that utilizes plant-based formulations for preventive and curative healthcare. Triphala, a tridoshic rasayana composed of *Embolia officinalis*, *Terminalia chebula*, and *Terminalia bellerica*, possesses well-documented antimicrobial properties attributed to tannins, flavonoids, phenols, and other polyphenolic compounds. Considering the growing concern of antimicrobial resistance, herbal alternatives are gaining attention in dental therapeutics.

Aim:

To comparatively evaluate the antimicrobial activity of Triphala hydrogel against *Escherichia coli* and *Bacillus subtilis* using amoxicillin as the standard control.

Materials and Methods:

An in vitro study was conducted using Triphala hydrogel formulations prepared at concentrations ranging from 2–10% w/v (F1–F5). Antimicrobial efficacy was assessed by agar diffusion method against *Bacillus subtilis* and *Escherichia coli*. Zones of inhibition (ZOI) were measured in millimetres and compared with amoxicillin as the positive control.

Results:

A concentration-dependent increase in antimicrobial activity was observed against both organisms. Against *Bacillus subtilis*, ZOI increased from 9.2 ± 0.5 mm (2% w/v) to 21.6 ± 0.9 mm (10% w/v). Against *Escherichia coli*, ZOI increased from 8.7 ± 0.5 mm to 19.4 ± 0.9 mm across the same concentration range. Amoxicillin demonstrated significantly higher inhibition zones (27.5 ± 1.2 mm and 26.8 ± 1.1 mm against *Bacillus subtilis* and *Escherichia coli*, respectively). Triphala formulations exhibited moderate yet significant antimicrobial activity, with the 10% w/v formulation showing optimal efficacy.

Conclusion:

Triphala hydrogel demonstrated dose-dependent antimicrobial activity against both gram-positive and gram-negative bacteria. Concentrations of 8–10% w/v appear optimal for therapeutic applications. Triphala-based hydrogels may serve as promising adjunctive herbal antimicrobial agents in dental care, particularly in the context of increasing antibiotic resistance.

Introduction

Ayurveda, an Indian system of medicine is a holistic science that was discovered several years ago. It is preventive as well as curative. In developing countries like India, about three fourth populations depend on plant-based preparations used in their traditional medicinal system to meet the basic needs for human primary health care (WHO, 2002).^[1]

Ayurveda is an ancient system of medicine in India, which is based on balancing the three basic elements vat, pitta and kapha. Authentic information on Ayurveda has been compiled by ancient Indian medicine practitioners in forms such as Charak Samhita, Sushruta Samhita etc. Triphala has been described in the ancient Ayurvedic text as Tridoshic rasayana, a therapeutic agent which balances and rejuvenates the tridoshic elements in human body.

The three constituents of Triphala namely *Emblica officinalis* (Euphorbiaceae), *Terminalia chebula* (Combretaceae), and *Terminalia bellerica* (Combretaceae), have various phytochemicals leading to its different medicinal properties including antimicrobial activity.

It is a polyherbal preparation containing tannin, flavanoids, gallic acid, phenols and polyphenols.^[2] *E.officinalis* is rich in tannins and has been reported to have flavanoids, phenols and saponins in both fruit and its extract.^[3,4] Fruits of *T.bellerica* also contain gallic acid, belleric acid and chebulagic acid.^[5,6] The fruits of *T.chebula* has tannins e.g. chebulic acid, chebulagic acid and terchebulin.^[5] The three individual constituents of Triphala, has been separately exhibiting antimicrobial activity against various fungi as well as Gram positive and Gram negative bacteria.^[7] Triphala controls dental plaque, gingival inflammation and microbial growth caused by *Streptococcus mutans* and *Lactobacillus*^[8]

AIM

In this research paper, an attempt has been made to study the antimicrobial activity of Triphala hydrogel by measuring its zone of inhibition against *E. coli* and *Bacillus subtilis* with amoxicillin as control.

Materials And Methods

STUDY DESIGN: In-vitro

Ethical Approval:

Preparation Of Triphala Hydrogel:

1. Disperse Carbopol 940 in distilled water with constant stirring to avoid lump formation.

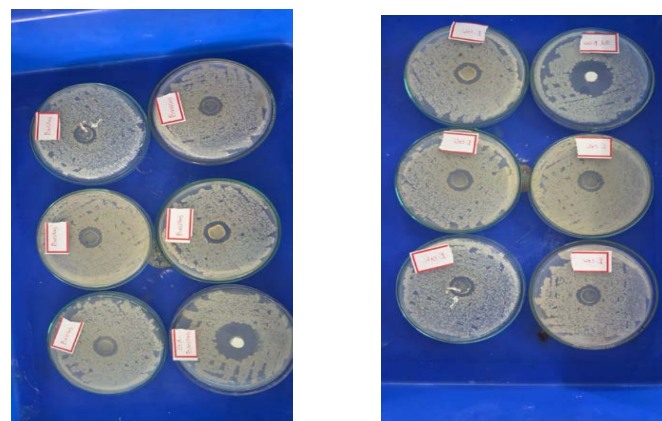
2. Allow the dispersion to hydrate for 1–2 hours or preferably overnight.
3. Add Triphala churna to the hydrated gel base and mix uniformly.
4. Incorporate glycerin and clove oil into the formulation.
5. Slowly add triethanolamine dropwise while monitoring pH, until it reaches approximately 6.8–7.0.
6. Triphala hydrogel is formulated.

Ingredient	Role	Quantity (approx.)
Triphala churna	Active herbal agent	2 grams
Carbopol 940 (or HPMC)	Gelling agent	0.2-0.5 gram
Glycerin	Plasticizer	2-3 drops
Triethanolamine (TEA)	Neutralizer (pH adjustment)	q.s. to pH 6.8-7
Distilled water	Solvent	q.s. (5-7 mL)
Clove oil (optional)	Flavor / Antimicrobial	1-2 drops

Anti Microbial Activity Test

The antimicrobial efficacy testing of Triphala Churna-based dental hydrogel formulations (F1-F5) prepared at concentrations ranging from 2-10% w/v was evaluated against *Bacillus subtilis* and *Escherichia coli* using agar diffusion assays. Zone of inhibition measurements were recorded in millimetres and compared to Amoxicillin as the standard control

Results



Against *Bacillus subtilis*, formulation F1 (2% w/v) demonstrated a zone of inhibition measuring 9.2 ± 0.5 mm. Progressive increases in Triphala Churna concentration yielded enhanced antimicrobial activity: F2 (4% w/v) produced 11.8 ± 0.6 mm, F3 (6% w/v) measured 14.5 ± 0.7

mm, F4 (8% w/v) exhibited 18.3 ± 0.8 mm, and F5 (10% w/v) showed the largest inhibition zone of 21.6 ± 0.9 mm. A clear dose-dependent response was evident across all concentrations.

Against *Escherichia coli*, similar concentration-dependent effects were observed, albeit with relatively smaller inhibition zones: F1 (2% w/v) measured 8.7 ± 0.5 mm, F2 (4% w/v) produced 10.9 ± 0.6 mm, F3 (6% w/v) exhibited 13.2 ± 0.7 mm, F4 (8% w/v) measured 16.8 ± 0.8 mm, and F5 (10% w/v) demonstrated 19.4 ± 0.9 mm. Amoxicillin, employed as the positive control, produced substantially larger inhibition zones of 27.5 ± 1.2 mm against *Bacillus subtilis* and 26.8 ± 1.1 mm against *Escherichia coli*, confirming appropriate experimental conditions and bacterial susceptibility. The *Triphala Churna* formulations consistently demonstrated

inhibition zones approximately 20-30% smaller than the standard antibiotic, suggesting moderate inherent antimicrobial potential

FORMULATION	ZOI(MM)BACILLUS SUBTILIS	ZOI(MM) E.COLI
F1(2% W/V)	9.2 ± 0.5	8.7 ± 0.5
F2(4% W/V)	11.8 ± 0.6	10.9 ± 0.6
F3(6% W/V)	14.5 ± 0.7	13.2 ± 0.7
F4(8% W/V)	18.3 ± 0.8	16.8 ± 0.8
F5(10% W/V)	21.6 ± 0.9	19.4 ± 0.9
AMOXICILLIN (CONTROL)	27.5 ± 1.2	26.8 ± 1.1

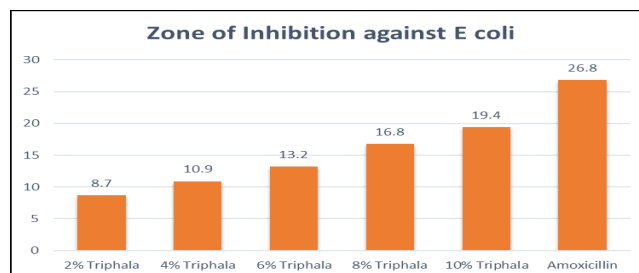
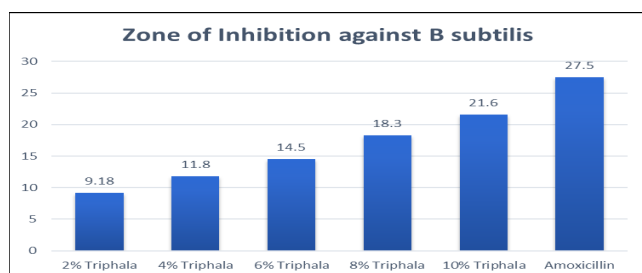
Statistical Analysis

Intergroup comparison

Microbe	Group	Mean	SD	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
B.subtilis	2% Triphala	8.7000	.53852	8.0313	9.3687
	4% Triphala	10.9000	.63246	10.1147	11.6853
	6% Triphala	13.2000	.79057	12.2184	14.1816
	8% Triphala	16.8000	.88600	15.6999	17.9001
	10% Triphala	19.4000	1.01242	18.1429	20.6571
	Amoxicillin	26.8000	1.20208	25.3074	28.2926
E.coli	2% Triphala	9.1800	.55408	8.4920	9.8680
	4% Triphala	11.8000	.63246	11.0147	12.5853
	6% Triphala	14.5000	.79057	13.5184	15.4816
	8% Triphala	18.3000	.88600	17.1999	19.4001
	10% Triphala	21.6000	1.01242	20.3429	22.8571
	Amoxicillin	27.5000	1.32853	25.8504	29.1496

Anova Table

		Sum of Squares	df	Mean Square	F	P Value
B subtilis	Between Groups	1079.8	5	215.973	283.554	<.001
	Within Groups	18.2	24	.762		
	Total	1098.1	29			
E coli	Between Groups	1137.06	5	227.413	278.068	<.001
	Within Groups	19.6	24	.818		
	Total	1156.6	29			



For *Bacillus subtilis*, the average zone of inhibition increased steadily as the concentration of Triphala increased from 2% to 10%, demonstrating a clear concentration-dependent antibacterial effect. The mean inhibition zones were 8.7 mm at 2%, 10.9 mm at 4%, 13.2 mm at 6%, 16.8 mm at 8%, and 19.4 mm at 10%, indicating progressive improvement in antimicrobial activity with increasing concentration. The standard deviation values were small across all groups, suggesting that the measurements within each group were consistent. The 95% confidence intervals were also narrow, which indicates that the average values are reliable estimates of the true effect. Amoxicillin showed the highest antibacterial activity with a mean zone of inhibition of 26.8 mm, clearly greater than all Triphala concentrations. To determine whether these differences were statistically meaningful, one-way ANOVA was performed. The analysis showed a very high F value (283.554) with a p value less than 0.001, confirming that the differences in antibacterial effect between the groups were not due to chance. This means that the concentration of Triphala significantly influences its antibacterial activity against *B. subtilis*, and although Triphala shows increasing effectiveness with higher concentrations, Amoxicillin remains superior in comparison.

For *Escherichia coli*, a similar concentration-dependent pattern was observed. The mean zone of inhibition increased consistently from 9.18 mm at 2% Triphala to 11.8 mm at 4%, 14.5 mm at 6%, 18.3 mm at 8%, and 21.6 mm at 10%, indicating that higher concentrations of Triphala produce stronger antibacterial effects. The standard deviation values were low across groups, demonstrating stable and reproducible results within each concentration level. The 95% confidence intervals were narrow and showed limited overlap between lower and higher concentrations, supporting the presence of real differences in antibacterial activity. Amoxicillin again produced the highest mean inhibition zone at 27.5 mm, showing superior effectiveness compared to all concentrations of Triphala. The one-way ANOVA test revealed a high F value (278.068) with a p value less than 0.001, confirming that the variation in antibacterial effect among the different groups was statistically significant. In simple terms, the antibacterial activity against *E. coli* increases significantly as the concentration of Triphala increases, but Amoxicillin demonstrates the strongest effect overall.

Discussion

The results obtained corroborated earlier findings regarding the antimicrobial properties of Triphala, a traditional Ayurvedic polyherbal formulation.^[9] The quantitative zone of inhibition measurements demonstrated a statistically significant dose-response relationship, with inhibition

zones increasing progressively from 9.2 mm to 21.6 mm for *Bacillus subtilis* and from 8.7 mm to 19.4 mm for *Escherichia coli* as Triphala Churna concentration increased from 2% to 10% w/v. This linear correlation suggested that bioactive constituent bioavailability increased proportionally with formulation concentration.

The observed antibacterial efficacy was attributed to the presence of bioactive compounds, including tannins, flavonoids, and alkaloids, which possess well-documented antimicrobial mechanisms. The differential inhibition responses observed between the two bacterial strains, with relatively smaller zones against *E. coli*, suggested varying susceptibilities between gram-positive and gram-negative bacteria to Triphala's bioactive constituents.

The 10% w/v formulation (F5) emerged as the optimal concentration, delivering superior antimicrobial activity (21.6 mm against *Bacillus subtilis* and 19.4 mm against *E. coli*) while maintaining adequate hydrogel structural integrity. These quantitative findings suggested that Triphala Churna at concentrations of 8-10% w/v represented a promising natural alternative or adjunctive agent for incorporation into dental hydrogel systems for oral health applications, particularly given growing antimicrobial resistance concerns and the clinical demand for herbal-derived therapeutic agents^[10]

Conclusions

These findings suggest that 10% w/v concentration may be optimal for therapeutic applications. The increasing prevalence of antimicrobial resistance and the demand for biocompatible herbal alternatives, Triphala-based hydrogels show promise as potential adjunctive antimicrobial agents for dental and oral healthcare applications.

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