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Determination of *in vitro* Antibacterial Activity of Triphala Churna on Selected Bacterial Strains

Lincy Sara Varghese
Department of Microbiology, Bishop Kurialacherry College for Women, Amalagiri, Kottayam, Kerala, India
Corresponding author: svlincy45@gmail.com

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ABSTRACT:
The rise of multidrug-resistant bacteria poses a significant threat to public health, driving the need to explore alternative therapies including herbal medicine. Triphala Churna, a classical Ayurvedic formulation composed of *Terminalia chebula*, *Terminalia bellirica*, and *Emblica officinalis*, is known for its diverse therapeutic properties including antimicrobial, antioxidant, and immunomodulatory effects. This study investigates the in vitro antibacterial efficacy of different solvent extracts (water, acetone, isopropanol) of Triphala Churna against seven pathogenic bacterial strains: *Escherichia coli*, *Salmonella typhi*, *S. paratyphi A*, *Pseudomonas sp.*, *Klebsiella sp.*, *Shigella sp.*, and *Staphylococcus aureus*. The extracts were prepared by cold maceration and antibacterial activity was determined using the disc diffusion method. The aqueous extract demonstrated the broadest antibacterial spectrum, inhibiting the growth of six out of seven test organisms with maximum inhibition observed against *S. typhi* (9.5 mm) and *S. paratyphi A* (9.5 mm). Isopropanol extracts also exhibited considerable activity against five strains, whereas acetone extract was only active against *S. aureus* (8 mm). *E. coli* showed resistance to all the three extracts. The findings support the antibacterial potential of Triphala Churna, especially in aqueous form, validating its ethnomedicinal use and offering a basis for future pharmacological investigations and therapeutic applications.

KEYWORDS: Triphala Churna, Antibacterial activity, Herbal medicine, Disc diffusion assay

I. INTRODUCTION:

Antimicrobial resistance (AMR) has emerged as a global health crisis, largely driven by the misuse and overuse of conventional antibiotics. This necessitates the discovery and evaluation of alternative antimicrobial agents. Traditional herbal medicine, particularly from Ayurvedic pharmacopoeia, presents a rich and underexplored reservoir of bioactive compounds with therapeutic potential. Among the plethora of Ayurvedic formulations, Triphala Churna has stood the test of time as a holistic remedy with documented benefits spanning gastrointestinal, metabolic, and immune health.

Triphala, literally meaning 'three fruits',is a polyherbal mixture comprising equal parts of the dried fruits of *Terminalia chebula* (Haritaki), *Terminalia bellirica* (Bibhitaki), and *Emblica officinalis* (Amalaki). It is traditionally used to rejuvenate the digestive system, detoxify the body, and promote overall longevity (1, 2). Modern pharmacological studies have validated its efficacy in conditions ranging from hyperlipidemia to oxidative stress and even microbial infections (3).

Each component of Triphala is pharmacologically active. *T. chebula* is rich in tannins and phenolics and shows antimicrobial, antioxidant, and anti-inflammatory properties. *T. bellirica* contains lignans and gallic acid, while *E. officinalis* is a known source of vitamin C, ellagic acid, and flavonoids. Collectively, these fruits provide a spectrum of bioactive compounds such as gallic acid, chebulinic acid, and chebulagic acid that contribute to Triphala's antimicrobial activity (4). Triphala strengthens the different tissues of the body, prevents aging, promotes health and immunity and also exhibits other immunomodulatory properties (5, 6). Triphala has traditionally been used to treat microbialinfections, such as wounds, ulcers, and dental caries (7, 8).

Several studies have reported the antiviral, antibacterial, antifungal and anti-allergic properties of the formulation. Singh et al. demonstrated that methanolic extracts of Triphala exhibit strong antibacterial effects, surpassing the individual fruit extracts (9). Similarly, Safiullah et al. documented significant antibacterial activities of aqueous and ethanolic extracts against pathogens like *Staphylococcus aureus*, *Shigella sonnei*, and *Pseudomonas aeruginosa* (10). However, data comparing the activity of different solvent extracts are limited.This study aims to evaluate the *in vitro*

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antibacterial efficacy of aqueous, isopropanol and acetone extracts of Triphala Churna against clinically relevant bacterial strains. Moreover, the present study includes a wider range of both Gram-positive and Gram-negative bacterial strains compared to previous reports, allowing for a more comprehensive evaluation of antimicrobial spectrum of Triphala Churna across clinically significant pathogens.

II. MATERIALS AND METHODS:

2.1 Collection of Sample

Triphala Churna was procured from a certified Ayurvedic medicine shop in Ettumanoor, Kottayam District, Kerala, India. The powdered formulation was stored in sterile containers and used for extract preparation.

2.2 Bacterial Strains

Seven bacterial strains were used in this study: *Escherichia coli*, *Salmonella typhi*, *Salmonella paratyphi* A, *Pseudomonas* sp., *Klebsiella* sp., *Shigella* sp., and *Staphylococcus aureus*. Cultures were obtained from the School of Biosciences, Mahatma Gandhi University, Kottayam, and maintained on nutrient agar at 4°C.

2.3 Preparation of Extracts

The extracts were prepared by cold extraction method. Five grams of Triphala Churna was suspended in 20 ml each of the solvents (water, isopropanol, acetone) for 10 days at room temperature with a sample to solvent ratio of 1:4. During this extraction period, the suspensions were agitated twice daily to ensure thorough mixing. Following extraction, the mixtures were filtered, transferred into sterile screw-capped containers, and stored at 4°C for preservation until further use.

2.4 Determination of Antimicrobial Activity

2.4.1 Preparation of Discs

Sterile sensitivity discs of 6 mm diameter were prepared from Whatman No. 1 filter paper, sterilized by autoclaving and stored at room temperature under aseptic conditions. Prior to the assay, the discs were soaked in the respective extracts and solvents for 5 minutes. Each disc was impregnated with approximately 20 µl of the respective extract, corresponding to 5 mg of Triphala Churna. Discs soaked in pure solvents served as negative controls. Ciprofloxacin antibiotic discs (HiMedia, Mumbai) were used as the positive control. The sample and solvent impregnated discs were finally air dried for 30 minutes under aseptic conditions and used for the assay.

2.4.2 Antibacterial Assay by Disc Diffusion Method

Pure isolated colonies of each bacterial strain grown on nutrient agar plates were inoculated into 2 ml peptone water broths and incubated at 37°C for 48 hours. The resulting broth cultures were utilized as inoculum for lawn cultures on Mueller-Hinton Agar (MHA) plates to evaluate the antibacterial activity of the extracts. Using a sterile cotton swab, 0.2 ml of the bacterial cultures in peptone water broths was evenly spread over the surface of the MHA to create a uniform lawn culture. The discs impregnated with the respective extracts and the solvents were placed on the inoculated agar plates, with two discs per plate. All experiments were conducted in duplicates. The plates were incubated at 37°C for 24 hours, after which the antibacterial activity of each extract against the test bacteria was quantified by measuring the diameter of the inhibition zones to the nearest millimeter. The data were recorded systematically and compared.

III. RESULTS:

Triphala, a balanced formulation of *Terminalia chebula*, *Terminalia belerica*, and *Emblica officinalis*, is widely recognized in traditional Indian medicine for its therapeutic efficacy. In the present study, the antibacterial activity of acetone, isopropanol, and aqueous extracts of Triphala Churna was evaluated against seven bacterial pathogens: *E. coli*, *Salmonella typhi*, *S. paratyphi A*, *Pseudomonas* sp., *Klebsiella* sp., *Shigella* sp., and *S. aureus*. The results are summarized in Table 1, with inhibition zones illustrated in Figures 1 to 4.

Among the solvents tested, the aqueous extract demonstrated the broadest antibacterial spectrum, inhibiting six of the seven pathogens, excluding *E. coli*. The isopropanol extracts also showed significant activity, except against *E. coli* and *Klebsiella* species while the acetone extract was selectively effective against *S. aureus* (8 mm). The maximum inhibition was observed for *S. typhi* with the isopropanol extract (10 mm), followed by *S. aureus*, *S. typhi*, and *S. paratyphi* A (9.5 mm each) with the aqueous extract. The minimal inhibition was recorded for *Pseudomonas* sp., particularly in the aqueous extract. The respective solvent controls showed no inhibitory effect on any of the tested bacterial strains, indicating that the observed antimicrobial activity was solely attributable to the

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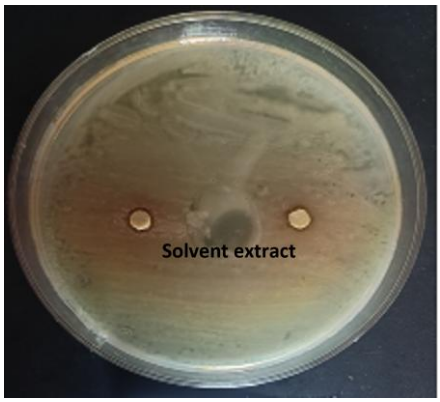
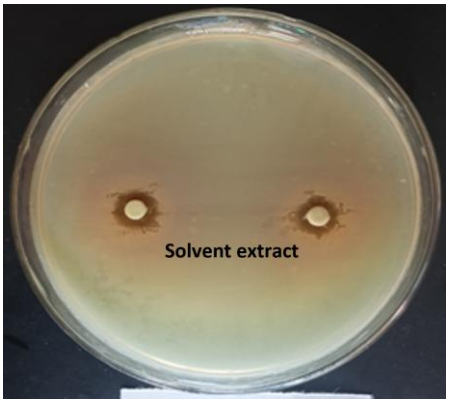
extracts. In contrast, the positive control, ciprofloxacin, effectively inhibited the growth of all test bacteria, producing markedly larger zones of inhibition. These findings validate the reliability and authenticity of the experimental results

Table 1. Antibacterial activity of Tribhala Churna against bacterial pathogens

Name of the test bacteria	Average Diameter of Zone of Inhibition of Growth in mm for		
	Acetone extract	Isopropanol extract	Aqueous extract
S. aureus	8	9.5	8.5
Pseudomonas sp.	0	8	7
E. coli	0	0	0
S. typhi	0	10	9.5
S. paratyphi A	0	8	9.5
Klebsiella sp.	0	0	7.5
Shigella sp.	0	9	8

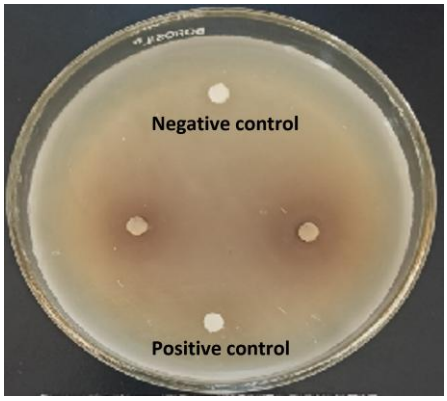


***Klebsiella* sp.**



S. aureus

E. coli



***S. paratyphi* A**

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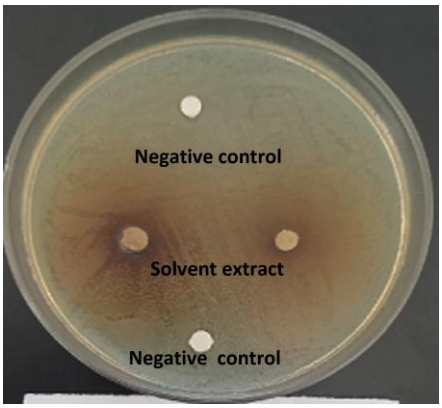
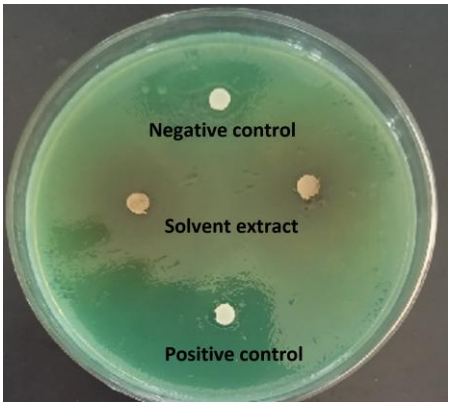
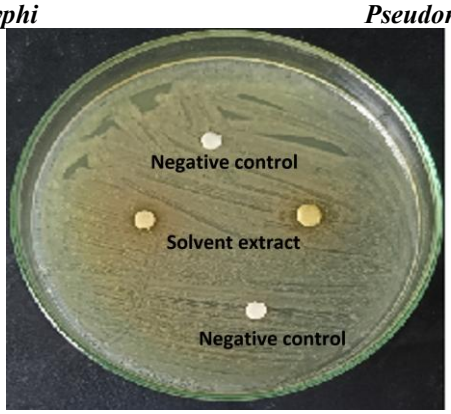


S. typhi

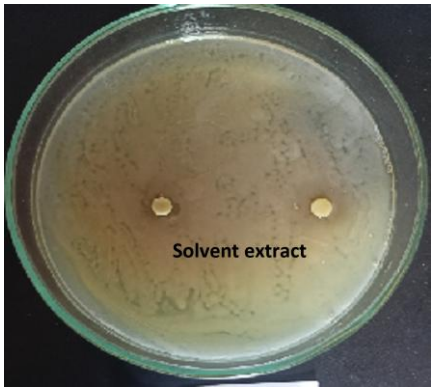


Pseudomonas sp.

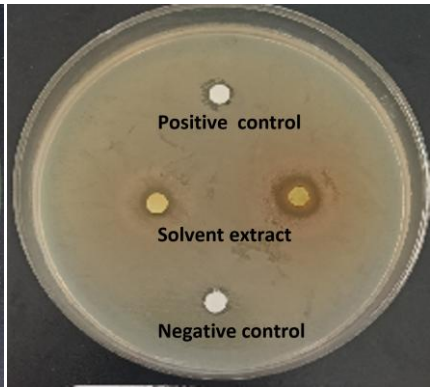


Shigella sp.

Fig. 1. Antibacterial activity of acetone extract of Tribhala Churna



Klebsiella sp.



S. aureus

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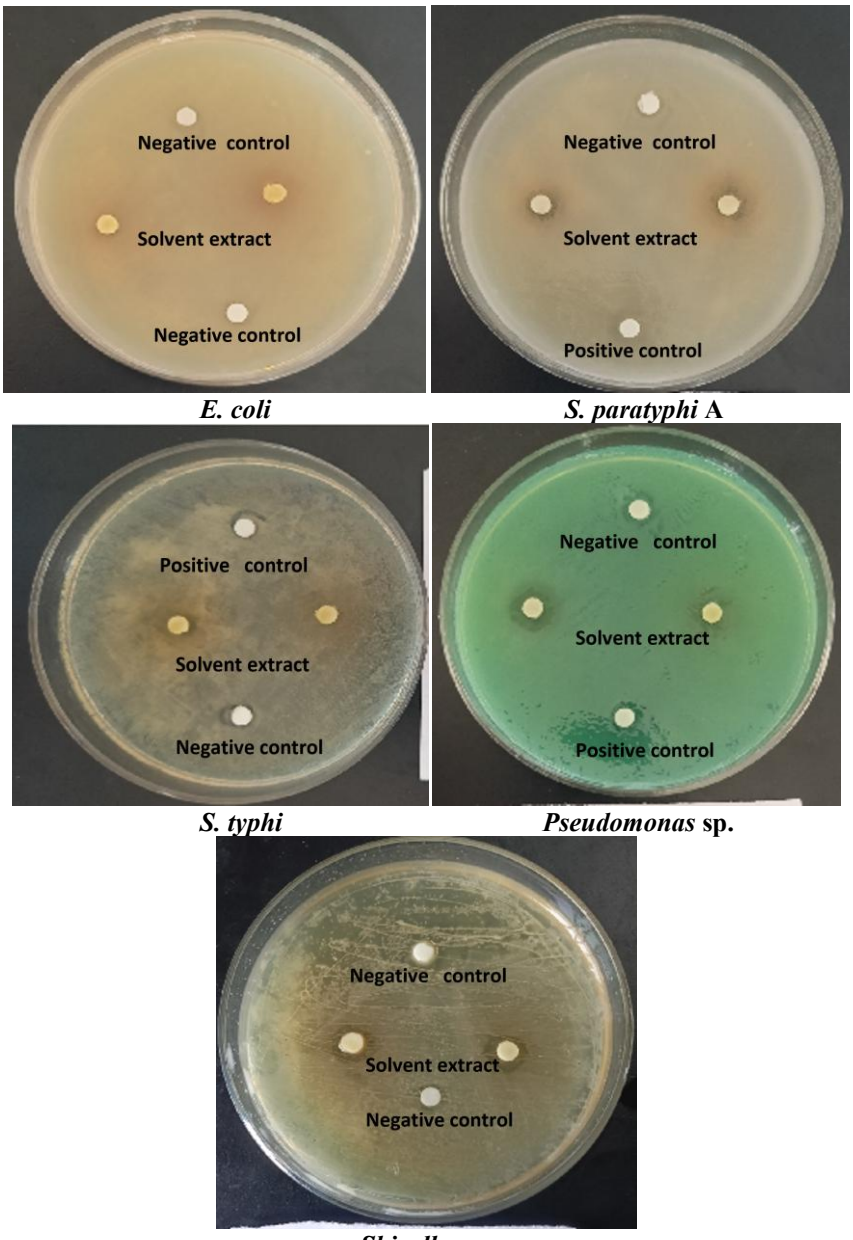

E. coli *S. paratyphi* A

S. typhi *Pseudomonas* sp.

Shigella sp.

Fig. 2. Antibacterial activity of isopropanol extract of Tribhala Churna

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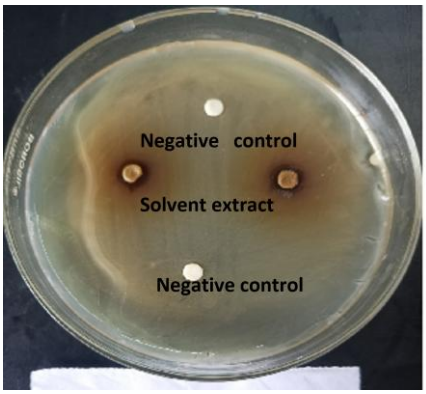
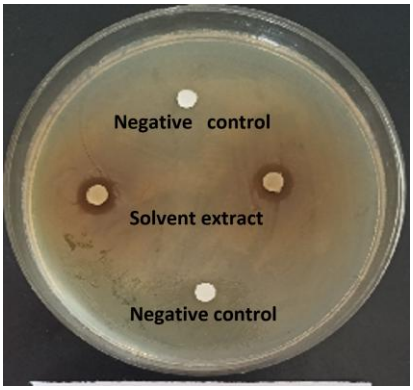
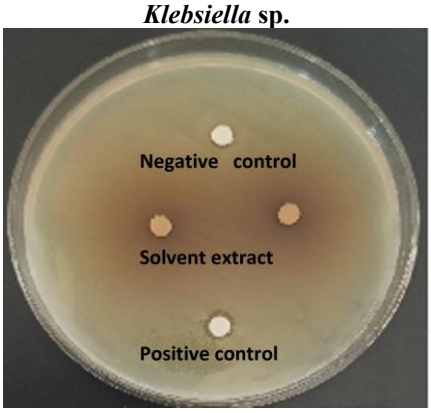


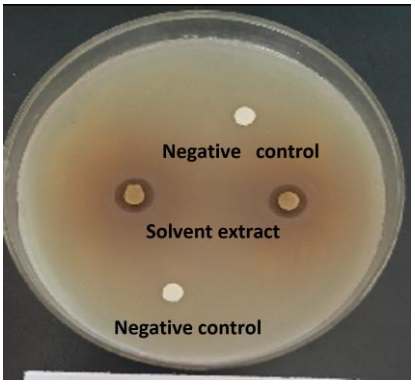
Klebsiella sp.



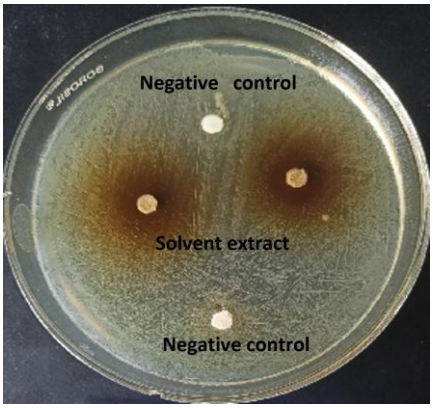
S. aureus



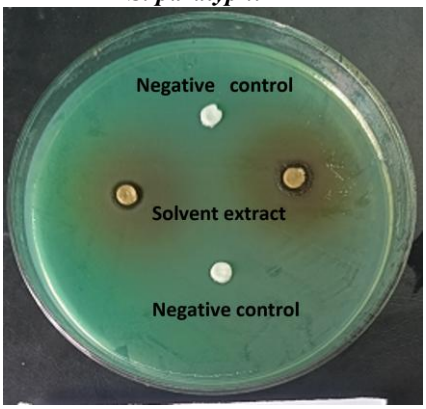
E. coli



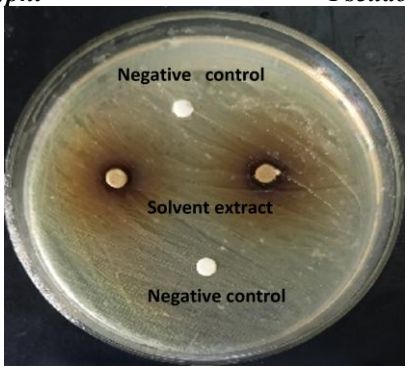
S. paratyphi A



S. typhi



Pseudomonas sp.



Shigella sp.

Fig.3. Antibacterial activity of aqueous extract of Tribhala Churna

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IV. DISCUSSION:

The present study reaffirms the antimicrobial potential of Triphala Churna, in agreement with previous reports (3, 9, 10). It is noteworthy that the present investigation encompassed a broader spectrum of bacterial species, including both Gram-positive and Gram-negative strains, than many earlier studies. This wider range enabled a more comprehensive assessment of antibacterial potential of Triphala Churna and provided clearer insights into the solvent-dependent variability in antimicrobial responses across different bacterial groups.

Among the three solvent extracts tested, the aqueous extract exhibited the broadest spectrum of antibacterial activity, inhibiting six of the seven test pathogens. This finding corroborates the results of Amala and Jeyaraj, who observed pronounced antibacterial activity of aqueous *Triphala* extracts against *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (11). Both studies suggest that water, being a highly polar solvent, efficiently extracts hydrophilic bioactive compounds such as phenolics, tannins, and flavonoids, which are known to disrupt bacterial cell membranes, precipitate proteins, and chelate essential metal ions involved in microbial metabolism.

The strong inhibition of *S. typhi*, *S. paratyphi A*, and *S. aureus* by the aqueous extract in the present study may be attributed to the susceptibility of their relatively permeable cell walls to these hydrophilic compounds. Safiullah et al. similarly reported significant activity of aqueous *Triphala* extracts against *Staphylococcus aureus*, *Shigella sonnei*, and *Pseudomonas aeruginosa*, further supporting the effectiveness of polar solvent systems in extracting antimicrobial constituents from *Triphala* (10).

In contrast, the isopropanol extract, which possesses intermediate polarity, exhibited broad-spectrum activity against both Gram-positive and Gram-negative strains, likely due to the presence of moderately polar bioactives such as alkaloids and certain polyphenols that interfere with enzymatic systems or compromise membrane integrity. In the present study, the isopropanol extract inhibited five bacterial strains, indicating that alcohol-soluble phytoconstituents play a substantial role in *Triphala*'s antibacterial effect. These observations are in line with Omran et al. who demonstrated strong antibacterial activity of ethanol extracts of *Triphala* against Gram-positive cocci, particularly *S. aureus* (12). However, compared to the higher inhibition zones reported by Omran *et al*., the relatively moderate zones in the current study may reflect differences in extraction duration, solvent polarity, or concentrations of active compounds used.

Interestingly, the acetone extract, being less polar, showed inhibitory activity only against *S. aureus*. This could be due to the ability of lipophilic compounds, such as terpenoids and low-polarity phenolics, to integrate into the lipid-rich cell membrane of Gram-positive bacteria, leading to increased permeability and cell lysis. The limited activity of the acetone extract may also be attributed to the selective solubility of active compounds governed by the solvent's polarity index. Similar selective inhibition of Gram-positive bacteria by less polar solvent extracts has also been reported by Amala and Jeyaraj and Singh et al. earlier (9, 11).

Although *E. coli* exhibited complete resistance to all extracts in the present study- contradicting the findings of Biradar et al. (13)- this observation aligns with the report of Omran et al. (12). The resistance of *E. coli* can be attributed to the presence of an outer lipopolysaccharide membrane and efficient efflux pump systems that restrict the accumulation of phytochemicals within the bacterial cell. These mechanisms confer a higher level of intrinsic resistance among Gram-negative bacteria compared to Gram-positive organisms. No antibacterial activity was observed for any of the solvent controls, confirming that the observed inhibition was due to the phytoconstituents present in the extracts. The positive control, ciprofloxacin, produced markedly larger inhibition zones against all test organisms, validating the reliability of the experimental conditions.

Collectively, these findings indicate that the antibacterial efficacy of Triphala Churna is influenced by both the solvent polarity and the structural characteristics of the bacterial strains tested. The aqueous extract proved most effective, likely due to its ability to solubilize hydrophilic antimicrobial compounds such as gallic acid, chebulagic acid, and chebulinic acid (4, 14). While the inhibition zones observed were moderate, this could be attributed to the cold maceration method employed, which typically yields lower concentrations of bioactives compared to hot extraction or Soxhlet techniques. Further studies are warranted to optimize extraction and evaluate dose-dependency and synergistic effects with antibiotics.

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V. CONCLUSION:

The present study confirms the antibacterial potential of Triphala Churna, particularly in its aqueous and isopropanol extracts, which effectively inhibited six out of seven tested bacterial pathogens. However, the relatively modest zones of inhibition indicate that cold extraction methods may not optimize the concentration of bioactive antibacterial compounds. The resistance of *E. coli* to all extracts highlights interspecies variability in susceptibility and suggests that the efficacy of *Triphala* may be organism-specific. These findings validate the traditional use of *Triphala* as an antimicrobial agent and underscore its potential application in the management of bacterial infections. Future research should focus on employing advanced extraction techniques such as hot extraction or Soxhlet extraction to enhance the yield of active constituents. Further quantitative investigations, including determination of the Minimum Inhibitory Concentration (MIC) and time-kill kinetics, are warranted to better define antimicrobial potency. Additionally, quantitative evaluations through assays like Minimum Inhibitory Concentration (MIC) and time-kill kinetics are necessary to precisely determine antimicrobial potency Exploring synergistic interactions between *Triphala* extracts and conventional antibiotics, as well as extending testing to clinical and multidrug-resistant strains, could provide valuable insights into its therapeutic potential. Collectively, these findings reinforce the relevance of Triphala Churna as a promising natural antimicrobial source and support its further development as an adjunct or alternative therapy in the fight against emerging antibiotic resistance.

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