"Antimicrobial and Antioxidant Activity of Triphala: An Ayurvedic Formulation"

Abstract

Triphala, an ancient Ayurvedic formulation composed of the dried fruits of *Emblica officinalis* (Amla), *Terminalia chebula* (Haritaki), and *Terminalia bellerica* (Bahera), is widely recognized for its broad-spectrum therapeutic applications. This study aimed to scientifically evaluate its **phytochemical composition**, **antimicrobial efficacy**, and **antioxidant potential** using different solvent extracts. Preliminary phytochemical screening revealed that the **ethanolic extract** was the most bioactive, containing alkaloids, tannins, glycosides, saponins, terpenoids, and phlobatannins. Antimicrobial testing against *Escherichia coli* and *Staphylococcus aureus* using the disc diffusion method demonstrated significant activity in ethanolic and aqueous extracts, with the ethanolic extract showing the highest inhibition zones. Furthermore, **DPPH radical scavenging assays** confirmed the antioxidant capacity of both ethanolic and aqueous extracts in a dose-dependent manner, although their efficacy was lower than that of standard ascorbic acid. These results substantiate the traditional use of Triphala and highlight its potential as a natural source of antimicrobial and antioxidant agents. Further phytochemical characterization and in vivo studies are recommended for clinical validation.

Keywords

Triphala, Antioxidant Activity, Antimicrobial Activity, Phytochemical Screening, Ayurvedic Medicine, Ethanolic Extract, DPPH Assay, Herbal Formulation, Natural Therapeutics, Escherichia coli, Staphylococcus aureus.

1. INTRODUCTION

Triphala, a traditional Ayurvedic formulation, has been extensively used for centuries in Indian medicine owing to its broad spectrum of therapeutic properties. Composed of the dried fruits of three medicinal plants—*Emblica officinalis* (Amla), *Terminalia chebula* (Haritaki), and *Terminalia bellerica* (Bahera)—Triphala is revered for its ability to promote digestive health, enhance immunity, and delay the aging process. Its multifaceted medicinal applications are increasingly being validated by modern scientific investigations, particularly for its antimicrobial and antioxidant potential.

The biological activities of Triphala are largely attributed to the presence of diverse phytochemicals. Phytochemicals are naturally occurring, biologically active compounds found in plants, excluding essential nutrients such as vitamins and minerals. These compounds act as the plant's defense mechanisms against pathogens and environmental stress, and they offer similar protective benefits when consumed by humans. Rich in flavonoids, tannins, phenolic acids, and other bioactive constituents, phytochemicals exhibit a variety of therapeutic properties, including antioxidant, anti-inflammatory, antibacterial, antiviral, and immune-boosting effects.

Each component of Triphala contributes uniquely to its overall pharmacological efficacy. *Emblica officinalis* (Amla) is a potent source of ascorbic acid (vitamin C), along with tannins, flavonoids, and polyphenols such as gallic acid and ellagic acid, all known for their antioxidant activity. *Terminalia chebula* (Haritaki) is rich in tannic acid, chebulinic acid, and betulinic acid, which possess significant antimicrobial and free radical scavenging properties. *Terminalia bellerica* (Bahera), on the other hand, contains ellagitannins, gallotannins, and a spectrum of polyphenolic compounds that further enhance the formulation's antioxidant and antimicrobial activities.

Given the rising global concern regarding antibiotic resistance and oxidative stress-related disorders, there is a pressing need to explore plant-based alternatives with minimal side effects. Triphala, with its rich phytochemical profile, presents a promising candidate for such therapeutic exploration. This study aims to evaluate the antimicrobial and antioxidant activities of Triphala, thereby providing scientific validation for its traditional usage and exploring its potential as a natural alternative for managing microbial infections and oxidative stress.

2. MATERIAL & METHOD

2.Materials

Triphala powder (100 g) was procured from Vindhya Herbals, Sanjeevani Ayurveda, Bhopal, and stored for further use. Extraction solvents included **chloroform, petroleum ether, and ethanol**, all of analytical grade. Microbial activity was assessed using **Nutrient Agar Media (NAM)** and **Nutrient Broth**, both with standard compositions and pH adjusted to 7.0.

2.1 Instruments

Key instruments used included a **Soxhlet extractor** for phytochemical extraction, **rotary vacuum evaporator** for solvent recovery, **digital pH meter**, **vertical autoclave**, **laminar airflow chamber**, **incubator**, and **antibiotic zone scale** for zone measurement.

2.2 Extraction Procedure

Soxhlet extraction was carried out using 25 g of Triphala powder and 250 mL of solvent at 50–60°C until clear extract was obtained. Solvent was then recovered via **rotary evaporation under vacuum**, and the residue was mixed with **chloroform water** and refrigerated at 4°C for further analysis.

2.3 Phytochemical Screening

Preliminary phytochemical screening of Triphala extracts was conducted following standard protocols (Brain & Turner, 1975; Evans, 1996) to identify key bioactive constituents. Various qualitative tests were performed using specific reagents to detect the presence of the following phytochemicals:

- **Alkaloids:** Detected using Mayer's reagent, indicated by a cream-colored precipitate.
- **Carbohydrates & Glycosides:** Identified by a red precipitate upon reaction with Fehling's solution.

- **Phenolic Compounds & Tannins:** Confirmed by a bluish-black coloration with ferric chloride.
- **Proteins & Amino Acids:** Indicated by a purplish-pink color upon addition of ninhydrin.
- **Terpenoids:** Detected by the formation of a red-brown interface with chloroform and concentrated H_2SO_4.
- **Phlobatannins:** Identified through the deposition of red precipitate after boiling with 1% HCl.
- **Saponins:** Confirmed by persistent frothing upon boiling with distilled water.
- **Flavonoids:** Detected by a yellow color with NaOH, which disappears after adding dilute HCl.

2.4 Antimicrobial Activity (Disc Diffusion Method)

Escherichia coli and *Staphylococcus aureus* were used to evaluate antimicrobial activity via the disc diffusion method. Inocula were prepared in sterile nutrient broth and incubated at 37°C. Nutrient Agar Media (NAM) was prepared and sterilized. Using the pour plate method, 1 mL of inoculum was added to Petri dishes, followed by 15 mL of NAM. Sterile filter paper discs loaded with 20 µL of Triphala extract were placed on the solidified media and incubated at 37°C for 24 hours. Zones of inhibition were measured in millimeters to assess antimicrobial efficacy.

2.5 Antioxidant Activity (DPPH Method)

Antioxidant activity was determined using the DPPH free radical scavenging assay. A 0.004% DPPH methanolic solution and ascorbic acid (standard) were prepared in varying concentrations (20–100 µg/mL). Triphala extract was similarly diluted. Each sample (2 mL) was mixed with 0.5 mL DPPH solution and incubated in the dark for 10 minutes. Absorbance was measured at 517 nm.

Calculation of Antioxidant Activity

The percentage of DPPH radical scavenging activity was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{(A_0 - A_t)}{A_0} \times 100$$

- A_0 = absorbance of the control
- A_t = absorbance in the presence of sample or standard

All tests were performed in triplicate, and the results were expressed as mean ± standard deviation.

3. RESULT

3.1 Phytochemical Screening

Preliminary phytochemical screening of Triphala extracts using various solvents revealed the presence of several bioactive compounds (Table 1). The ethanolic extract exhibited the presence of alkaloids, carbohydrates and glycosides, tannins, saponins, terpenoids, and phlobatannins. Chloroform extract showed positive results for alkaloids and terpenoids, while the aqueous extract confirmed the presence of tannins, saponins, and terpenoids. No phytochemicals were detected in the petroleum ether extract.

These findings affirm the presence of secondary metabolites in Triphala, which are known for their therapeutic potential. The ethanolic extract, being the richest in phytochemicals, was subjected to further antimicrobial and antioxidant testing.

Phytochemical tests	**Triphala**			
	Chloroform	**Ethanol**	**Petroleum ether**	**Aqueous**
Alkaloids (Mayers Reagent)	+	+	-	-
Carbohydrates & glycosides (Fehling Solution)	-	+	-	-
Phenolic compounds & tannins (Ferric Chloride solution)	-	+	-	+
Proteins/Aminoacids (Ninhydrin test)	-	-	-	-
Saponins	-	+	-	+
Terpenoid	+	+	-	+
Phlobatannins	-	+	-	-
Flavanoides	-	-	-	-

3.2 Antimicrobial Activity

The antimicrobial efficacy of Triphala extracts was evaluated using the disc diffusion method against *Escherichia coli* and *Staphylococcus aureus*. The ethanolic extract exhibited the highest

antimicrobial activity, with zones of inhibition measuring 10 mm against *E. coli* and 9 mm against *S. aureus*. The aqueous extract showed moderate activity with inhibition zones of 9 mm and 7 mm, respectively. The chloroform and petroleum ether extracts displayed negligible or no activity.

When compared with the standard antibiotic streptomycin, which showed inhibition zones of 18 mm (*E. coli*) and 13 mm (*S. aureus*), the ethanolic extract demonstrated moderate but significant antimicrobial potential. This activity may be attributed to the presence of phenolic compounds and other bioactive constituents in the extract.

Extract Type	Microorganism	Zone of Inhibition (mm)
Ethanolic	*E. coli*	10
	S. aureus	9
Aqueous	*E. coli*	9
	S. aureus	7
Streptomycin	*E. coli*	18
	S. aureus	13

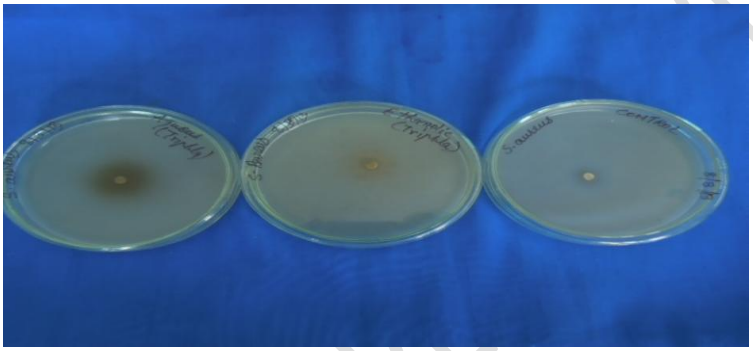


Fig: Zone of inhibition of triphla for *S. aureus*

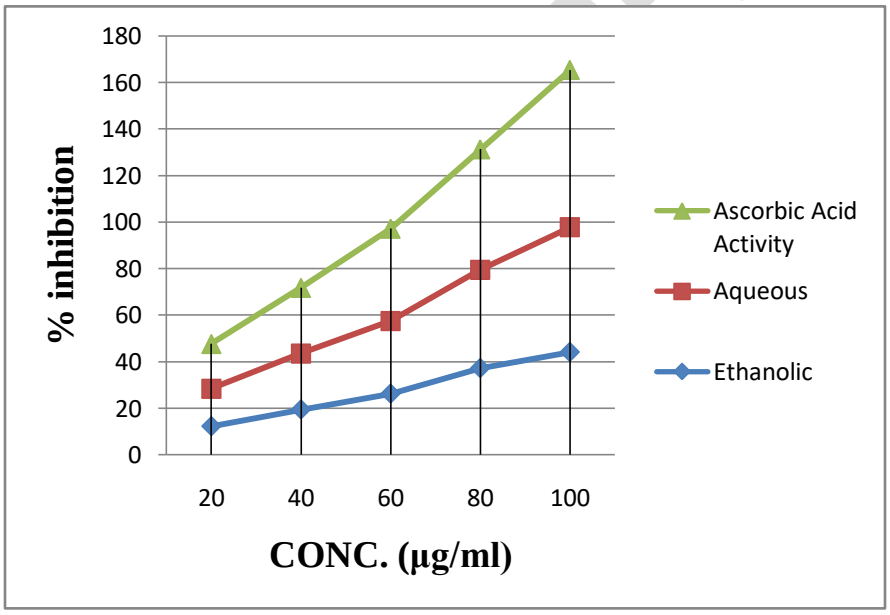
3.3 Antioxidant Activity

The antioxidant potential of Triphala was evaluated using the DPPH free radical scavenging assay. Both ethanolic and aqueous extracts demonstrated dose-dependent radical scavenging activity, although lower than the standard antioxidant, ascorbic acid.

At the highest tested concentration (100 µg/mL), the aqueous extract showed a scavenging activity of 53.6%, followed by the ethanolic extract with 44.2%, compared to 67.6% for ascorbic acid. This suggests a significant antioxidant potential, likely due to the presence of phenolic compounds, flavonoids, and other phytochemicals.

Fig: Antioxidant activity of extract of triphala

Concentration (µg/mL)	Ethanolic Extract (%)	Aqueous Extract (%)	Ascorbic Acid (%)
20	12.3	16.2	19.2
40	19.5	24.15	28.15
60	26.3	31.3	39.6
80	37.3	42.3	51.7
100	44.2	53.6	67.6



These findings highlight Triphala as a promising source of natural antioxidants with potential applications in food and pharmaceutical industries. The presence of flavonoids, tannins, alkaloids, glycosides, and phenolics may contribute to its free radical scavenging activity. Further isolation and characterization of the active compounds are warranted to understand their mechanisms and enhance the formulation of polyherbal therapeutics.

4. DISCUSSION

This study validated the traditional medicinal use of *Triphala* by confirming its phytochemical richness, antimicrobial, and antioxidant properties through various solvent extractions. The ethanolic extract exhibited the highest concentration of bioactive compounds—including alkaloids, tannins, glycosides, and phenolics—correlating with its superior antimicrobial and antioxidant effects. Antimicrobial activity was strongest against *E. coli* and *S. aureus* with the ethanolic extract, while antioxidant potential was observed in both ethanolic and aqueous extracts, though less than that of ascorbic acid. These findings highlight *Triphala*'s therapeutic potential and suggest its suitability as a natural source for antimicrobial and antioxidant agents.

5. CONCLUSION

The present study affirms the pharmacological potential of **Triphala**, validating its traditional Ayurvedic applications with modern scientific evidence. Among the various solvent extracts analyzed, the **ethanolic extract** demonstrated the most potent antimicrobial and antioxidant activities, likely due to its rich phytochemical content, especially phenolic compounds, tannins, and glycosides. The findings support the role of Triphala as a promising **natural alternative to synthetic antimicrobial and antioxidant agents**, with possible applications in the pharmaceutical and nutraceutical industries. However, **further chemical characterization** and **in vivo investigations** are essential to isolate active constituents and determine their mechanisms of action and clinical safety profiles.

6. REFERENCES

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