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Original Article

Triphala: Is it a Competent Guardian Against Oral Pathogens- An *In Vitro* Study

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Triphala is an ayurvedic herbal formulation consisting of the dried fruits of three medicinal plants *Terminalia chebula*, *Terminalia belerica*, and *Phyllanthus embelica* which has antimicrobial, anti-oxidant, and anti-inflammatory property. The aim of this study was to evaluate the efficacy of Triphala against oral pathogens. The standard stock culture of microorganisms was isolated from 20 patients with different types of dental infections. The efficacy of Triphala against microbial type culture collection was evaluated using agar gel diffusion. Minimum inhibitory concentration (MIC) values were obtained using broth dilution method. MIC of Triphala against *Actinobacillus actinomycetemcomitans*, *Clostridium* bacilli, *Streptococcus mutans* and *Staphylococcus aureus* isolates was 25%, 25%, 12.5%, and 12.5%, respectively. Triphala seems to be a promising alternative for the control of oral diseases in terms of antimicrobial response.

Keywords: Ayurvedic herbal formulation, Triphala, antibacterial effect, oral bacteria

India has an ancient history of traditional herbal medicine. Herbal medicines are being used increasingly as dietary additives to treat or prevent many diseases. Although effective treatment of bacterial infections may be obtained with conventional antibiotics, but there is an increasing problem of antibiotic resistance, with cases of immediate hypersensitivity reactions, and toxicity which require new solutions. Hence, nowadays, herbal drugs are considered as attractive alternatives to synthetic antibiotics (1-3).

Triphala is a traditional ayurvedic herbal formulation which contains the dried fruits of three medicinal plants: *Terminalia chebula*, *Terminalia belerica* and *Phyllanthus embelica* also known as 'three myrobalan' (4). *Phyllanthus embelica* contains ascorbic acid, astragalin flavonol, gallic acid benzenoid, emblicol, phyllemblic acid, emblicanin A, emblicanin B, pedunculagin, punigluconin tannin, and ellagic acid coumarin. *Terminalia chebula* contains arjungenin, arjunglucoside I, gallic acid benzenoid, chebulic acid, ellagic acid coumarin, corilagin, punicalagin, terchebulin, and terflavin A tannin. *Terminalia belerica* contains beta sitosterol, gallic acid, ellagic acid, ethyl acetate, galloyl glucose and chebulagic acid (5).

The present study has been performed to

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evaluate the antimicrobial activity of Triphala against oral pathogens.

Materials and methods

Patients and sample collection

20 patients exhibiting mild to moderate supragingival calculus were selected. Visible supragingival calculus was removed using hand scalers. Patients who were clinically and radiographically diagnosed with periapical abscess and periodontal abscess were selected for purulent discharge collection. Fine needle aspiration of suppuration was performed using 22 gauge needle.

All clinical samples were immediately transferred onto thioglycollate broth (Figure 1a), were further incubated for 4 h, and then subcultured in anaerobic jars.

An approval for this study had been obtained from the ethical committee of Rungta College of Dental Sciences and Research, Bhilai, Chattisgarh, India. Written informed consent was also taken from all patients before involving them in the study.

Isolation of strains from clinical samples

After 4-6 h, samples which were inoculated onto thioglycollate medium were further subcultured on Mutans-Sanguis agar, and incubated at 37 °C for 48 h (Figure 1b). The colonies formed were further identified by Gram staining method, and biochemical tests such as catalase test, esculil test, starch hydrolysis, raffinose and mannitol fermentation tests to confirm the colonies identity.

Preparation of the extracts

Triphala powder (Patanjali Yogpeeth Co, India) was used. The aqueous product of Triphala churna was prepared by suspending 100 g in 100 ml of double distilled water, followed by 45 min boiling. The preparation was further cooled, and filtered. The filtrate of Triphala churna thus obtained was used for antimicrobial activity test. The commercially available ciprofloxacin (30 µg) and ofloxacin (5 µg) were also used. An aqueous product was prepared by suspending 1 g of each antibiotic in double distilled water.

Antimicrobial activity assessment

Antibacterial properties of Triphala were detected using disc diffusion method. Microdilution

Figure 1. Isolation of strains from clinical samples. a: clinical samples transferred onto thioglycollate broth; b: sub culture of colonies in Mutans-Sanguis agar.

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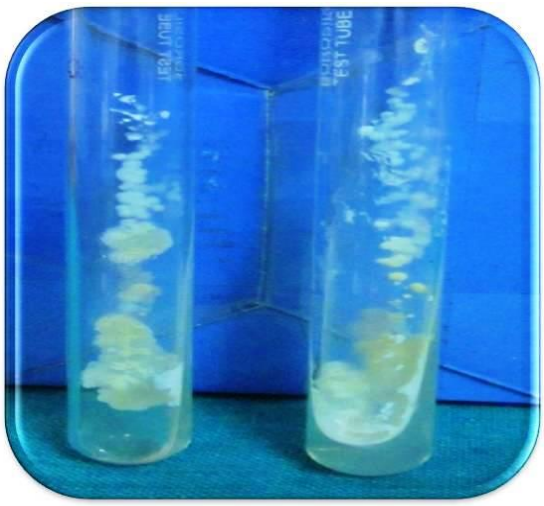

Figure 2. Isolates culture in molten Mueller- Hinton agar. The clinical isolates were grown in Mueller-Hinton broth and incubated at 37 °C for 24 h.

and agar dilution method were performed to measure the minimum inhibitory concentration (MIC).

The clinical isolates were grown in Mueller-Hinton broth, and incubated at 37 °C for 24 h (Figure 2). 0.1 ml of the culture was seeded into 25 ml molten Mueller- Hinton agar, mixed and poured into a sterile petri plate and allowed to solidify (Figure 3a). The wells were bored in seeded agar using an 8 mm borer. Then, at the particular concentrations (50%, 25%, 12.5%) of Triphala extract, and antibiotics, collected samples were added into each well (Figure 3b). After standing at room temperature for normalization, plates were incubated incubated at 37 °C for 24 h. The zone of inhibition was measured and recorded after the completion of the incubation period (Figure 4).

Minimum inhibitory concentration (MIC)

Micro-broth dilution method was used to measure the MIC values. Equal volumes of various concentrations of the extract and Mueller Hinton broth were mixed in micro-tubes to make a 0.5 ml solution. 0.5 ml of McFarland standard of the organism suspension was added to each tube. The tubes were incubated aerobically at 37°C for 24 h. Two control tubes were maintained for each test batch. These included tube-containing extract without inoculums and the tube containing the growth medium with the inoculums. The minimum bactericidal concentration (MBC) was determined by subculturing the test dilution on Mueller Hinton agar and further incubating for 24 h. The highest dilution that yielded no single bacterial colony was taken as the MIC and MBC.

Statistical analysis

The collected data were statistically analyzed by using Student "t" test to evaluate the differences. A p-value less than 0.05 was considered to be statistically significant and $P < 0.001$ was considered to be highly significant.

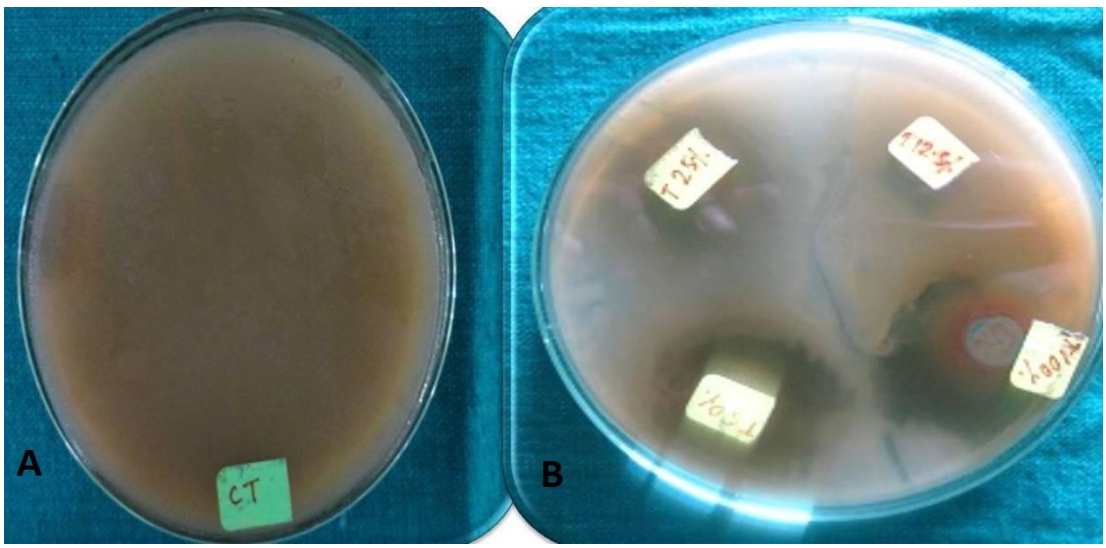

Figure 3. Microdilution and agar dilution method for measurement of the minimum inhibitory concentration. a: culture microdilution preparation; b: wells bored in seeded agar in which 100%, 50%, 25%, 12.5 % of Triphala was poured.

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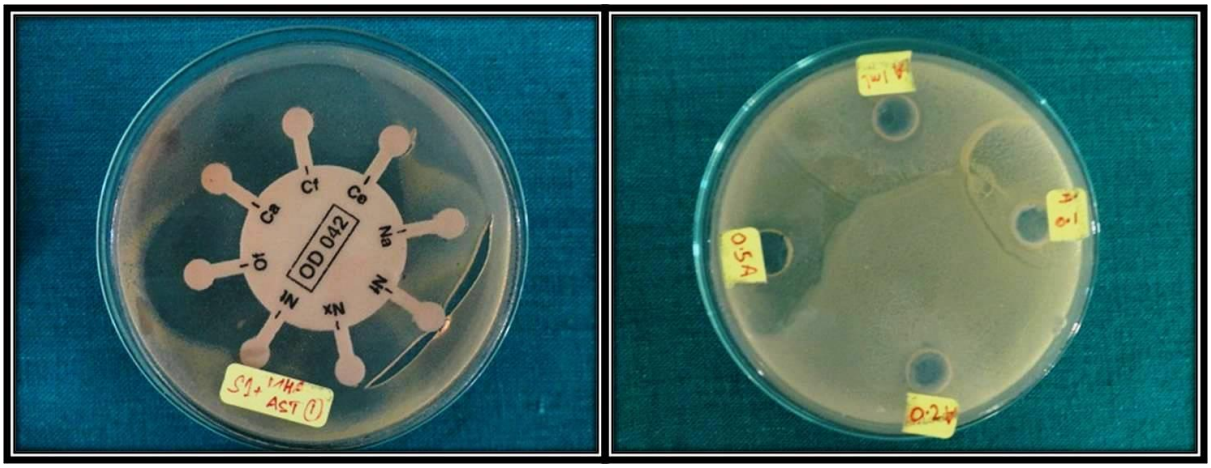

Figure 4. Antimicrobial activity assessment. Zones of bacterial growth inhibition were recorded.

Results

Identification of bacterial isolates

The samples collected were analyzed by staining, specific media culture (cooked meat media, PDA agar) and biochemical tests. The results of which confirmed that they contained strains of *Actinobacillus actinomycetemcomitans*, *Clostridium* bacilli, *Streptococcus mutans*, and *Staphylococcus aureus*.

Antibacterial activity of Triphala

Triphala showed antibacterial properties at higher concentrations (100%, 50%, 25%, $P < 0.001$). At lower concentration (12.5%) there was no effect against the tested bacteria. The 2% DMSO which was used as a dilutent, showed no inhibitory activity on any of these bacteria.

The average zone of inhibition measured against tested bacteria for Triphala, ciprofloxacin, and ofloxacin is shown in Table 1.

MIC of Triphala against *Actinobacillus actinomycetemcomitans*, *Clostridium* bacilli, *Streptococcus mutans*, and *Staphylococcus aureus* was 25%, 25%, 12.5%, and 12.5%, respectively.

Discussion

Most of the infections in oral diseases are due to the microbial flora of the oral cavity. Periodontal diseases are mostly associated with anaerobic Gram negative bacilli such as *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythus*, and *Fusobacterium species*. For bacteremia and endocarditis prevention, antibiotic administration is recommended prior to invasive dental procedures (6-7). However, antibiotics may have side effects such as immediate hypersensitivity reactions, toxicity, and multi-drug resistance for some bacteria. During the last century, bacterial resistance development caused the increase of morbidity and mortality. Relatively, the cost of bacterial infectious treatments increased, and the global health situation worsened with the development of transportation possibilities. To

Table 1. Zone of inhibition against microbes using different antibiotic preparations and Triphala powder

Antibiotics	Zone of inhibition (mm)			
	A. actinomycetemcomitans	*C.* bacilli	*S. mutans*	*S. aureus*
Triphala (100%)	7.9	7.3	7.8	7.6
Ciprofloxacin (30 µg)	7.4	7.1	7.8	7.3
Triphala (50%)	4.8	4.4	4.6	4.1
Ofloxacin (5 µg)	4.6	7.1	7.8	7.3

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overcome drug resistance as well as side effects associated with the antibiotics, many attempts has been made to study the antimicrobial properties of Triphala in comparison with antibiotics or synthetic antibacterial compounds (8-10)

In the present study, Triphala's strong antimicrobial effect against *Actinobacillus actinomycetemcomitans*, *Clostridium* bacilli, and *Staphylococcus aureus* was detected. Tambekar et al. found that Triphala was effective against most of the enteric bacteria tested, with the maximum antibacterial activity against *Staphylococcus aureus* (11).

Tannic acid is one of the major constituent of the ripe fruit of *Terminalia chebula*, and *Terminalia belerica*. Tannic acid is considered to be bacteriostatic or bactericidal to some Gram positive and Gram negative pathogens (12).

Triphala was also shown to be effective against *Actinomycete commitans*. Bajaj and Tandon found that Triphala was as effective as chlorhexidine mouthwash and had similar effect on gingival health. They also concluded that Triphala has better inhibitory effect on microbial count than chlorhexidine (13).

Desai et al. administrated Triphala and chlorhexidine after scaling and root planning. They found that both oral hygiene index and plaque index were significantly reduced in Triphala and chlorhexidine groups which showed similar effects on gingival health. In patients who used chlorhexidine mouthwash, teeth staining was observed but there were no teeth staining in patients who used Triphala as mouthwash (14).

Triphala has potent anticariogenic and antimicrobial activity according to the study conducted by Jagadish et al. who reported that upon Triphala usage at different concentrations, there was 83%, and 86% growth reduction of *Streptococcus mutans* in the presence of 5%, and 10% Triphala, respectively (15). Jagtap and Karkera reported that oral rinsing extracts of *Terminalia chebula* inhibited the growth and adherence of *Streptococcus mutans* (16).

Conclusively, Triphala has significant antimicrobial activity. Future *in vivo* studies are required to explore its antibacterial activity against various oral pathogens on a larger sample size, before recommending its usage in the clinical practice.

Conflict of interest

The authors declared no conflict of interest.

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