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Preparation Of Mouthwash Using Triphala Aqua - Ethanolic Extract - A Comparative Study On Antimicrobial And Cytotoxic Effects

Research Article

Indra G[1], Maragathavalli G[2*], Rajeshkumar S[3]

[1] Post graduate resident, Department of Oral Medicine and Radiology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai - 77, India.
[2] Professor, Department of Oral Medicine and Radiology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai - 77, India.
[3] Associate Professor, Department of Pharmacology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai - 77, India.

Abstract

Objective: The objective of the study was to prepare mouthwash using triphala aqua-ethanolic extract and to compare and evaluate the antimicrobial and cytotoxic effects.
Materials and method: This study includes triphala churna powder which is the key ingredient and absolute ethanol as the solvent for preparing the mouthwash and subjected to antimicrobial efficacy and cytotoxic effects. For antimicrobial activity, four microorganisms, S.aureus, S. mutans, E. faecalis and C. albicans are used and checked for zones of inhibition. For cytotoxic assay, brine shrimp is used to check for cytotoxicity after 24 hours.
Result: The cytotoxicity assessment of the number of live nauplii after 24 hours revealed that there were at least 8 nauplii alive in each group. Zone of inhibition for 25µL showed 40% inhibition of S.aureus, in 50µL the S.aureus showed 32% inhibition and in 100µL it showed 30% inhibition.
Conclusion: Triphala aqua-ethanolic mouthwash has proven to have excellent antimicrobial properties and hence can be used in dentistry. Elaborate studies should be done to evaluate the toxic potential before using in human beings.

Keywords: Triphala; Antimicrobial Activity; Cytotoxicity; Mouthwash; Plant Extract.

Introduction

In India, the traditional medicines such as AYUSH (Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy) have been in practice for centuries and focus on healing of the mind and body and in treating different ailments.[1]

Triphala is an ayurvedic polyherbal medicine mainly consisting of three ingredients namely, Amalaki (Phyllanthus emblica), Bibhitaki (Terminalia bellirica), and Haritaki (Terminalia chebula) in equal proportions.[2] The phytochemicals present in it are components rich in gallic acid, tannins, chebulagic acid, ellagic acid, phenols and glycosides.[3] Triphala is reported to be an effective antibacterial, antifungal, antioxidant, chemopreventive, antidiabetic, radioprotective, antimutagenic, analgesic, anticancer and antipyretic agents. [3-11]

In dentistry, triphala are proven to have good anticariogenic efficacy, used as a root canal irrigant, anti-collagenase activity, antifungal, antioxidant and as mouth rinse.[12] Triphala have also shown to be a good denture cleanser. It is because of the gallic acid component in the triphala accountable for the anticandidal efficacy.[13]

Triphala is commercially available as choorna and tablets. Maximum dosage of Triphala extract is 2000 mg per day. Concentrate tablets of Triphala contain around 500 mg to 750 mg depending on the manufacturer.[12, 14]

***Corresponding Author**:
Maragathavalli G,
Professor, Department of Oral Medicine and Radiology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai - 77, India.
Tel: +91 9445171146
E-mail: drgopalvalli@ gmail.com

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The aim of the study was to prepare mouthwash using triphala aqua-ethanolic extract and to compare and evaluate the antimicrobial effects against *S. aureus, S. mutans, E. faecalis and C.albicans* and cytotoxicity in live nauplii for 24 hours.

Material and Methods

Study design: In-vitro study.

Chemicals used

1. Triphala churna powder – key ingredient
2. Absolute ethanol - Used as the solvent.
3. Distilled water - Used as a vehicle.
4. Sodium benzoate - Used as a preservative.
5. Peppermint oil - Used as a flavouring agent.
6. Sucrose – Used as a sweeter.
7. Sodium lauryl sulphate – Used as a foaming agent.

Preparation of mouthwash solution

2g of Triphala churna was mixed with 100mL of distilled water in 250 mL conical flask. The flask was kept in the heating mantle at 60-70°C for 30 min. The extract was filtered and used for the preparation of mouthwash. This plant extract concentrate was mixed with sterile distilled water to get a mouth rinse of 10% (w/v) concentration. 0.3g of sucrose, 0.001 sodium benzoate and 0.01g of sodium lauryl sulphate is added and dissolved in 10 mL of distilled water. Then 600 µL of triphala churna extract is added along with 50 µl of peppermint oil which is used as a flavouring agent. (Figure 1)

Antimicrobial activity

Antimicrobial activity of respective extract against the strain Staphylococcus aureus, Streptococcus mutans, Candida albicans and Enterococcus faecalis. Mueller-Hinton agar (MHA) agar was utilized for this activity to determine the zone of inhibition. MHA were prepared and sterilized for 45 minutes at 120lbs. Media was poured into the sterilized plates and let it stabilize for solidification. The wells were cut using the well cutter and the test organisms were swabbed. The extract with different concentrations were loaded and the plates were incubated for 24 hours at 37°C. After the incubation time the zone of inhibition was measured. (Figure 2)

Cytotoxicity assay

The cytotoxicity of Triphala reinforced with aqua-ethanolic extract was assessed using Brine shrimp lethality assay. 12 well ELISA plates were taken and to each plate 6-8 ml of saltwater was added; followed by adding 10 nauplii to each well. Triphala reinforced with aqua-ethanol was added to each well at different concentrations (5 µL, 10 µL, 20 µL, 40 µL, 80 µL) and was then incubated for 24 h. After 24 h, the total number of live and dead nauplii was counted and the mortality rate was checked. (Figure 3)

% death = (Number of dead nauplii/Number of dead nauplii − number of live nauplii) x 100

Results And Discussion

After 24 hours, the cytotoxic effect based on each concentration revealed the following result. In 5µL all the 10 nauplii were alive,

Figure 1. Preparation of plant extract.

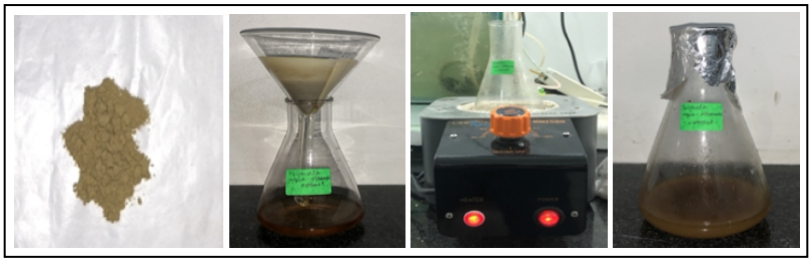


Figure 2. Antimicrobial activity checked in C. albicans, E. faecalis, S. mutans and S.aureu. (From left to right)

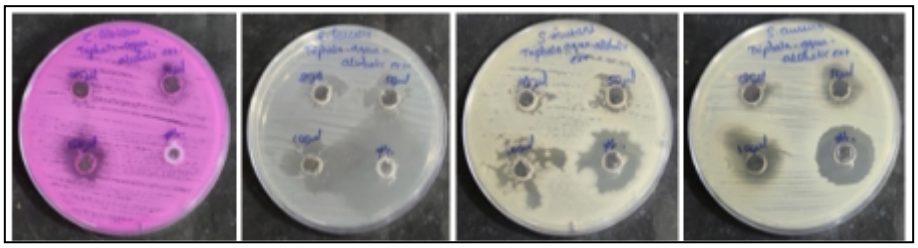


Figure 3. Cytotoxic effect carried out in ELIZA plate with live nauplii.

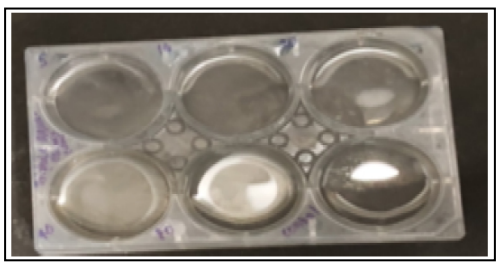


Figure 4. Cytotoxic effect based on concentration.

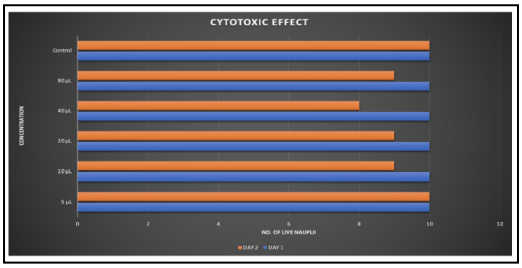
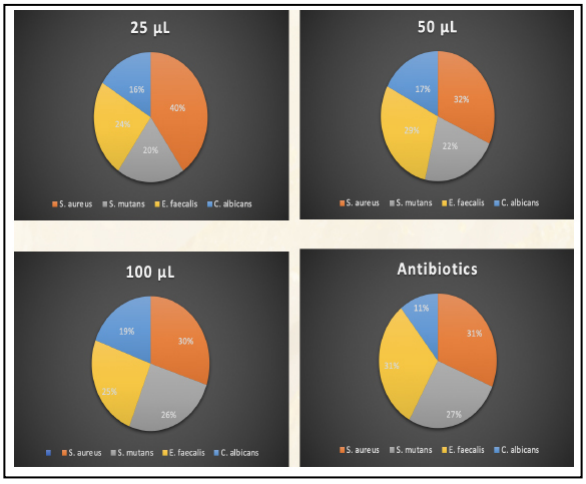


Figure 5. Antimicrobial activity at different concentrations.



in 10µL, 20µL and 80µL 9 nauplii were alive, and in 40µL 8 nauplii were alive. (Figure 4).

Zone of inhibition for 25µL showed 40% inhibition of S.aureus, in 50µL the *S.aureus* showed 32% inhibition and in 100µL it showed 30% inhibition. Whereas in 25µL the *S. mutans* showed 20%, *E. faecalis* showed 24% and *C. albicans* showed 16% inhibitions. In 50µL, the *S. mutans* showed 22%, *E. faecalis* showed 29% and *C. albicans* showed 17% inhibitions. In 100µL, the *S. mutans* showed 26%, *E. faecalis* showed 25% and *C. albicans* showed 19% inhibitions. (Figure 5)

It is understood that by now numerous studies have been carried out in the past to assess the effectiveness of triphala in dentistry. Triphala as such has proven to be good for health and is used for its therapeutic effects. Before administration of any newly prepared mouthwash, their effectiveness towards microorganisms, their properties such as antioxidants, anti-inflammatory and cytotoxicity has to be explored.

Gold standard mouthwashes such as chlorhexidine have proven to be more effective against microorganisms, however they tend to stain the teeth and restoration, provide altered taste sensations paving ways for new researches using herbal medicines.[2, 15, 16] A study conducted by Naiktari RS et al., reported that triphala showed a significant reduction in plaque index, gingival index and oral hygiene index with no staining when compared to chlorhexidine mouthwash.[17] A similar study comparing the efficacy of 6% triphala mouthwash with 0.2% chlorhexidine reported that when triphala used twice showed a reduction of 17% and 44% reduction against oral streptococcal agent [18]. Our study compared the effectiveness of antimicrobial agents in four different microorganisms namely, *S. aureus, S. mutans, E. faecalis and C.albicans*. The zone of inhibition showed the effective antimicrobial activity against the above mentioned microorganisms.

Cytotoxicity is performed to analyse the toxic ability of the mouthwash to the cells. Giannelli et al. suggest that mouth washes like chlorhexidine are highly cytotoxic in vitro and advise a more cautious use of them.[19] Numerous nauplii were taken into the study in an ELISA plate and checked after 24 hours. Our study showed that 92% of the nauplii were alive proving it to be less cytotoxic to the cells. However it is to the best of our knowledge that most studies determining the cytotoxicity of chlorhexidine have been done in the cell culture models and animals but human studies are few.[20, 21]

Numerous in vitro studies have been conducted in the past to analyse the cytotoxic effects and antimicrobial activity of mouthwashes prepared using plant extracts, showing remarkable results against microbial agents and cytotoxic nature of the cells proving that it is mandatory to perform a laboratory based trial before administering it to the patients.[22-26]

Acknowledgement And Declaration

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Conclusion

To conclude, the authors conclude that tripahal has an excellent antimicrobial efficacy and minimal cytotoxic effects when used as mouthrinse. However, larger clinical trials are required in human beings to quantitatively analyse the result.

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