

Research article**Antibacterial and anti-candida activity of chlorhexidine gluconate, Triphala and Munamal pothu (bark of *Mimusops elengi*)**

GK Wijesinghe¹, PDTA Jayarathna¹, TDCP Gunasekara¹,
N Fernando¹, N Kottegoda^{2,3}, MM Weerasekera¹

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Abstract

Introduction and Objective: Chlorhexidine is used as an oral mouthwash to reduce oral colonization related to dental disease. Triphala (An ayurvedic preparation from the dried fruits of *Emblica officinalis*, *Terminalia bellirica* and *Terminalia chebula*) and *Mimusops elengi* (Munamal) are used as mouthwashes in Ayurvedic medicine and are known to have antimicrobial activity.

This study was aimed to determine the effect of 0.2% Chlorhexidine gluconate, Triphala and *Mimusops elengi* (Munamal) on *Candida* species and three common bacterial pathogens.

Methods: The effect of 0.2% chlorhexidine gluconate, Triphala and *Mimusops elengi* (Munamal) aqueous extracts against *Candida spp.*, *Staphylococcus aureus*, and methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa* were determined using an agar well diffusion method. Minimum inhibitory concentration (MIC) of Triphala and Munamal aqueous extracts for the microorganisms were determined using broth micro dilution method with alamar blue modification.

Results: 0.2% Chlorhexidine gluconate showed a zone of inhibition for all test microbial strains. Triphala demonstrated activity against all tested microbial species except *Candida glabrata*, *Candida dubliniensis* and *Escherichia coli*. Munamal Pothu demonstrated activity against *Staphylococcus aureus* and MRSA only. Chlorhexidine gluconate inhibited the growth of *Candida* (*C. albicans* and *C. tropicalis*) at 1/512 dilution (MIC = 0.004 mg/ml for both organisms). The MIC of Triphala for *C. albicans* and *C. tropicalis* was 32.5 mg/ml. Anti-candida activity of Chlorhexidine gluconate was achieved at very low concentrations.

¹Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka

²Department of Chemistry, Faculty of Applied Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka

³Sri Lanka Institute of Nanotechnology (SLINTEC), Pitipana, Homagama, Sri Lanka

Address for correspondence: Dr. Manjula M. Weerasekera, Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka. Telephone: +094714432870

Email: mmweera@sjp.ac.lk; mmweera@yahoo.com  <https://orcid.org/0000-0002-8194-2205>

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Conclusions: Triphala as a potential oral mouthwash agent with activity against *C. albicans* and *C. tropicalis* requires further investigation.

Key words: *Candida*, *Chlorhexidine*, *Triphala*, *Mimusops elengi* (Munamal)

Introduction

Candida species, *Pseudomonas* species, *Staphylococcus aureus* and *Escherichia coli* are common microorganisms associated with serious superficial and systemic infections in man.^{1,2} *S. aureus* is a major pathogen that causes a diverse range of infectious diseases.² *P. aeruginosa* is most commonly associated with chronic wound infections, ear infections and lung infections in patients with cystic fibrosis.³ These three pathogens cause community as well as hospital acquired infections while the ability of *Pseudomonas spp.* to form biofilms contribute to their success as a pathogen.^{4,5,6} MRSA is a major cause of nosocomial infections.⁷ Available data show that MRSA accounts on average 57% of *S. aureus* isolates causing nosocomial infection in intensive care units (ICUs).⁷

Fungal species, mainly yeasts and *Aspergillus* species, are commonly associated with patients with diabetes mellitus.⁸ *C. albicans* accounts for > 90% of oral and systemic candida infections followed by *C. tropicalis*, *C. parapsilosis*, *C. glabrata* and *C. krusei*.^{9,10}

Ayurvedic rasayana consisting of Amulaki/Nelli (*Emblica officinalis*), Bibhitaki/Bulu(*Terminalia bellirica*) and Halituki/Aralu (*Terminalia chebula*) commonly known as “Triphala” is effectively used in Ayurveda.^{11,12} *Mimusops elengi*(Munamal)¹³ is a plant known to have antimicrobial activity which is also used in ayurvedic medicine. In our study we investigated the effect of these ayurvedic preparations Triphala and Munamal and chlorhexidine on planktonic microorganisms *in - vitro*.

Methods

Test strains and culture conditions

Five *Candida* type strains, (*C. albicans*: ATCC10231; *C. tropicalis*: ATCC13803; *C. parapsilosis*: ATCC22019; *C. glabrata*: ATCC90030; *C. dubliniensis*: MYA 580), three Gram positive cocci (*S. aureus*: ATCC25923; *S. epidermidis*: ATCC14990; MRSA: a clinical isolate), and two Gram negative bacilli (*E. coli*: ATCC25922; *P. aeruginosa*: ATCC27853) were used for this study. Stock cultures of *Candida* species were maintained on Sabouraud Dextrose Agar (SDA, Sigma-Aldrich, USA) slants and were subcultured onto SDA plates and incubated at 35 °C for 24 hours. Other stock cultures were maintained on nutrient agar (NA)(Sigma-Aldrich, USA) slants and were subcultured onto NA plates and incubated at 35 °C for 24 hours in order to obtain 24 hours fresh culture isolates.

Preparation of herbal antifungal agents:

Dried fruits of *Terminalia chebula* (aralu), *Terminalia bellirica*(bulu), *Phyllanthus emblica*(nelli), and dried bark of *Mimusops elengi* (Munamal pothu) were purchased from local vendors and identified and authenticated by the Botany Division, Bandaranayake Memorial Ayurvedic Research Institute, Sri Lanka.

Preparation of Triphala: The dried plant materials were washed with running tap water and air dried for 48 hours. Each dried fruit (20.0 g) was measured. The mixture of three dried fruits was boiled by addition of 6 cups (1440 ml) of distilled water, until the final volume of the extract was 240 ml, following the standard Ayurvedic protocol.

Preparation of *Mimusops elengi* extract: Dried *Mimusops elengi* bark (60.0 g) was used to prepare the aqueous extract using the same protocol.

An aliquot of prepared herbal aqueous extracts was freeze dried to determine the concentrations of working solutions.¹⁴ The prepared decoctions were filtered and stored at 4 °C up to two weeks until use. Chlorhexidine gluconate (Sigma Aldrich, USA) (0.2%) was used as a control.

Determination of the effect on planktonic cells

Inhibitory activity of 0.2% Chlorhexidine gluconate and the two herbal plant extracts (Triphala and *Mimusops elengi* extract) on planktonic test organisms were determined using an agar well diffusion method as described by Magaldi, with few modifications.¹⁵ Standard suspensions (0.5 MacFarland) of test organisms were prepared in sterile normal saline and inoculated on Mueller-Hinton agar (MHA) separately to obtain a confluent growth. Five wells of 6 mm diameter each were prepared in each agar plate and the bottoms of the prepared wells sealed with 50 µl of sterile MHA. 200 µl of working solutions of 0.2% chlorhexidine gluconate, aqueous extracts of Triphala and *Mimusops elengi* were added into the wells separately with sterile distilled water as a negative control. 1.25 mg/ml Fluconazole (Sigma-Aldrich, USA) was used as positive control for *Candida*. 30 mg/l gentamicin solution was used as positive control for Gram negative test organisms and 30 mg/l vancomycin for Gram positive cocci tested. The diameter of the zone of inhibition was measured after overnight incubation of the agar plates at 37 °C.

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) of *Candida* was determined using the CLSI M-27A broth microdilution method with alamar blue modification. Suspensions of *C. albicans* and *C. tropicalis* (1×10^6 cells/ml) were prepared in sterile RPMI 1640 using 48-hour fresh *Candida* cultures.

The final concentration of Triphala extract was 65 mg/ml and *Mimusops elengi* extract 12 mg/ml.

Serial two-fold dilutions (1/2, 1/4, 1/8, 1/16, 1/32 and 1/64) of antifungal agents were prepared using sterile distilled water. Prepared *Candida* suspensions (100 µl) were inoculated in triplicate to a sterile 96 well polystyrene microtiter plate and 100 µl of each dilution of antifungal agent was added to each well. A 100 µl of 0.02% Alamar blue stain was added to each well as an indicator. Plates were covered with a lid and sealed with parafilm and MIC was read after incubating the plate at 37 °C for 48 hours. Any zone of inhibition (ZOI) in the agar well diffusion assay was considered as evidence of activity of the relevant ayurvedic preparation against each test organism. All tests were performed in triplicate.

Results

Activity of chlorhexidine gluconate and two ayurvedic preparations

The results of activity of working solutions of 0.2% Chlorhexidine gluconate, Triphala and *Mimusops elengi* aqueous extracts against test organisms are presented in Table 1.

Chlorhexidine gluconate (0.2 mg/ml) showed a zone of inhibition for all ten test microbial strains. Among all *Candida* strains, Triphala demonstrated zones of inhibition only against *C. albicans*, *C. tropicalis* and

Table I: ZOI exhibited by 0.2% chlorhexidine gluconate, Triphala and *Mimusops elengi* extract against planktonic test organisms.

Organism	Zone of inhibition in millimetres*		
	Chlorhexidine gluconate (0.2 w/v)	Triphala (65 mg/ml)	<i>Mimusopselengi</i> (12mg/ml)
<i>C. albicans</i>	22 ± 1.25	16 ± 1.43	No ZOI
<i>C. tropicalis</i>	20 ± 2.20	09 ± 1.12	No ZOI
<i>C. parapsilosis</i>	28 ± 1.00	18 ± 1.50	No ZOI
<i>C. glabrata</i>	24 ± 1.00	No ZOI	No ZOI
<i>C. dubliniensis</i>	25 ± 2.25	No ZOI	No ZOI
<i>S. aureus</i>	23 ± 1.50	22 ± 1.00	13 ± 1.50
<i>S. epidermidis</i>	28 ± 1.00	20 ± 2.50	No ZOI
MRSA	17 ± 1.25	17 ± 1.00	13 ± 2.00
<i>E. coli</i>	18 ± 2.00	No ZOI	No ZOI
<i>P. aeruginosa</i>	20 ± 1.50	17 ± 1.00	No ZOI

*All experiments were done in triplicates. Data are mean ± standard deviation of three individual experiments

C. parapsilosis.

Triphala showed a ZOI against all tested Gram positive cocci and *P. aeruginosa* but not against *E. coli*.

Munamal pothu extract demonstrated activity only against *S. aureus* and MRSA.

MIC of the 3 test products against planktonic *C. albicans* and *C. tropicalis*

Table 2: MIC of the 3 test products

Organism	Product tested	MIC (mg/ml)
<i>C. albicans</i>	Chlorhexidine gluconate	0.004
	Triphala	32.5
	<i>Mimusops elengi</i> extract	-
<i>C. tropicalis</i>	Chlorhexidine gluconate	0.004
	Triphala	32.5
	<i>Mimusops elengi</i> extract	-

*Three individual tests were performed in triplicate

As shown in Table 2, Chlorhexidine gluconate exhibited a MIC of 0.004 mg/ml for both planktonic *Candida* species. The MIC of Triphala for both *Candida* species was 32.5 mg/ml. *Mimusops elengi* extracts did not exhibit any activity against these 2 species of candida.

Discussion

Chlorhexidine gluconate (0.2%), is a recommended, commercially available mouth rinse with reported activity against oral bacteria.¹⁶ In the present study also it showed an activity against free living planktonic cells which indicated its effectiveness in preventing microbial colonization and viability. However long term use of this antiseptic as an oral rinse has side effects, including staining of teeth, alteration of taste sensation and development of antimicrobial resistance.¹⁷ The use of alternative herbal extracts may help overcome these side effects. *Mimusops elengi* is known to have antimicrobial activity.¹³ It is also used for treating or controlling oral complications such as dental caries and gum bleeding. However, according to the present study this extract had no inhibitory effect against *Candida* or other bacterial species except *S. aureus* and MRSA.

Triphala is a common ayurvedic preparation used for a broad range of oral and non-oral infections. It is reported to have anti-inflammatory, antioxidant, and antimicrobial activity against a wide spectrum of microorganisms including bacteria, yeasts and dermatophytes.^{18,19,11} The active phytochemicals contributing to antimicrobial activity are tannic acid, chebulic acid, and flavonoids. Its antimicrobial activity against *C. albicans* and dermatophytes have been reported using the agar well diffusion assay.¹⁸ In this in-vitro study Triphala was found to show activity against *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *S. aureus*, MRSA, *S. epidermidis*, and *P. aeruginosa* planktonic cells. Antibiofilm activity of Triphala has been reported which suggests its usefulness as a general antiseptic with low side effects. Triphala has been shown to be effective in reducing biofilms of both *C. albicans* and *Enterococcus faecalis* in one study.¹²

The MIC of Triphala for *C. albicans* and *C. tropicalis* was found to be 32.5 mg/ml in the current study. Further investigations are needed to determine the MIC of Triphala against the other *Candida* species. The current study demonstrates the antibacterial and anti-candida activity of Triphala. However, the role of Triphala against a wider spectrum of microorganisms, including oral colonizers would be useful in determining whether this ayurvedic product could be effectively used as an herbal mouth rinse to prevent oral pathogens including *Candida* species.

A limitation of this study is that only the aqueous extract of both Triphala and *Mimusops elengi* were tested for antimicrobial activity. It would be useful to test the ethanolic extracts of both and compare the antimicrobial activity of aqueous and ethanolic extracts.

Conclusion

The findings of this study further strengthen the effectiveness of 0.2% chlorhexidine gluconate as an antimicrobial agent. Triphala is a potential antimicrobial agent against *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *S. aureus*, *S. epidermidis*, MRSA and *P. aeruginosa*. *S. aureus* and MRSA are sensitive to Munamal extract.

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References

1. Chen T, YuWH, Izard J, et al. The Human Oral Microbiome Database: a web accessible resource for investigating oral microbe taxonomic and genomic information. *Database* 2010; Article ID baq013. doi: <https://doi.org/10.1093/database/baq013>.
2. Archer GL. *Staphylococcus aureus*: a well-armed pathogen. *Clinical Infectious Diseases* 1998; 26:1179-1181. doi: <https://doi.org/10.1086/520289>.
3. Klaus Kirketerp-Møller, Peter Ø Jensen, Mustafa Fazli et al. Distribution, organization, and ecology of bacteria in chronic wounds. *Journal of Clinical Microbiology* 2008; 46:2717-2722. doi: <https://doi.org/10.1128/JCM.00501-08>.
4. Yoshikawa TT, Bradley SF. *Staphylococcus aureus* infections and antibiotic resistance in older adults. *Clinical Infectious Diseases* 2002; 34(2):211-216. doi: <https://doi.org/10.1086/338150>.
5. Flemming HC and Wingender J. The biofilm matrix. *Nature Review Microbiology* 2010; 8(9):623-633. doi: <https://doi.org/10.1038/nrmicro2415>.
6. Khan HA, Ahmad A, Mehboob R. Nosocomial infections and their control strategies. *Asian Pacific Journal of Tropical Biomedicine* 2015; 5(7):509-514. doi: <https://doi.org/10.1016/j.apjtb.2015.05.001>
7. National Nosocomial Infections Surveillance (NNIS) System Report; data summary from January 1992 through June 2002. *American Journal of Infection Control* 2003; 31:481-498. doi: <https://doi.org/10.1016/j.ajic.2004.10.001>.
8. Wijesuriya TM, Kottahachchi J, Gunasekara TD, et al. Aspergillus species: An emerging pathogen in onychomycosis among diabetics. *Indian Journal Endocrinology and Metabolism* 2015; 19(6):811–816. doi: <https://doi.org/10.4103/2230-8210.167565>.
9. Dilhari A, Weerasekera MM, Siriwardhana A et al. Candida infection in oral leukoplakia; an unperceived public health problem: *Acta Odontologica Scandinavica*. 2016; 74(7):565-569. doi: <https://doi.org/10.1080/00016357.2016.1220018>
10. Cannon RD, Holmes AR, Mason AB, Monk BC. Oral Candida: clearance, colonization, or candidiasis. *Journal of Dental Research* 1995; 74(5): 1152-1161. doi: <https://doi.org/10.1177/00220345950740050301>.
11. Biradar YS, Jagatap S, Khandelwal KR, Singhania SS. Exploring of antimicrobial activity of Triphala Mashi-an ayurvedic formulation. *Evidence-Based Complementary and Alternative Medicine* 2008; 5(1):107–113. doi: <http://dx.doi.org/10.1093/ecam/nem002>
12. Jyothi KN, Gopal A. Comparison of antimicrobial efficacy of 0.3% Propolis, 10% Neem, 10% Triphala and 5% Sodium hypochlorite on *Candida albicans* and *E. faecalis* biofilm formed on root dentin: An in vitro study. *Journal of Dental Sciences*. 2016; 4(3):90-94. doi: <https://doi.org/10.1016/j.jds.2016.03.001>
13. Deshpande RR, Ruikar A, Panvalkar PS, et al. Comparative evaluation of different concentration of *Mimusops Elengi* (L) extract as an antimicrobial against salivary microflora. *Journal of Biomedical Science and Research*. 2010; 2:51–4. No doi
14. Silva L, Borghetti F, de Lima EC. A simple procedure for the purification of active fractions in aqueous extracts of plants with allelopathic properties. *Acta Botanica Brasilica*. 2013; 27(1):50-53. doi: <https://doi.org/10.1590/S0102-33062013000100007>

15. Magaldi S, Mata-Essayag S, Hartung de Capriles C, et al. Well diffusion for antifungal susceptibility testing. *International Journal of Infectious Diseases* 2004; 8(1):39–45.
doi: <https://doi.org/10.1016/j.ijid.2003.03.002>.
16. Talebi S, Sabokbar A, Riazipour M, Saffari M. Comparison of the in vitro effect of chemical and herbal mouthwashes on *Candida albicans*. *Jundishapur Journal of Microbiology* 2014; 7(12): e12563.
doi: <https://doi.org/10.5812/jjm.12563>
17. McCoy LC, Wehler CJ, Rich SE, et al. Adverse events associated with chlorhexidine use: Results from the department of veterans affairs dental diabetes study. *Journal of American Dental Association* 2008; 139:178-83 doi: <https://doi.org/10.14219/jada.archive.2008.0134>.
18. Gomes PLR, Hewageegana S, Kottahachchi J, et al. In vitro study to determine antimicrobial activity of selected ayurvedic preparations against bacteria and fungi causing superficial skin infections, Sri Lanka. *Journal of Infectious Diseases* 2013; 3(1):32-39.
doi: <http://dx.doi.org/10.4038/sljid.v3i1.4717>.
19. Salam R, Sarker B K, HaqM R, Khokon JU. Antimicrobial activity of medicinal plant for oral health and hygiene. *International Journal of Natural and Social Sciences* 2015; 2 (1):1-15.No doi