







ORAL HEALTH PROTECTION THROUGH AN AYURVEDA POLY HERBAL FORMULATION- AN IN VITRO STUDY

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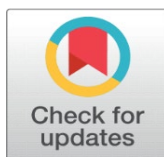
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ABSTRACT

Introduction: Natural medicines prescribed by the traditional Ayurvedic medical system of Sri Lanka have been extensively used to treat and prevent oral diseases.

Objective: Here, we investigated the antimicrobial effect of Ayurveda polyherbal formulation, mentioned in authentic text as Gandusha (mouth wash) consisting of Jasminum officinale leaves, Terminalia chebula fruits, Tinospora cordifolia stem, Desmodium triflorum whole plant and Glycyrrhiza glabra roots.

Methods: Anti-microbial susceptibility of the poly herbal preparation against common oral pathogens (Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Enterococcus faecalis) was tested using agar well diffusion method. Minimum inhibitory (MIC) was expressed in mg of freeze-dried extract per millilitres of agar solution.

Results: The plant extract has considerable antimicrobial activity against Staphylococcus aureus and Enterococcus faecalis. The lowest MIC of 250mg/ml was shown against Enterococcus faecalis and Staphylococcus aureus.

Conclusions: The polyherbal formulation is effective in mitigating the bacterial growth and hence enhance the oral health protection.

Keywords: Oral Health, Poly Herbal Formulation, Plant Extract

1. INTRODUCTION

In Susruta Samhita, there are numerous descriptions on periodontal diseases, oral mucosal diseases, and dental caries. [Singhal \(1972\)](#) Among oral diseases, periodontal disease and dental caries are major oral health hazards [WHO Technical Report Series. \(1995\)](#). Dental plaque is the main etiological factor for periodontal disease and dental caries [William et al. \(2001\)](#). Therefore, since ancient time Ayurveda and Sri Lankan traditional medical system has paid a significant attention on preventive and curative aspects of oral diseases. Further, Charaka Samhita has emphasized on modalities of tooth brushing, and oral cleansing through mouth rinses to maintenance of oral hygiene in *Dinacharya* (daily personal hygienic measures) [Agnivesha \(2008\)](#). Therefore, Ayurveda medical science recommends special local therapeutic measures such as *Gandusha* (Mouth wash), *Kavala* (Gargles) and *Pratisarana* (local application) to prevent dental plaque formation [Sharangadhara. \(2013\)](#).

Anti-bacterial behaviour of the medicinal plants is attributed to potential bioactive compounds that are meant to reduce the bacterial load in the oral cavity thus preventing plaque formation [Kadam et al. \(2011\)](#).

This research is aimed at investigating antimicrobial effect of an Ayurveda polyherbal formulation mentioned in authentic text as a *Gandusha* (mouthwash). The ingredients of this poly herbal formulation are leaves of *Jasminum officinale*, fruits of *Terminalia chebula*, stem of *Tinospora cordifolia*, whole plant of *Desmodium triflorum* and roots of *Glycyrrhiza glabra*. This poly herbal preparation is long been used in Sri Lankan Ayurveda as an effective oral treatment. However, none of the research studies have been carried out to prove its' clinical effectiveness on scientific basis. Therefore, this study was aimed to fill the gap of knowledge from Ayurveda presumption to modern scientific evidence. Further, Ayurveda Materia medica have been proven their safety and efficacy through several thousand years of use.

2. METHODS

2.1. STUDY DESIGN AND SETTING

This is a laboratory based In-vitro experimental study carried out at the microbiology laboratory, Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, University of Sri Jayewardenepura, Sri Lanka. All the plant materials were identified and authenticated by the Department of *Dravyaguna*, Institute of Indigenous Medicine, University of Colombo, Sri Lanka.

2.2. PREPARATION OF AQUEOUS EXTRACT

Aqueous extract was prepared using dried pericarp of *T. chebula*, dried stem of *T. cordifolia*, roots of *G. glabra*, 75 g each, tender leaves of *J. officinale* and whole plant of *D. triflorum*, 150 g of each. The ingredients were boiled in a clay pot under low heat with 12 cups of water (2880 ml) to reduce the volume up to 2 cups (480 ml) according to Ayurveda *Gandusha* preparation method [Sharangadhara. \(2013\)](#). The polyherbal extract was filtered, and filtrate was used for the assessment of in-vitro antibacterial efficacy. [Figure 1](#)

Figure 1

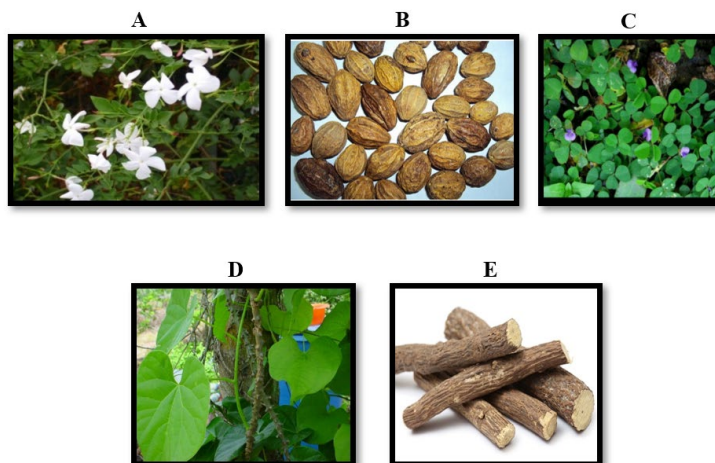


Figure 1 Ingredients of Poly Herbal Preparation

A. *J. officinale* B. *T. chebula* C. *D. triflorum* D. *T. cordifolia* E. *G. glabra*

2.3. PREPARATION OF POSITIVE CONTROLS (ANTIBIOTIC SOLUTIONS) FOR ABST

- 1) Amoxicillin – 10 mg was dissolved in sterile distilled water (5 ml). Concentration of amoxicillin was 2 mg/ml.
- 2) Penicillin –Commercially available and concentration was 10000 units/ml.
- 3) Gentamicin –Commercially available as liquid and concentration was 40mg/ml.

2.4. PREPARATION OF BACTERIAL CULTURE MEDIA

Mueller Hinton Agar Media (MHA) - Mueller Hinton agar powder (26.6 g) and bacteriological agar powder (2.1 g) was dissolved completely in 700 ml of distilled water [Kanchan et al. \(2019\)](#). The mouth of the conical flask was wrapped using aluminium foil and was sterilized by autoclaving and allowed to cool at room temperature. Cooled MHA medium was poured into sterilized disposable culture plates and allowed to solidify at room temperature.

Molten Agar –Agar (0.4 g) was dissolved with 20ml distilled water, autoclaved at 120° C for 15 minutes and kept in a water bath at 60°C.

2.5. MICROORGANISMS AND CONTROLS

Bacterial species representative of key pathogens causing oral diseases and widely accepted as testing strains for antimicrobial activity were chosen. Two pathogenic Gram-positive bacteria, *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 29212); two pathogenic Gram-negative bacteria, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were used.

All microbial cultures were collected from Quality Control Laboratory, Department of Microbiology, Medical Research Institute, Colombo, Sri Lanka and the Department of Microbiology, National Hospital, Colombo, Sri Lanka.

2.6. STANDARD MICROBIAL CULTURE PREPARATIONS

Staphylococcus aureus (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Enterococcus faecalis* (ATCC 29212) were subcultured by using freshly prepared MHA plates separately to obtain 24-hour fresh cultures for the antimicrobial assessment. They were incubated overnight at 37°C. At the end of the incubation period, small colonies were taken from sterilized wire loop and dissolved in normal saline (0.9%). The bacterial suspension was adjusted by adding sterilized normal saline until the turbidity matched with of 0.5 McFarland standard [Khan et al. \(2013\)](#). Four bacterial suspensions were prepared separately from each culture collection and labelled accordingly.

2.7. DETERMINATION OF ANTI-MICROBIAL ACTIVITY BY AGAR WELL DIFFUSION METHOD

The study was based on anti-microbial susceptibility test by using ATCC cultures as “standard microorganisms”, sterile normal saline as a “negative control” and antibiotic solutions as “positive controls” using agar well diffusion method.

The inoculum was smeared over the MHA plate using a sterile cotton swab in three directions, rotating the plate approximately 60° to ensure uniform microbial growth. Five wells were made in the agar surface. The wells were made with 8mm in diameter and 4mm in depth. The bottom of the wells was sealed by adding a drop of molten agar into each using sterile plastic Pasteur pipette. All the procedures were carried out aseptically. The wells contained.

- 1) Aqueous extract (75µl) as the test sample
- 2) Amoxicillin solution (75µl) of and penicillin solution (75µl) as positive controls against gram positive microorganisms (*S. aureus* and *E. faecalis*).
- 3) Sterile distilled water (75µl) as the negative control.
- 4) Gentamicin (75µl) antibacterial preparation as positive control against gram negative microorganisms (*E. coli* and *P. aeruginosa*).

The plates were labelled systematically for better identification before incubation.

The plates were wrapped with para films carefully without disturbing the solutions in the wells and kept for 15 minutes to diffuse the antibacterial solutions, the plant extract and the sterile distilled water to the media and then incubated at 37°C for 24 hours. At the end of the incubation period, diameter of inhibition zones was measured for the plant extract, positive control, and negative control for each microorganism. In agar well diffusion method, any zone of inhibition observed was considered as significant. The well diffusion assay was done in triplicates for each isolate and average inhibition zone was calculated [Peiris et al. \(2019\)](#).

2.8. DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) OF THE POLY HERBAL PREPARATION

The antimicrobial activity of the poly herbal preparation was quantified by determining the

MIC using the pour plate method [Sanders \(2012\)](#).

Mueller Hinton agar powder (26.6g) and bacteriological agar powder (2.1g) were dissolved in 700ml of distilled water and the ingredients were sterilized.

Two-fold serial dilutions were made by taking 6 ml from the herbal preparation with an initial concentration of 250mg/ml and diluting with 6 ml of distilled water to obtain a dilution of 125mg/ml. The same procedure was followed to obtain the dilution series of the poly herbal preparation. As a result, the poly herbal extract with initial concentration of 250 mg/mL was diluted to a final concentration of 3.906 mg/ml.

Molten MHA (20ml) was cooled to 50°C and mixed well with 3.0ml of each doubling dilutions (250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 15.63 mg/ml, 7.81 mg/ml, 3.91 mg/ml) and poured into sterile culture plates and allowed to dry. Inoculum was prepared as a suspension in a 0.9% normal saline and the turbidity of the inoculum was adjusted to McFarland 0.5 standard. A grid containing 5 squares was drawn on the back of the glass culture plates using marker pen. A volume of 5.0 µl of each suspension of organisms (*Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*) and the negative control (sterile normal saline) were kept on the surface of the agar plate in each grid using micropipette. Plates were then covered and left for overnight incubation at 37 ° C. The lowest concentration of the extract that inhibit the visible growth of a microorganism after overnight incubation (when compared with the control sterile normal saline) was determined as MIC and was carried out in triplicates and average MIC was calculated [Gunasekara et al. \(2017\)](#).

2.9. STATISTICAL ANALYSIS

The results were represented as the mean of three independent replicates ± standard deviation. Statistical analysis of data was carried out by SPSS (Statistical Package for Social Sciences) version 21.

3. RESULTS

3.1. ANTIMICROBIAL ACTIVITY OF THE POLY HERBAL PREPARATION

The poly herbal preparation has considerable antimicrobial activity against *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 29212) [Table 1](#). It was unable to inhibit the growth of *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC25922). However, the preparation showed a lower zone of inhibition for the tested bacteria in comparison to the standard antibiotic (amoxicillin, penicillin, and gentamycin). Among the tested microorganism, inhibitory activity of plant extract was found to be most active against *Enterococcus faecalis*. [Figure 2](#), [Figure 3](#)

Table 1

Table 1 The Zone of Inhibition of Poly Herbal Preparation (Mean ± SD) In Comparison with Standard Antibiotics				
Antimicrobial agent	<i>Enterococcus faecalis</i> (ATCC 29212)	<i>Escherichia coli</i> (ATCC 25922)	<i>Pseudomonas aeruginosa</i> (ATCC 27853)	<i>Staphylococcus aureus</i> (ATCC 25923)
Herbal preparation	17.00 ± 1.00	0.33 ± 0.58	1.00 ± 0.00	16.00 ± 0.00
Amoxicillin	39.33 ± 1.16	30.00 ± 0.00	15.00 ± 1.00	49.33 ± 1.16
Gentamycin	25.00 ± 0.00	30.00 ± 0.00	38.33 ± 1.53	29.67 ± 0.58
Penicillin	30.67 ± 1.16	23.33 ± 1.16	30.00 ± 0.00	50.00 ± 2.00

Figure 2

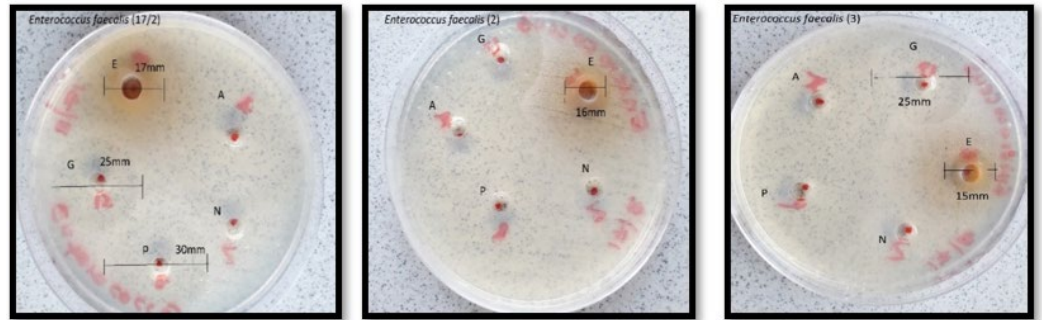


Figure 2 Triplicates of *Enterococcus faecalis*

Figure 3

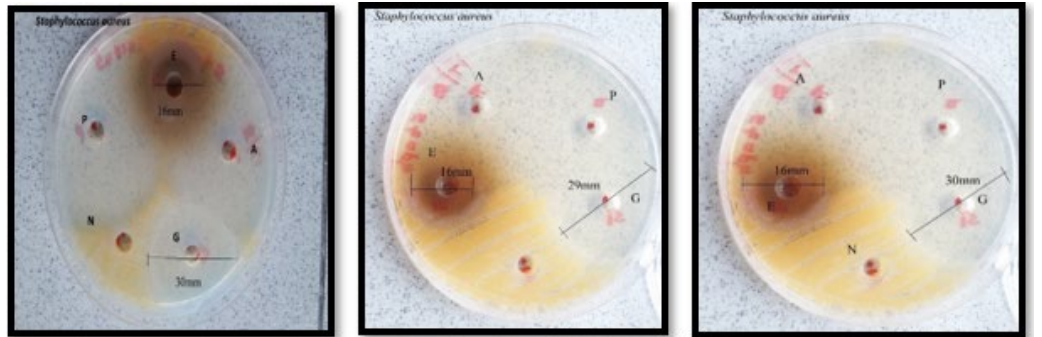


Figure 3 Triplicates of *Staphylococcus aureus*

P – Penicillin N – Normal saline G – Gentamicin A – Amoxicillin E – Extract

3.2. MIC OF THE POLY HERBAL PREPARATION

As depicted in the [Table 2](#), the effective concentration of herbal preparation for its maximum antimicrobial activity is 250mg/ml. In this concentration, the plant extract has inhibitory activity against *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Staphylococcus aureus*.

Table 2

Table 2 MIC of the Polyherbal Preparation					
Concentration of herbal preparation (mg/ml)	<i>Escherichia coli</i> (ATCC 25922)	<i>Pseudomonas aeruginosa</i> (ATCC 27853)	<i>Enterococcus faecalis</i> (ATCC 29212)	<i>Staphylococcus aureus</i> (ATCC 25923)	Negative control
250	G	NG	NG	NG	G
125	G	G	NG	NG	G
62.5	G	G	G	NG	G
31.25	G	G	G	G	G
15.63	G	G	G	G	G
7.81	G	G	G	G	G
3.91	G	G	G	G	G

G: Growth

NG: No growth

4. DISCUSSION

Although medicinal plants have been used from ancient times as natural remedies in Sri Lankan Ayurveda and are considered as alternatives to complement the synthetic medications, in-depth evidence based scientific investigations must be conducted to establish the scientific rationale for the efficacy. Thus, in this study, microbial susceptibility was evaluated for a reputed poly herbal preparation that is used as an effective treatment against periodontal disease and dental caries.

The study provides evidence to prove that the poly herbal formulation has moderate antibacterial action against the gram-positive bacteria *Enterococcus faecalis*, and *Staphylococcus aureus* that is responsible for oral mucosal diseases, periodontal disease, and dental caries.

The antimicrobial effect of individual ingredients of the polyherbal formulation was confirmed by previous scientific research data. The indigenous medical literature has stated that the extract of leaves from *J. officinale* is used to treat mouth ulcers due to its' *vrana ropana* (ulcer healing), *krimighna* (anti-microbial), *sothaghna* (reduce swelling) and *sophaghna* (anti-inflammatory) actions [Ayurveda Pharmacopoeia \(2002\)](#). Another study has confirmed that the ethanolic extracts of all part of the plant extract is effective against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa* [Shahbaa and Al-Khazraji \(2015\)](#).

[Raza et al. \(2010\)](#) have reported the antimicrobial activity of water extract of *T. chebula* fruit against *Staphylococcus aureus* [Raza et al. \(2010\)](#). In another study it was revealed that different solvent fractions of *T. chebula* fruit is effective against *Staphylococcus aureus* and *Escherichia coli* [Bag et al. \(2009\)](#). Further, the study has claimed that *Staphylococcus aureus* strains were found to be more susceptible to a hot aqueous extract and *Escherichia coli* strains to ethanol extract [Bag et al. \(2009\)](#). It has been reported that, *T. chebula* has the ability in decreasing oral diseases, such as gingival inflammation, mouth ulcers and plaque formation [Gupta et al. \(2016\)](#). The indigenous medical literature has also made strong evidence reputed that it has *krimighna*, *vrana ropana*, *raktasthambhana* and *sophaghna* actions according to pharmacodynamics properties of *T. chebula*.

Crude methanol extract of *T. cordifolia* was reported to contained antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* [Singh et al. \(2016\)](#). Phytochemical study of aqueous extract of *T. cordifolia* has confirmed that it contains alkaloids, flavonoids, glycosides and tannins [Sharma et al. \(2019\)](#). It further proved that the Ayurveda pharmacodynamics of *sothahara*, *sophahara*, *raktasthambhana*, *vrana ropana* and *vrana shodana* properties to be associated with *T. cordifolia* [Ayurveda Pharmacopoeia \(2002\)](#).

G. glabra was found to show potential antibacterial efficacy against primary plaque colonizers and periodontal pathogens [Sharma et al. \(2016\)](#). These results further confirmed its' *sophahara*, *sothaghna*, *vrana sodhana*, *ropana* and *krimighna* properties.

Several studies have reported that the crude extract of the whole plant of *D. triflorum* to possess analgesic, anti-inflammatory, ulcer healing and anti-microbial actions. Methanolic extract has shown considerable analgesic and anti-inflammatory activities [Liu et al. \(2013\)](#). Another study has confirmed that methanolic extract has considerable anti-bacterial activity against gram positive

organisms such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* [Sharma et al. \(2013\)](#).

Therefore, the evidence based scientific data has confirmed that the ingredients in the polyherbal preparation has an array of bioactivities such as antimicrobial, anti-inflammatory, analgesic, and cleansing properties thus serving as an effective remedy. Further the existing literature significantly proved the pharmacological actions mentioned in Ayurveda texts on each ingredient of poly herbal *Gandusha* formulation to be effective against pathogens causing oral diseases. Moreover, this type of herbal preparation is much beneficial than antibiotics due to the non-emergence of resistant by pathogens. They are cost effective and therefore economically affordable. The plant materials are widely available in abundance and easy preparation method has led to wide use among people who rely on Ayurvedic treatments.

5. CONCLUSION

The poly herbal combination mentioned in Ayurveda consisting of *J. officinale* leaves, *T. chebula* fruits, *T. cordifolia* stem, *D. triflorum* whole plant and *G. glabra* roots has a potential for inhibiting oral pathogens. It therefore improve the oral hygiene and provide scientific rationale for its wide use among people who rely on Ayurvedic treatments. However, further studies especially in the clinical setting are required to confirm efficacy.

CONFLICT OF INTERESTS

None.

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