

Detection of Antimicrobial Property of Ayurvedic Toothpaste formulation on common Oral Microbial Flora: In-Vitro Study

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Abstract

Background: Oral hygiene maintenance is most important task to nullify the effect of microbes and oral diseases. There are various options available for oral hygiene maintenance but the use of herbal alternatives tops the list.

Aim: To develop a dentifrice using Amla, Turmeric, Neem with other herbs and to study the effects of these on Streptococcus mutans, Lactobacillus acidophilus & Candida albicans.

Material & Method: This was an in-vitro model using the well method of microbial culture. Pure strains of the micro-organisms were purchased from National Chemical

Laboratory (NCIM), Pune and Microbial Type Cell Culture (MTCC), Chandigarh. Formulated Ayurvedic toothpaste along with Colgate Charcoal, Colgate vedshakti & Dantkanti toothpaste were diluted in 1:1 ratio with distilled water individually & poured in respective wells. Pure bacterial strains were inoculated on their respective culture media with lawn culture technique and incubated. Anti-microbial zone exhibited by each of the toothpaste was measured in millimetres after 24 and 48 hours. Tests were done in triplicate. Statistical analysis was done by ANOVA.

Results: The maximum zone of inhibition was obtained for dantkanti toothpaste while ayurvedic toothpaste and

colgate vedshakti had shown almost similar zone of inhibition for streptococcus mutans, lactobacillus and candida albicans.

Conclusion: It can be concluded that purely ayurvedic toothpaste can be used as alternative to other commercially available toothpaste with herbal extracts for maintaining oral hygiene.

Keywords: Ayurvedic toothpaste, oral hygiene

Introduction

Oral hygiene is maintenance of cleanliness of mouth which prevents dental, gingival as well as periodontal disorders [1].

Toothpaste is an oral hygiene aid used with a toothbrush. They have a complex composition with multiple ingredients for preventing gum and periodontal diseases, caries as well as ingredients or factors to maintain the proper consistency, texture, flow, flavor of toothpaste like abrasives, humectants binders. [1]

In spite of artificial ingredients found in common toothpaste; one can also use natural ingredients available for taking care of oral cavity & preventing it from various diseases. Using natural products can be easy as they are readily available at low cost, in low income or developing countries where dental & gum diseases are highly prevalent this alternative becomes a need of hour. [2] So to obtain cost effective oral hygiene one can use toothpaste with natural ingredients or herbs like amla, neem, babool, turmeric, meswak, tulsi, tomar, which have been mentioned in ayurvedic text & have been used since ancient times because of their antimicrobial as well as anti-inflammatory properties [3].

Dental caries is one of the most common diseases which is caused by combination of different microorganisms. Streptococcus mutans (S. mutans) initiates dental caries & is supported by other bacteria especially Lactobacillus acidophilus (L. acidophilus) which act as secondary

invader / progressor for caries. [4-6] Other than bacterial species; fungal species like Candida albicans (C. albicans) are also present in plaque over teeth surface. [4] which act as opportunistic infective agent causing candidiasis and caries [7].

Keeping the above in mind this study aimed to develop a dentifrice using Amla, Turmeric, Neem with other herbs and effects of these on S. mutans, L. acidophilus & C. albicans.

Materials and Methods

Materials for Formulation of Ayurvedic Toothpaste

- An Ayurvedic toothpaste will be formulated using : (Fig 1.)
 - Activated Charcoal powder
 - Turmeric powder
 - Honey
 - Neem (Leaves /Branch) extract
 - Gum acacia/ babool(Gum)
 - Tomar (Zanthoxylum alatum) (Buds)
 - Mint
 - Tulsi (Basil leaves) extract
 - Meswak (Stem /Branch)
 - Amla (Fruit)
 - Aloe vera gel
 - Mousari (Mimusops elengi) (Fruit)
 - Paan (Betel leaves) extract
 - Hingot (Fruit)
 - Fenugreek/ methidana
 - Water (q.s)



Figure 1 : Materials required for toothpaste formulation.

Method for formulation of toothpaste

Activated Charcoal powder, turmeric powder, honey, gum acacia, tomar, amla, mousari, meswak, fenugreek and mint extract were brought from local market while leaves of neem, tulsi, paan and aloe vera were obtained from plant nursery present at Indore.

Aqueous extracts of neem, tulsi, paan, aloe vera & meswak were obtained by grinding the respective leaves/branches in mechanical grinder and filtering it, extracts were stored in different plastic bottles at cool place.

Tomar, gum acacia, amla, hingot, fenugreek and mousari were grinded to obtain the powderd form.

In the first step for mixing the toothpaste; extracts of neem, tulsi, paan, mint, aloe vera & honey were mixed in measured quantity.

Powdered contents (except hingot & fenugreek powder) were added in solution of extracts. At last hingot & fenugreek powder were added in paste.

Formed paste was stored in a jar until further proceedings.

Materials for Culture

Culture plate with Blood Agar for *S. mutans* & *L. acidophilus* and Sabouraud Dextrose agar for *C. albicans*.

Pure strains of *S. Mutans*, *L. acidophilus* and *C. albicans*.

Ayurvedic toothpaste, Distill Water and Colgate Charcoal, Colgate vedshakti and Dantkanti toothpaste.

Method

The study was designed as an in vitro model using the well method of microbial culture. Distilled water was used as the negative control along with three dentifrices (Colgate total Charcoal, Colgate vedshakti and Dantkanti) which were readily available in the market. The standard strains of microorganisms were obtained from National Chemical Laboratory (NCIM) Pune, NCIM no. 3102 (*C. albicans*) and NCIM 5306 (*L. acidophilus*) and Microbial Type Culture Collection (MTCC), Chandigarh, MTCC NO. 497 (*S. mutans*) & were revived on respective medium. Dentifrices were prepared in 1:1 dilution using distilled water. The standard strains were inoculated into the respective broth and incubated for 4 hrs. The prepared inoculums were adjusted to 0.5 McFarland turbidity standard according to National Committee on Clinical Laboratory Standard guidelines. The suspension of *S. mutans*, *L. acidophilus* and *C. albicans* were lawn cultured on Blood Agar and Sabouraud Dextrose agar respectively, using sterile cotton swab. Sterile template was used for preparing wells in agar plates & filled with 100 μ l of the 1:1 diluted dentifrices (Fig 2).

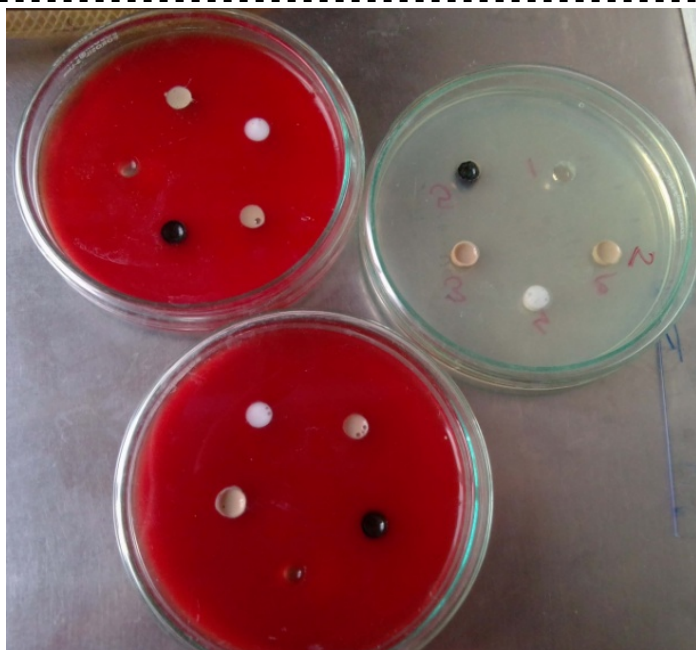


Figure 2 : Prepared agar plates; inoculated with respective microorganisms and wells filled with distilled water and diluted toothpastes.

The culture plates were incubated aerobically at 37°C for 48 hrs. After incubation; the diameter of zone of inhibition were measured in millimeters after 24 hrs & 48 hrs. and statistically analysed. [6]

Result & discussion

Result: In this study, the zone of inhibition were measured after 24 h and 48 h incubation and compared for effectiveness of toothpaste. For *S.mutans*, maximum zone of inhibition was obtained for colgate total charcoal (positive control) i.e. 14.5 and 16 mm followed by Dankanti toothpaste (13 mm & 13mm) while there were comparatively same results were obtained for ayurvedic toothpaste (11.5 mm & 12 mm) and colgate vedshakti (10.5 mm & 12 mm), distilled water was used as negative control in which zone of inhibition was absent (0 mm & 0 mm). (Fig 3)(Chart 1)



Figure 3 : Antimicrobial Zones of inhibition on Streptococcus mutans culture plate. 1.Distilled water 2.Ayurvedic toothpaste 3.Dantkanti 4.Colgate total Charcoal 5. Colgate Vedshakti.

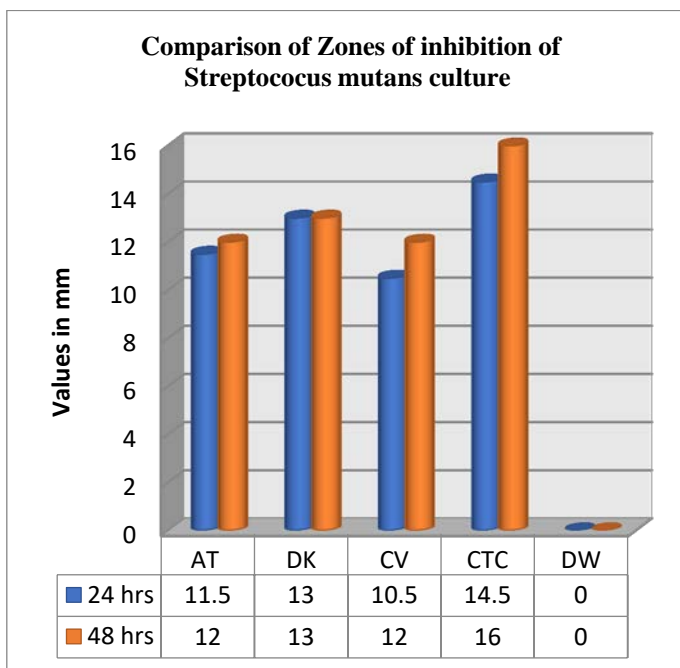


Chart 1 : AT – Ayurvedic Toothpaste, DK – Dantkanti, CV – Colgate vedshakti, CTC – Colgate Total Charcoal, DW – Distilled water.

For *L.acidophilus* ; zone of inhibition in decreasing order; were found in colgate total charcoal i.e. positive control (10.5 mm & 11.5 mm), ayurvedic toothpaste (11 mm & 11 mm), Dantkanti (10 mm & 10.5 mm) and Colgate Vedshakti (9.5 mm & 10.5 mm) and negative control;

distilled water (0 mm & 0 mm) after 24 h and 48 h respectively. (Fig 4)(Chart 2)

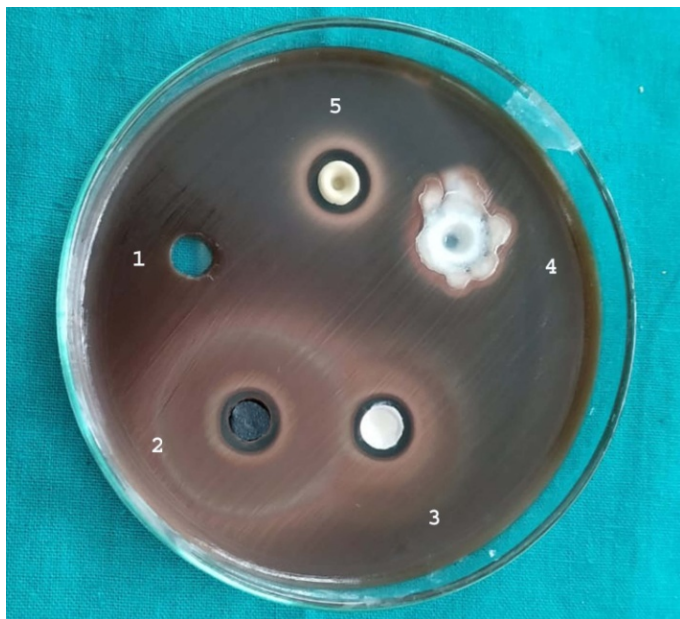


Figure 4 : Antimicrobial Zones of inhibition on Lactobacillus acidophilus culture plate. 1.Distilled water 2.Ayurvedic toothpaste 3.Dantkanti 4.Colgate total Charcoal 5. Colgate Vedshakti.

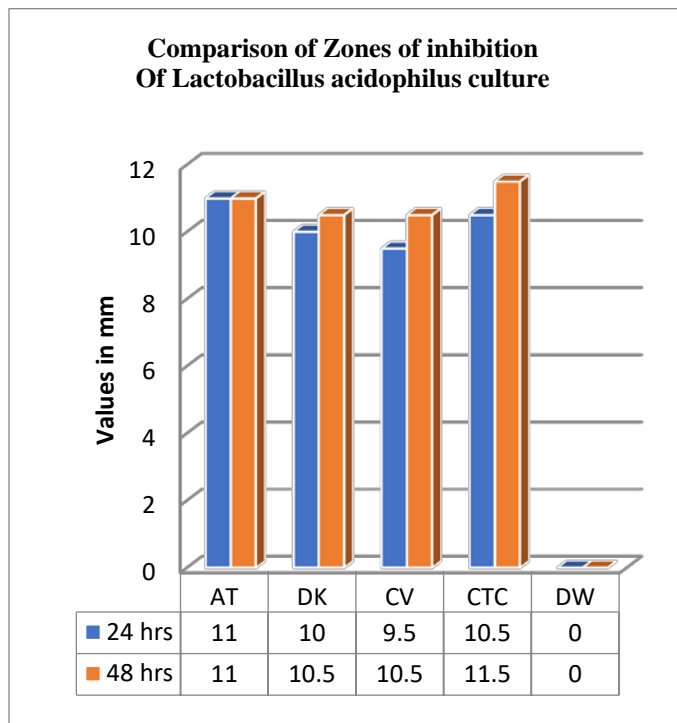


Chart 2 : AT – Ayurvedic Toothpaste, DK – Dantkanti, CV – Colgate vedshakti, CTC – Colgate Total Charcoal, DW – Distilled water.

For C.albicans, maximum zone of inhibition were obtained for Dantkanti and Colgate vedshakti (20.5 mm & 22 mm) followed by Colgate total charcoal (20 mm & 20 mm) and Ayurvedic Toothpaste (18 mm & 20mm), no zone of inhibition was found with distilled water after 24 h & 48 h respectively. (Fig 5)(Chart 3)

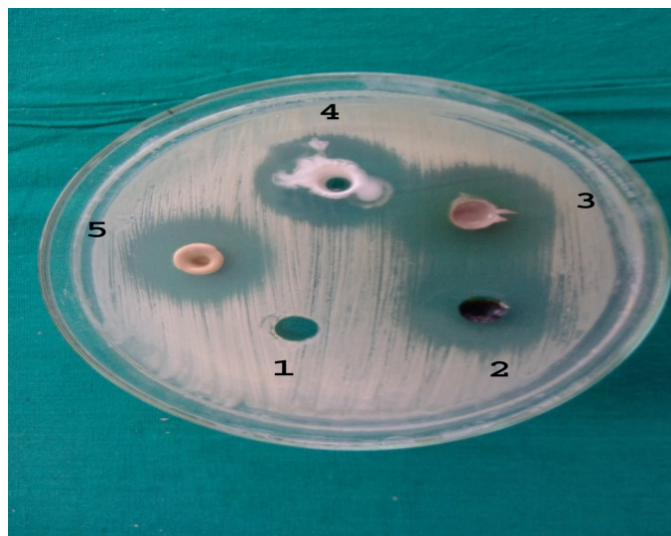


Figure 5 : Antimicrobial Zones of inhibition on Candida albicans culture plate. 1.Distilled water 2.Ayurvedic toothpaste 3.Dantkanti 4.Colgate total Charcoal 5. Colgate Vedshakti.

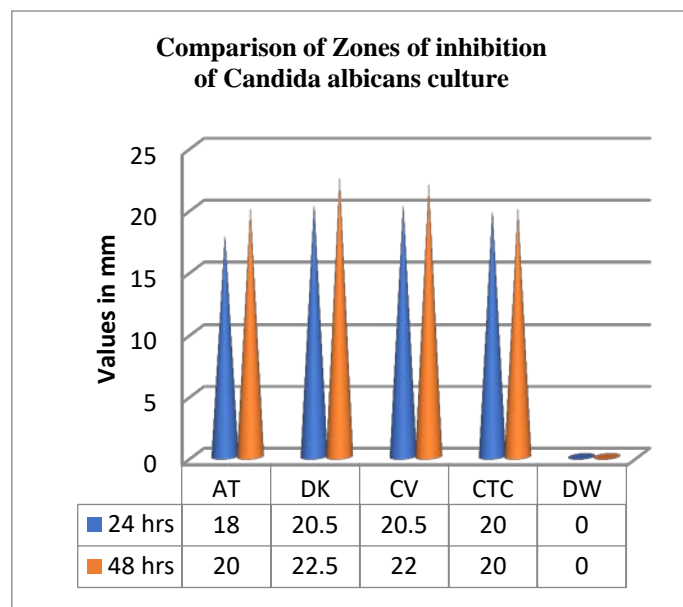


Chart 3: AT – Ayurvedic Toothpaste, DK – Dantkanti, CV – Colgate vedshakti, CTC – Colgate Total Charcoal, DW – Distilled water.

The data were analyzed using the 'Data-Analysis ToolPak' on Microsoft Excel 2013 (Microsoft Corporation) with the help of two ways ANOVA with replications followed by post-hoc analysis for pairwise comparison. Level of significance was considered to be 5%. No significant difference was found with the results of ayurvedic toothpaste and other commercially available toothpastes.

Discussion

In our study we found that ayurvedic toothpaste containing different herbs have equal amount of medicinal values as they contain antimicrobial property. These results were similar to Venisha Pandita et al who conducted a systemic review in 2014 on role of herbs in periodontal care and Concluded that the herbs and natural plants have medicinal effects which make them of medicinal value. These herbs can be used as anti-inflammatory, antiseptic, antimicrobial agent as well as they can be used for healing purpose. [3]

We found that Colgate total charcoal was effective against Candida, Streptococcus and Lactobacillus species, (in decreasing order of diameter of zone of inhibition) contrary to comparative in vitro study of J. Sunitha et al on antimicrobial effect of herbal dentifrices who found that Colgate total & Dabur Babool are more effective against Lactobacillus species than Streptococcus mutans.[8] This could be attributed to the difference in composition of toothpaste used in these two studies.

In our study, the ayurvedic toothpaste containing extract of miswak herb was found to be equally effective against caries causing organism along with candida species. Similar results were presented by Mariem O. Wassel et al in a research to prove antibacterial activity of propolis, miswak, and chitosan in dental varnishes against Streptococcus mutans in which they concluded that all

natural herbs have certain amount of antimicrobial effect over caries causing organism. [2]

In our study, we found that ayurvedic toothpaste with herbal extracts was effective against oral microbial flora causing intraoral disturbances like Amrita Parida et al also proved similar results in research on antimicrobial effects of Ocimum Sanctum on oral microbes & concluded that Tulsi (Ocimum Sanctum) acts as anti-inflammatory agent and also has antimicrobial property against Prevotella intermedia, Streptococcus mutans, Candida albicans, Lactobacillus acidophilus, Streptococcus mitis & Peptostreptococcus etc. [9]

Conclusion

There was no significant difference found between antimicrobial property of other marketed toothpaste and newly formulated Ayurvedic toothpaste. Hence, it can be concluded that purely ayurvedic toothpaste is as effective as other commercially available toothpaste with herbal extracts, thereby producing a cheaper & healthier option to people.

Clinical Applicability

The ayurvedic toothpaste was found to be more effective in eliminating C. albicans as compared to S. mutans and L. acidophilus. Hence we would like to propose the usage of this toothpaste in medically compromised individuals who have a higher tendency for developing opportunistic infections.

Abbreviations

S.mutans - Streptococcus mutans, L.acidophilus - Lactobacillus acidophilus, C.albicans – Candida albicans

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