

# Antifungal and antibacterial activities of polyherbal toothpaste against oral pathogens, *in vitro*

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## Article Info

**Article type:**  
Original article

## Article History:

Received: 23 April 2018

Revised: 20 July 2018

Accepted: 06 August 2018

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## ABSTRACT

**Background and Purpose:** Herbal toothpastes are more secure and efficacious and less poisonous due to containing natural chemicals as compared with the synthetic toothpastes. The present study aimed to formulate a polyherbal toothpaste using accessible medicinal plants in Iran and evaluate its efficiency in the protection of oral hygiene and prevention of dental caries.

**Materials and Methods:** The developed toothpaste was made of the leaf extracts of *Artemisia dracuncululus*, *Satureja khuzestanica* (Jamzad), and *Myrtus communis* (Linn), combined at four different dilutions, namely 1:4 (25%), 1:1 (50%), 3:4 (75%), and (100%), with sterile distilled water. The product was tested against five microorganisms, including *Streptococcus mutans*, *Lactobacillus casei*, *S. sanguis*, *S. salivarius*, and *Candida albicans*, using agar well diffusion method.

**Results:** After 24 h of incubation, the maximum mean diameters of inhibition zone against *L. casei* and *C. albicans* were obtained as 17-30 and 10-25 mm, respectively. Furthermore, the minimum mean diameter of inhibition zone against *S. salivarius* was estimated as 15-20 mm.

**Conclusion:** The formulated toothpaste showed potent inhibitory activities against Gram-positive bacteria and *C. albicans*. Therefore, more studies are required to accurately investigate the efficacy of the formulated toothpaste.

**Keywords:** Antibacterial, Antifungal, Oral pathogens, Polyherbal toothpaste, Yeast

## ➤ How to cite this paper

Sadeghi-Nejad B, Moghimipour E, Yusef Naanaie S, Nezarat S. Antifungal and antibacterial activities of polyherbal toothpaste against oral pathogens, *in vitro*. *Curr Med Mycol*. 2018; 4(2): 21-26. DOI: [10.18502/cmm.4.2.65](https://doi.org/10.18502/cmm.4.2.65)

## Introduction

One of the most common chronic oral infections in the world is dental caries [1]. Oral pathogenic microorganisms have been the cause of dental plaques, dental caries, as well as gingival and periodontal diseases [2]. *Streptococcus mutans* is one of the main opportunistic pathogens of dental caries, which is responsible for the formation of dental plaque and caries [3]. Other microorganisms associated with this oral condition include *Escherichia coli*, *S. aureus* [4], and *Candida* species.

*C. albicans* is the most frequent yeast isolated from the oral cavities with poor oral hygiene [2]. The formulation ingredients of commercial toothpastes are mostly chemical substances, such as fluoride and whitening agents [2]. The literature contains evidence regarding the adverse effects of fluoride and bleaching agents (e.g., peroxide-based agents) used in the commercial toothpastes [5].

With this background in mind, the present study aimed to formulate a polyherbal toothpaste without any

fluoride or whitening agents and evaluate its antimicrobial properties. The main ingredients of this toothpaste included the leaf extracts of *Artemisia dracuncululus* L. (ADL), *Satureja khuzestanica* (Jamzad; SKJ), and *Myrtus communis* (Linn; MCL). The MCL or myrtle, belonging to the Myrtaceae (Lamiaceae) family, is an aromatic evergreen small tree with small foliage and numerous branches [6]. In ancient medicinal herbs, myrtle leaves and flowers were used for the treatment of respiratory problems, dysentery, urinary tract infections, and candidiasis as a mouthwash [7].

According to the literature, the essential oil of MCL has pharmacological activities, including antioxidant [8], antimicrobial [9, 10], and antifungal activities [11, 12]. There is evidence regarding the inhibitory activity of the essential oil of MCL against clinically isolated oral pathogenic microorganisms [13]. *Artemisia* is a small and continual aromatic shrub from the Asteraceae family [14], which is called "Tarkhon" in Iran. In traditional medicine, this plant is used for the

remedy of stomach pains, fever, and diabetes and is known to have anti-inflammatory, anti-parasitic, antioxidant, and antimicrobial activities [15].

On the other hand, SKJ, belonging to the Lamiaceae family, is extensively grown in the northern Khuzestan and southern Lorestan provinces of Iran [16]. This plant has traditionally been used for relieving tooth pain, strengthening the gum, and healing the wound in the southern part of Iran [17]. Moreover, this herb has been applied for antimicrobial [18] and antifungal [19] purposes, as well as the treatment of infectious diseases [20-21]. With regard to the previous studies reporting the antimicrobial properties of polyherbal toothpaste [22, 23], the current study was conducted to formulate a new polyherbal toothpaste containing the aqueous herbal extracts available in Iran and evaluate its antimicrobial potency against oral pathogens.

## Materials and Methods

### Preparation of extracts

The ADL was purchased from the local market, and MCL and SKJ were prepared with the aids of the Agricultural and Natural Resources Center, Ahvaz, Iran. The collected plants were air dried in shade. About 10 g of each powdered air dried plant was added to 100 ml sterile distilled water (1:1 W/V) in a glass beaker for maceration, and then incubated on a rotary shaker for 72 h [24]. In the next step, the filtration of suspension was accomplished using Whatman filter paper No.1. The filtrated aqueous extracts were evaporated and dried at the room temperature. The extracts were stored in air-tight containers at -20°C until future use.

### Microorganisms and inoculum preparation

The antimicrobial activity of four anaerobic bacteria (Gram-positive) isolated from clinical isolates and *Candida* species was assessed. A total of twelve anaerobic bacteria, including *S. sanguis*, *S. salivarius*, *S. mutans*, and *L. casei*, were prepared from the frozen stock cultures obtained from the Department of Medical Microbiology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. In addition, five *C. albicans* strains were isolated from patients with periodontitis and gingivitis referring to the Educational

Clinics of Dentistry School, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

These samples were subcultured, and then diluted in a sterile normal saline solution (0.9%) to obtain a concentration of  $5 \times 10^5$  CFU/ml for fungal strains and a colony forming unit of  $10^6$  (CFU/ml) for bacterial strains, adjusted with the turbidity of 0.5 McFarland [25, 26].

### Formulation of polyherbal toothpaste

The ingredients of the fluoride-free polyherbal toothpaste was prepared according to the procedure adopted by Sekar and Zuhilmi Abdullah [22] with some modifications (Table 1).

### Screening polyherbal toothpaste for antimicrobial activity

The formulation of the toothpaste was accomplished using three plants, namely ADL, MDL, and SKL, which were previously confirmed to have antimicrobial activities by in vitro assays. The dentifrice solution was prepared according to the previously reported procedures [2, 27].

The solution was tested against five microorganisms, including *S. mutans*, *L. caseie*, *S. sanguis*, *S. salivarius*, and *C. albicans*, by using agar well diffusion method following the previous studies [28, 29]. The agar plates inoculated with bacteria were kept in an anaerobic cabinet supplied with CO<sub>2</sub> at 37°C for 24, 48, and 72 h. On the other hand, those agar plates inoculated with *C. albicans* were incubated at 30°C for 48 h [30].

### Statistical analysis

Statistical analysis was performed in SPSS software (version 20.0). The mean diameters of the inhibition zones were calculated. P-value less than 0.05 was considered statistically significant.

### Quality parameters of formulated polyherbal toothpaste

The organoleptic investigation of polyherbal toothpaste, including color, taste, odor, and texture, were carried out by sensational and visual surveys according to the modified procedure of Sekar and Zuhilmi Abdullah [22].

**Table 1.** Ingredients of the formulated polyherbal toothpaste

Components	Amounts g/%	Property
<i>A. dracunculus</i> leaf extract	0.0625	Active ingredient
<i>S. khuzestanica</i> leaf extract	0.0625	Active ingredient
<i>M. communis</i> leaf extract	0.0625	Active ingredient
Hydroxypropyl methyl cellulose	3	Gelling agent
Sodium lauryl sulphate	5	Surfactant
Calcium carbonate	25	Abrasive
Glycerin	5	Anti-crusting agent
Methyl paraben	0.5	Preservative
Propyl paraben	0.25	Preservative
Sodium saccharine	0.3125	Sweetener
Peppermint oil	0.75 (2-3 drops)	Flavoring agent
Demineralized water	60.5	Vehicle
Total	100 ml	

## Results

Table 2 summarized the inhibition zones produced by four polyherbal toothpaste dilutions of full strength, 3:4, 1:1, and 1:4 against *S. mutans*, *S. salivarius*, *S. sanguis*, *L. casei*, and *C. albicans*. The mean values of the microbial inhibition zones are shown in Table 2. The results demonstrated that *L. casei* showed the highest sensitivity against the dilutions of polyherbal toothpaste ranging from 5-30 mm in 24 h, followed by *C. albicans* (10-25 mm), *S. mutans* (5-25 mm), *S. salivarius* (10-25 mm), and *S. sanguis* (5-20 mm) at different dilutions of the toothpaste (figures 1 and 2; Table 2). All dilutions of polyherbal toothpaste were effective in inhibiting the

growth of the tested bacterium and fungus; however, they had no inhibitory effect on *L. casei*, *S. sanguis*, and *S. mutans* at the dilution of 1:4.

The polyherbal toothpaste showed a significant antimicrobial activity against all tested bacterium and yeast ( $P < 0.001$ ). Table 3 tabulates the results of one-sample t-test for each dilution of toothpaste against all the tested strains. The comparison of antimicrobial activities against tested microorganism in different concentrations is demonstrated in Table 4.

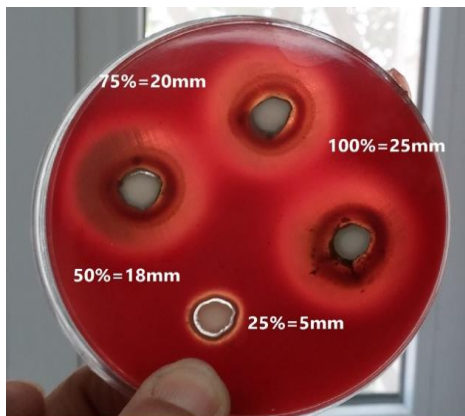
Table 5 exhibits the results of the physicochemical parameters of the formulated toothpaste.

**Table 2.** Antimicrobial activity of polyherbal toothpaste against five dental caries pathogens by agar well diffusion method

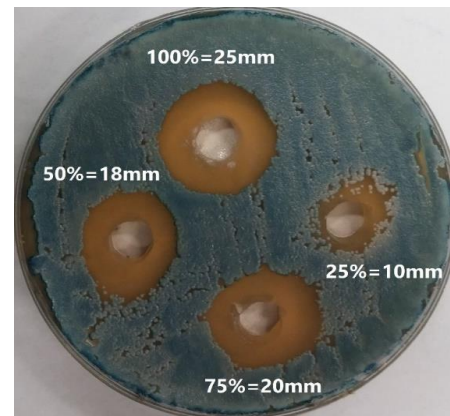
Dilutions of pt	Mean diameter of growth inhibition zones (mm)				
	<i>S. salivarius</i>	<i>S. sanguis</i>	<i>S. mutans</i>	<i>L. casei</i>	<i>C. albicans</i>
1:4 (25%)	<sup>a</sup> 12.33±2.51	3.33±1.52	3.33±2.08	4.00±1.00	7.33±2.51
1:1 (50%)	18.33±1.52	12.33±2.51	15.00±3.00	17.33±2.51	15.00±3.00
3:4 (75%)	19.00±3.60	15.00±3.00	17.66±2.51	24.00±3.60	17.33±2.51
FS (100%)	20.66±4.04	17.66±20.51	21.00±3.60	25.00±5.00	20.00±5.00

PT: polyherbal toothpaste, FS: full strength

<sup>a</sup>Values including the diameter of the well (7 mm) are means of three replicates.



**Figure 1.** Inhibition zones increased by polyherbal toothpaste against *Streptococcus mutans* on blood agar at four different dilutions



**Figure 2.** Inhibition zones increased by polyherbal toothpaste against *Candida albicans* on Sabouraud dextrose agar at four different dilutions

**Table 3.** Results of one-sample t-test for each dilution of toothpaste against all tested strains

Dilution of polyherbal toothpaste	Mean±SD	Significant (2-tailed)
1:4 (25%)	6.06±3.97	$P < 0.05$ 0.001
1:1 (50%)	15.60±3.06	$P < 0.05$ 0.001
3:4 (75%)	18.60±4.04	$P < 0.05$ 0.001
FS (100%)	20.86±4.27	$P < 0.05$ 0.001

Significance level:  $< 0.05$ , FS: full strength

**Table 4.** Comparison of antimicrobial activity against tested microorganisms in different concentrations

Dilution of polyherbal toothpaste	Mean±SD	Significant (2-tailed)
Pair 1 Microbs-PMT 1:4 (25%)	3.66±4.48	$P < 0.05$ 0.007
Pair 2 Microbs-PMT 1:1 (50%)	13.20±3.46	$P < 0.050$ 0.001
Pair 3 Microbs-PMT 3:4 (75%)	16.20±4.12	$P < 0.050$ 0.001
Pair 4 Microbs-PMT FS (100%)	18.46 ±4.32	$P < 0.050$ 0.001

Significance level:  $< 0.05$ , FS: full strength

**Table 5.** Organoleptic investigation of the formulated polyherbal toothpaste

Parameter	Result
Color	Bright green
Taste	Slightly Bitter
Odor	Mint
Texture	Smooth
Moisture content	39%
Foaming identity	23 ml
pH	8
Storage constancy	After 45 days, no observation was made regarding the separation of the liquid and solid phases of the toothpaste.

## Discussion

A good oral hygiene maintenance is the key to prevent oral diseases. The novelty of the herbal toothpaste developed in the current study owes to its natural compounds as aqueous extract. Therefore, it can be claimed that this toothpaste can be used as a completely natural product without the complications of the commercial products. The results revealed that our developed toothpaste had different degrees of effectiveness against the five tested microorganisms. In this regard, the formulated toothpaste exerted a highly significant effect against all tested oral pathogens.

As the results indicated, *L. casei* and *S. sanguis* had the highest and lowest sensitivities to the formulated polyherbal toothpastes, exhibiting inhibition zones of 17-30 and 15-20 mm, respectively. In a clinical study, a commercially available toothpaste was reported to have inhibitory effects on some pathogenic oral microorganisms, such as *S. mutans*, *Micrococcus* species, *Proteus vulgaris*, *S. aureus*, and *C. albicans* [29].

There are a number of polyherbal toothpastes playing an important role in dental prophylaxis and improvement of oral health [31]. In a study conducted by Sekar and Zulhilmi Abdullah, *S. aureus* was reported as the most sensitive species to polyherbal toothpaste with an inhibition zone of 10-15 mm, followed by *E. coli* (9-12 mm), *Bacillus cereus* (7-12 mm), and *Pseudomonas aeruginosa* (9-11 mm) [22]. In another study, the polyherbal dentifrices containing Neem, Pudina, Long, Babul, Turmeric, and Vajradanti showed significant antimicrobial activities against *E. coli*, *S. aureus*, *S. mutans*, and *C. albicans* [23].

In a study conducted by Sharma et al. (2014), the methanolic extract of polyherbal formulation showed the highest and lowest activities against *S. mutans* and *C. albicans*, respectively [32]. In the present study, the formulated toothpaste demonstrated a good activity against *C. albicans* in various concentrations (i.e., 100%, 75%, 50%, and 25%) and exhibited a strong activity against *L. casei*, *S. mutans*, *S. sanguis*, and *S. salivarius* in 100%, 75%, 50% concentrations.

Moreover, the presence of carvacrol (2-methyl-5-[1-methylethyl]-phenol) (96.9%) as the main component of *S. khuzestanica* essential oils (SKEO) may be responsible for the antimicrobial properties of the present toothpaste. It was also reported that the SKEO has remarkable antibacterial effects, particularly against the resistant *S. aureus* [33]. In another study, *S. khuzestanica* leaf extract was reported to show a significant antibacterial activity against *S. mutans* in the range of 300-500 µg/ml [34].

Zomorodian et al. investigated the antimicrobial activity of seven essential oils against the common species accounting for oral infections, such as *S. mutans*, *S. sanguis*, *S. salivarius*, *E. faecalis*, *S. aureus*, *C. albicans*, *C. glabrata*, *C. dubliniensis*, and *C. krusei*. The results of the mentioned study revealed that SKJ and *A. sieberi* were active against all tested oral

pathogens. In addition, in the mentioned study, SKJ showed the highest antimicrobial activities, while *A. sieberi* exhibited the lowest antimicrobial activity [35].

The consistency between the results of the aforementioned study and those of our research can be due to the utilization two plants used in the mentioned research for the formulation of our polyherbal toothpaste. Moreover, there is evidence confirming the antimicrobial effects of the essential oil of MCL on clinically isolated oral pathogens. All isolates were sensitive to MCL at 125-1000 µg/ml by agar disk diffusion with inhibition zones of 8.1-41.25 mm in diameter. Furthermore, all *S. pyogenes*, *S. mutans*, and *C. albicans* strains showed sensitivity at 62.5 µg/ml.

The *S. mutans*, as the main etiological agent of dental caries, has been recorded as the second most sensitive pathogen to MCL (21). Previous studies have also reported that the main compounds of the essential oil of MCL are terpenoid compounds (i.e., 1,8- cineole, α-pinene, myrtenyl acetate, limonene, linalool, and α-terpinolene), flavonoids and tannin that are responsible for the inhibitory effects of the MCL in the formulated toothpaste [36].

In addition, Sharafati Chaleshtori demonstrated that the essential oil of ADL has an antibacterial activity against *S. aureus*, *Alcaligenes faecalis*, *Providencia rettgeri*, *Serratia marcescens*, *Shigella dysenteriae*, *Listeria monocytogenes*, and *Klebsiella oxytoca* [37]. Based on these observations, the efficiency of polyherbal toothpaste can be ascribed to the presence of variant phytochemical compounds in plant extracts.

The novelty of the present study is the use of the aqueous extracts of selected plants instead of chemical solvents, such as acetone, ethanol, and methanol. Moreover, the extracts utilized in our polyherbal toothpaste had no side effects as confirmed by an *in vivo* study previously performed by the authors of the current study.

## Conclusion

Further studies are recommended to make it as one of the commercial herbal toothpaste for the treatment of oral microbial infections.

## Acknowledgments

The authors are grateful to the Department of Medical Microbiology, Ahvaz Jundishapur University of Medical Sciences for providing bacterial samples. The current research protocol was supported by Abadan School of Medical Sciences, Ethics Committee of Iran with a code of 93 U-020 and reference ID of IRCT2015112825240N2.

## Author's contribution

B. S.: study design, microbiological study, data collection, writing and editing the article, and study supervision. E. M.: participation in the design of the formulated herbal toothpaste. S.Y. N.: preparation and

authentication of the selected herbal medicine. S. N.: participation in the collection of specimens.

### Conflicts of interest

None declared.

### Financial disclosure

The authors declare no financial interests related to the materials of this study.

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