

# Evaluation of the effect of Jaftex herbal mouthwash on the growth of *Candida albicans* and *Candida tropicalis*

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## ABSTRACT

**Background:** Increased resistance of *Candida albicans* to standard antifungal agents has caused special attention to medicinal plants. The aim of this study was to evaluate the effect of Jaftex mouthwash on the growth of *C. albicans* and *Candida tropicalis*. **Methods and Material:** In this *in vitro* study, standard strains of *C. albicans* and *C. tropicalis* were used. Jaftex mouthwash was prepared with the active ingredient (10g/100cc) of aqueous extract of oak fruit hull (Jaft), *Zataria multiflora*, and *Satureja bachtiarica*. The mouthwash was diluted in half, 8 different concentrations were obtained. 10 µl volume of each dilution was poured on discs mounted linearly on the culture medium inoculated with the target fungus. After 24 h, due to the slow growth rate of these fungi, the Petri dishes were incubated at 37°C and the mean minimum inhibitory concentration (MIC) was determined for each fungus. The modified E. test method was used to measure the MIC of Jaftex mouthwash for the two fungi. The experiment was repeated three times for each fungus and the mean value was measured. **Results:** The mean value of MIC for *C. albicans* and *C. tropicalis* was 0.0625 (mg/mL) and 0.0833 (mg/mL), respectively. *Candida albicans* appeared to be more sensitive to Jaftex, but no statistically significant difference was observed. **Conclusion:** Jaftex mouthwash inhibits the growth of *C. albicans* and *C. tropicalis*. The use of this mouthwash is recommended for treatment of oral candidiasis.

**Keywords:** *Candida albicans*, mouthwash, multifloral, oak tree, satureja

## Introduction

Oral candidiasis is a fungal infection caused by pathogens and commonly affects the oral mucosa.<sup>[1]</sup> The incidence of fungal infections has been increased over the past decades.<sup>[2]</sup> *Candida albicans* species coexist with 75% of healthy individuals.<sup>[3]</sup> Physiological defense mechanism and immune system of healthy individual can control and prevent the growth and spread of *C. albicans*.<sup>[4]</sup> However, predisposing factors such as poor oral hygiene, endocrine diseases, weakened immune system, poorly

adapted dentures, and dry mouth increase the incidence of oral candidiasis.<sup>[5]</sup> *Candida albicans* is the most common pathogen isolated from the human oral cavity and has the highest virulence in humans followed by *Candida tropicalis*.<sup>[4,6]</sup> *Candida albicans* colonization has been found in various surfaces of the oral cavity including palate, tongue, and dental caries and plaque.<sup>[7]</sup> Dental prostheses are more prone to the growth of candidiasis. *Candida tropicalis* has been found in these colonies and has shown resistance to clinical antifungal agents.<sup>[8]</sup> In recent years, *C. tropicalis* has attracted widespread attention due to increased prevalence and elevated mortality.<sup>[9]</sup>

Mouthwashes are the most commonly used solutions used in dentistry to prevent and control *C. albicans* infection.<sup>[10]</sup> Mouthwashes contain water and some active ingredients such as antibiotics and antifungals and anti-inflammatory components.<sup>[11]</sup>

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The increased resistance of *C. albicans* to standard antifungal drugs has led to special attention to medicinal plants as natural compounds.<sup>[12]</sup>

Medicinal plants play a vital role in the treatment of diseases due to their antimicrobial and antifungal activity against human pathogens. Herbal mouthwashes possess antibacterial activity against oral pathogens and also alleviate the pain without systemic side effects.<sup>[13]</sup>

Jaftex is a novel herbal mouthwash that has been prepared in the Medicinal Plants Research Center of Ahvaz Jundishapur University of Medical Sciences (AJUMS). The antibacterial effects of this mouthwash have been proven in the previous studies.<sup>[14,15]</sup> Jaftex mouthwash is a combination of aqueous extract of oak fruit hull (Jaft) as a base and aqueous extract of *Zataria multiflora* and *Satureja bachtiarica*.<sup>[14]</sup> The inner layer of the oak is called Jaft and has medicinal and industrial applications. Jaft is recommended for treatment of microbial and viral diseases.<sup>[14]</sup> Zomordian *et al.*<sup>[16]</sup> showed that *Zataria multiflora* and *Satureja bachtiarica* inhibited the growth of *C. albicans* and *C. tropicalis*. The aim of the present research was to evaluate the antifungal effect of Jaftex mouthwash on the growth of *C. albicans* and *C. tropicalis*.

## Materials and Methods

### Ethical considerations

This study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (Ethics ID: IR. AJUMS.REC. 1398.884).

### Strains and culture conditions

This *in vitro* study was conducted in the Microbiology Laboratory, Department of Microbiology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, during October to December 2019.

Standard strains of *C. albicans* (PTCC: 5027) and *C. tropicalis* (PTCC: 1643) were prepared as lyophilized ampoules from the collection center of fungi and bacteria of Pasteur Institute (Tehran, Iran). A volume of 2 cc of Sabouraud Dextrose Agar (*SDA*) culture medium (Merck, Germany) was transferred to lyophilized vials and mixed, accordingly. Some of this microbial suspension was then transferred to *SDA* culture medium (Merck, Germany) and placed at 37°C for 24 h to be activated (incubated). The grown colonies were then cultured and stored in the freezer and stored at -70°C for the next experiment. After 24 h of incubation, 0.5 McFarland standard fungal suspension ( $1.5 \times 10^8$  CFU/mL) was prepared from the fungal culture medium.

### Preparation of mouthwash

Jaftex mouthwash (IR Patent No: 139350140003008118) was prepared in the Medicinal Plants Research Center of AJUMS. The active ingredients of Jaftex mouthwash included a total of

10 gr of Jaft, *Satureja bachtiarica*, and *Zataria multiflora*. The extraction process of each of the plants was as follows.

Jaft was washed with distilled water and dried at laboratory temperature and powdered using powder grinder machine. The prepared powder was poured into a double-layer bag. The bag was placed in a glass Erlenmeyer flask contained 150 mL of distilled boiling water. The solution was shaken at low speed for 24 h at laboratory temperature. After filtering the extract with a double-layer cloth, the solution was passed through Whatman® Grade 1 filter paper (Merck, Germany) and centrifuged for 10 min at 2400 rpm. The resulting clear liquid was then stored at 4°C in a closed dark container.

Similarly, *Satureja bachtiarica* and *Zataria multiflora* extracts were prepared as Jaft extraction method. In the final step, the aqueous extracts of three plants were mixed with each other and the volume of prepared solution was adjusted to 100 mL by distilled water and stored in the refrigerator.

Jaftex mouthwash was serially diluted using two-fold dilution in sterile test tubes. Eight standard sterilized test tubes were numbered. Then, 1 mL of distilled water was poured into tubes No. 2 to 8 using a sampler. In the next step, 2 mL of mouthwash was poured into tube No. 1 and 1 mL was taken from tube NO.1 and poured into tube No. 2; then, the tube was shaken and 1 mL was taken from the tube No. 2 and poured into tube No. 3 and these processes continued until the original concentration reduced by one half. To perform this test, the target fungus was cultured in the culture medium and immediately 8 blank discs were placed in a straight line from top to bottom on the medium inside the petri dish. A volume of 10 µl of each dilution was taken and poured onto discs that placed linearly on the culture medium inoculated with the target fungus. Disc No. 8 was a blank disc that was placed as a negative control on the culture medium and contained distilled water solvent. The Petri dishes were kept in an incubator at 37°C for 24 h.

### Minimum inhibitory concentration (MIC) measurement

The modified E-test method was used to determine the MIC of Jaftex mouthwash. In the modified E test, several *AB BIODISKS* infused with different dilutions of the mouthwash are used instead of strips. In fact, modified E test is a simulated version of the standard E- test.<sup>[17]</sup> The MIC values were determined at the points where dense colonial growth traversed the disc. The test was performed three times for each culture.<sup>[18]</sup> Equivalent concentrations were calculated for each disc, accordingly.

## Results

In this study, to determine the MIC of Jaftex mouthwash, eight concentrations of this mouthwash against *C. albicans* and *C. tropicalis* were investigated. Equivalent concentration was calculated for each disc. The amount of substance in 10 microliters of solution was reported as MIC in milligrams per

milliliter. The results for *C. albicans* and *C. tropicalis* after three replications are reported in Table 1. The mean MIC results of Jaftex mouthwash in both groups are shown in Table 2. The mean value of MIC for *C. albicans* and *C. tropicalis* was 0.0625 (mg/mL) and 0.0833 (mg/mL), respectively [Table 2].

Mann-Whitney test was used to compare the mean MIC value of the two fungi. The mean MIC value for *C. albicans* was lower than *C. tropicalis*, i.e. *C. albicans* was more sensitive to Jaftex compared to *C. tropicalis*, but no significant difference was observed between the two groups [Figure 2]. The antifungal activity of Jaftex mouthwash against *C. albicans* and *C. tropicalis* using modified E-test are presented in Figures 1 and 2.

### Discussion

In recent years, *Candida* species infection have been augmented, dramatically.<sup>[19]</sup> *Candida albicans* is the most important cause of oral candidiasis. However, other species including *C. tropicalis*, may be involved in the development of symptomatic oral candidiasis in HIV-positive individuals.<sup>[20]</sup>

In recent years, herbal agents have been widely used in oral care products. There is a lot of clinical evidence of the use of herbal products in mouthwashes.<sup>[21]</sup> The aim of this study was

to investigate the antifungal effect of Jaftex herbal mouthwash on the growth of *C. albicans* and *C. tropicalis*. Moreover, the minimum concentration required by Jaftex mouthwash to inhibit the growth of these two fungi by modified E-test was specified. The findings of this study showed the potential antifungal activity of Jaftex mouthwash against the growth of standard strains of *C. albicans* and *C. tropicalis*. In this study, the mean value of MIC for *C. albicans* and *C. tropicalis* were 0.0625 and 0.0833 mg/mL, respectively. *Candida albicans* was more sensitive to Jaftex, but no statistically significant difference was observed.

Since the present study was the first study examined the antifungal effect of this herbal mouthwash, no prior studies regarding the antifungal effect of Jaftex mouthwash were found to be compared in the literature; however, its application as an antibacterial mouthwash has been examined in the previous studies.<sup>[14,15]</sup> In a study, the effect of Jaftex mouthwash on the number of microorganisms in the saliva of dental students was investigated. The results of the research showed that this mouthwash significantly reduced the salivary microorganisms. Jaftex mouthwash showed less antimicrobial activity compared to chlorhexidine, but the difference was not statistically significant.<sup>[15]</sup>

Babadi et al.<sup>[22]</sup> reported that Jaftex mouthwash was more effective in inhibiting the growth of oral bacteria than Matrica and Persica

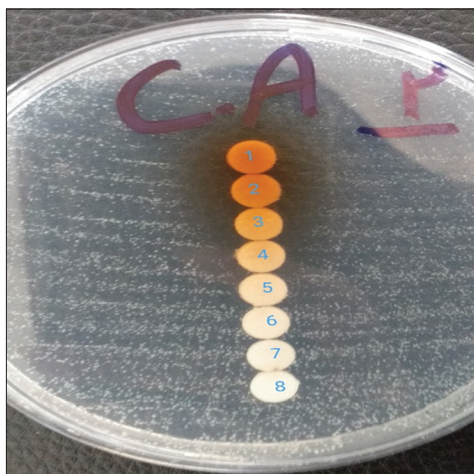


Figure 1: Antifungal activity of Jaftex mouthwash against *Candida albicans*



Figure 2: Antifungal activity of Jaftex mouthwash against *Candida tropicalis*

Table 1: Results of the effect of Jaftex mouthwash on *Candida albicans* and *Candida tropicalis* after three repetitions by modified E-test method

Groups	Test	MIC mg/ml							
		1	0.5	0.75	0.125	0.0625	0.03125	0.015625	0
		Disc 1	Disc 2	Disc 3	Disc 4	Disc 5	Disc 6	Disc 7	Disc 8
<i>Candida albicans</i>	First	*-	-	-	-	*+	+	+	+
	Second	-	-	-	-	+	+	+	+
	Third	-	-	-	-	+	+	+	+
<i>Candida tropicalis</i>	First	-	-	-	-	+	+	+	+
	Second	-	-	-	+	+	+	+	+
	Third	-	-	-	-	+	+	+	+

\* (-) no fungal growth, (+) fungal growth

**Table 2: Mean and standard deviation of MIC of Jaftex herbal mouthwash against *Candida albicans* and *Candida tropicalis* by modified E-test**

Groups	n	Mean±SD of MIC	Test statistics	P
<i>Candida albicans</i>	3	0.0625±0.0000	3.000	0.317
<i>Candida tropicalis</i>	3	0.0833±0.0208		

herbal mouthwashes. It is noteworthy that, Jaftex mouthwash is a combination of oak Jaft, Satureja bachtiarica, and Zataria multiflora.<sup>[14]</sup>

The antifungal activity of the Jaft on the growth of *C. albicans* and *C. tropicalis* has not been studied so far. Sharifi *et al.*<sup>[23]</sup> reported that the hydroalcoholic extract of the Jaft inhibited the growth of Saprolegnia fungus. The results of the study showed that tannins, which are more found in the Jaft extract, had antifungal properties. Furthermore, tannins showed to be toxic to yeasts and filamentous fungi and had the potential to prevent their growth by several mechanisms, including deposition of microbial proteins and keeping food proteins out of the reach of microbes.

The antifungal activity of Satureja bachtiarica and Zataria multiflora against Candida growth has been investigated in several studies and their results are consistent with the results of the present study.<sup>[16-20]</sup> Zomorodian *et al.*<sup>[16]</sup> examined the antibacterial activity of oily extracts of seven medicinal plants, including Zataria multiflora and satureja against several common oral microorganisms, including *C. albicans* and *C. tropicalis*. The results of the study showed the highest antibacterial activity of Satureja khuzestanica, Zataria multiflora, and Satureja bachtiarica, respectively. The reason could be explained due to phenolic major or alcoholic monoterpenes. Pirbalouti *et al.*<sup>[20]</sup> investigated the antifungal effect of several plant extracts, including Satureja bachtiarica against the growth of *C. albicans* isolated from the vagina using disc diffusion method. The results of this study indicated that Satureja bachtiarica oil extract had stronger antifungal effect compared to other plants. Satureja bachtiarica contains a large amount of phenol.

Naeini *et al.* in an *in vitro* study examined the effects of essential oils and extracts of 50 medicinal plants, including Zataria multiflora on the standard strain of *C. albicans* using disc diffusion method. They reported that Zataria multiflora essential oil and its extracts had a strong anti-candida effect and the essential oil was more active than those of the extract. In this study, Zataria multiflora was one of the plants that showed stronger anti-candida effects compared to nystatin, amphotericin B, and clotrimazole.<sup>[24]</sup> Gonoudi *et al.*<sup>[25]</sup> examined the antifungal efficacy of Nystatin and Zataria Multiflora in the reduction of *C. albicans* colony count and concluded that both agents showed similar efficacy and caused a significant reduction in *C. albicans* CFU.

Zataria multiflora and Satureja khuzestanica are rich in phenolic monoterpenes, which include carvacrol and thymol.<sup>[26]</sup> Thymol and carvacrol are chemically isomers.<sup>[27]</sup> Carvacrol is one of

the main constituents of Zataria multiflora. Carvacrol is an isothymol that prevents the activity of ATPase enzymes and increases the non-specific permeability of the cell membrane of microorganisms. Thus, the susceptibility of microorganisms to the entry site will be increased.<sup>[28]</sup> Thymol is a topical disinfectant. This compound has antifungal properties.<sup>[29]</sup>

It is recommended to compare the antifungal effect of this mouthwash with chemical drugs such as Nystatin mouthwash, azoles, and herbal mouthwashes.

## Conclusion

Jaftex mouthwash inhibits the growth of *C. albicans* and *C. tropicalis*. The combination of the aqueous extracts of Jaft, Satureja bachtiarica, and Zataria multiflora in the form of mouthwash may increase the antifungal activity of these plants. The use of this mouthwash is recommended for the treatment of oral candidiasis.

## Key Messages and recommendations

The present study revealed the antifungal potential of Jaftex mouthwash against the growth of *C. albicans* and *C. tropicalis*. We recommend the use of this mouthwash for maintaining good oral hygiene.

## Acknowledgement

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## Authors' contribution

Mansour Amin: Methodology, Data curation, Formal analysis, Writing - original draft. Fatemeh Babadi: Methodology, Data curation, Formal analysis, Writing - original draft. Najme Baghipour: Methodology, Data curation, Formal analysis, Writing - original draft. Batool Sadeghi-nejad: Methodology, Writing - original draft.

## Ethical considerations

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## Conflicts of interest

There are no conflicts of interest.

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