



Preparation and Evaluation of a Herbal Mouthwash Containing Oak Husk of *Quercus brantii* and *Zataria multiflora*

Shohreh Alipour^{1,2}, Shadab Dehshahri^{3,*} and Afshin Afsari⁴

¹Department of Quality Control, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

²Pharmaceutical Science Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

³Department of Pharmacognosy, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

⁴Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran

*Corresponding author: Department of Pharmacognosy, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran. Tel/Fax: +98-7132305410, Email: dehshahri@sums.ac.ir

Received 2017 January 04; Revised 2017 May 16; Accepted 2017 July 02.

Abstract

Background: Considering increasing antibiotic resistance and periodontal diseases incidence, the need of a safe, effective, and economical alternative mouthwash seems necessary. Combination of Persian oak husk of *Quercus brantii* (Jaft) with astringent and antibacterial properties of its tannins and *Zataria multiflora* leaves with anti-bacterial activity related to its essential oil seems to be more effective.

Objectives: The aim of the present study was to prepare and evaluate a herbal mouthwash formulation containing the extract of Oak husk and essential oil of *Zataria multiflora*.

Methods: The essential oil of *Zataria multiflora* leaves was obtained by hydro-distillation. Oak husk powder of *Quercus brantii* was extracted by infusion and then the extract was freeze-dried. Determination of tannin amount in the extract was performed by the Folin-Denis method. The essential oil analysis was performed by GC/MS. Formulations were prepared at five different concentrations of tannin (0.1%, 0.2%, 0.5%, 1%, and 2%) with 0.15% v/v of essential oil in the base of polyethylene glycol 400 and propylene glycol. The selected formulations were subjected to screening against *Candida albicans*, *Lactobacillus acidophilus*, *Staphylococcus aureus*, and *Streptococcus mutans*, using standard protocol of cell viability in comparison to the Persica mouthwash. Stability tests, such as tannin assay and pH, were done on days zero and forty-five after preparation of the formulations.

Results and Conclusions: The best formulations with 0.2% and 0.5% of tannin, which were more stable and showed significantly more antibacterial effects than Persica mouthwash may be safe and effective for future clinical studies.

Keywords: Mouthwash, Thymol, *Zataria multiflora*, *Quercus brantii*

1. Background

The most prevalent oral infectious diseases, such as dental caries, periodontal inflammations, and gingivitis are produced by dental biofilm formation in the oral cavity (1). Dental plaque is a multifaceted biofilm that builds up on the surface of teeth, including more than 500 bacterial species. Moreover, it has recently been shown that oral microorganisms can cause other severe or chronic infectious diseases (2, 3). The value of mouth and teeth hygiene has been known from ancient times until now. It has been documented that Dioscorides, Greek physician, prepared a mouth wash mixture from the extract of plants, milk, oil or vinegar (4). Mouthwashes are oral solutions or liquids used to rinse the mouth in order to remove bacteria, to act as an astringent, to deodorize oral cavity, and for their ther-

apeutic effect by relieving infection or preventing dental caries so they are considered as one of the most effective and safe delivery systems to decrease oral microbes (2, 5). Chlorhexidin is a golden chemical antiplaque, however, it may cause side effects in long usage (6). To overcome the side effects of artificial chemical drugs, it is recommended to search for naturally occurring substances, such as plant extracts, which offer well-tolerated, delicate, and low cost drugs with lower side effects (6, 7). Various herbal extracts such as clove oil, green tea, *Aloe vera*, *Punica granatum*, and white oak bark have shown therapeutic effects in the oral cavity when used as mouthwashes (4, 7).

Quercus brantii from the Fagaceae family has many medical and traditional uses. It has shown antibacterial and anti-inflammatory activities. Tannin content of oak husk (Jaft) showed astringent properties and it is used tra-

ditionally for treatment of hemorrhoid, diarrhea, gastric ulcer, and inflammations (8, 9).

Zataria multiflora, locally named in Persian as Avishan-e-Shirazi, belongs to the Lamiaceae family. Essential oil of *Zataria multiflora* has shown anti-bacterial and antifungal effects (10). The most important compounds of essential oil of *Zataria multiflora* are thymol, carvacrol and paracimol (11, 12).

2. Objectives

In this study a stable formulation containing both oak husk extract and *Zataria multiflora* essential oil was designed and evaluated and according to the author's knowledge, this is the first report on such formulation. Preparing a formulation containing tannins from oak husk as astringent agents and the essential oil of *Zataria multiflora* with antibacterial activity, will introduce a combination, which is more effective and comfortable to administer in patients.

3. Methods

3.1. Material

Propylene glycol (PG), poly ethylene glycol 400 (PEG400), glycerin, and ethanol were purchased from Merck (Germany). Dried oak husk of *Quercus brantii* and *Zataria multiflora* were bought from a local store in Shiraz, Fars province, Iran. The plant samples were authenticated by an expert botanist and voucher specimens were preserved with the code PM 712 (*Zataria multiflora*) and PM 713 (*Quercus brantii*) at the herbarium of the faculty of pharmacy, Shiraz University of Medical Sciences.

3.2. Oak Husk Dried Extract

The Oak husk of *Quercus brantii* was powdered mechanically and 200 g of the 180 mesh powder was soaked in deionized water at 80°C to 90°C on the heater for about 10 minutes in order to infuse. The volume was adjusted to 1000 mL by distilled water. It was shaken for one hour in order to complete extraction. The extract was filtered and then freeze-dried.

3.3. Essential Oil Extraction

The essential oil was obtained by hydro-distillation of the fresh powdered leaves of *Zataria multiflora*, using a Clevenger-type apparatus for about four hour. The essential oil was stored in the freezer until use.

3.4. Tannin Colorimetric Assay

Tannin colorimetric assay was performed by the Follin-Denis method (13). Tannic acid was used as standard. To prepare different dilutions of standard tannic acid, 0 to 10 mL of tannic acid (0.1 mg/mL) was added to separate 100-mL volumetric flasks and then each volume was adjusted to 100 mL by 5 mL of Follin-Denis reagent, 10 mL of sodium carbonate solution (35% w/v) (14), and distilled water. Then, each sample was mixed and kept in the dark for 30 minutes.

3.5. Tannin Calibration Curve Validation

Different concentrations of standard tannic acid (8, 6, 5, 4, 3, and 2 µg/mL) was prepared from stock solution using a serial dilution. Absorbance levels were measured by ultra violet/visible (UV) spectrophotometer (Agilent, USA) at 760 nm. All concentrations were prepared on three different days. Each concentration was examined in triplicates to find the inter-day and intra-day variation. The mean data was used to prepare a calibration curve. Linearity, inter-day, and intra-day precision and accuracy were determined for the calibration curve.

3.6. Essential Oil Analysis

Gas chromatography-mass spectroscopy (GC/MS) analyses of the essential oil were carried out by an Agilent 7890A series gas chromatograph interfaced with an Agilent 7000 mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) in electron impact mode with an ionizing voltage of 70 eV. A silica DB1 column (30 m, 0.32 mm diameter, 0.25-mm film thickness) was used. The flow rate of carrier gas (helium) was 1.2 mL/minute. The injector temperature was 250°C. The oven temperature was increased at a rate of 3°C per minute from 60°C to 280°C and finally maintained for four minutes. The volatile compounds were identified by comparison of their retention indices, referring to compounds known from the literature database, and also by comparing their mass spectra fragmentation with the Wiley Library 7 and NIST data base (15). The yield of essential oil was determined.

3.7. Selection of Mouthwash Base

Different mixture of solvents shown in Table 1 were made as the base for formulations of mouthwash, according to previous studies (not published). To select a suitable base for the mouthwash, different bases with different percentages were examined (16). The formulations were tested for different parameters, such as transmittance percentage, turbidity state, and phase state.

Table 1. Different Composition of Base Formulation^a

	PG	PEG400	Glycerin	Ethanol	Water
B1	-	30	30	10	30
B2	-	40	45	5	10
B3	-	45	50	5	-
B4	30	30	30	10	-
B5	45	45	-	10	-
B6	-	30	30	-	40
B7	50	50	-	-	-

^aValues are expressed as percentage.

3.8. Preparation of Mouthwash Formulations

Five different formulations (F1-F5) with different tannin concentrations (0.1%, 0.2%, 0.5%, 1% and 2%) were prepared by adding 0.33, 0.66, 1.3, 2.6, and 5.3 g of dried extract of oak husk. Essential oil was added at fixed amount of 0.15% v/v (equal to 0.064% thymol) to all formulations, according to reported antiplaque concentration of thymol for bacterial enzymes suppression (15). The final volume was adjusted to 100 mL by selected base for better homogenization. Samples were sonicated for 10 minutes using a bath sonicator.

3.9. Quality Control Tests for Selected Formulations

Quality control tests, including mouthwash pH, tannin content percentage, and essential oil yield were done on days 0 and 45, after preparation of formulations (15).

3.10. Anti-Microbial Activity of Formulations

Microorganisms, including *Staphylococcus aureus* ATCC 29737, *Streptococcus mutans* ATCC 35668, *Lactobacillus acidophilus* ATCC 4356, and *Candida albicans* ATCC 10231 were obtained from Iran collection of yeast and bacteria center. Fresh cultures of microorganisms were grown in their appropriate media and then microbial suspensions with 0.5 McFarland concentrations were prepared in Mueller-Hinton broth for bacteria and Sabouraud dextrose broth for *C. albicans*. Then 50 μ L was added to each well in a 96-well microplate, separately, that contained 50 μ L of broth media and 50 μ L of each formulation (F2, F3) with different dilutions (1, 1:10 and 1:100). The microplate was incubated at 37°C for 18 hours. The microplate was placed in the incubator at 37°C for 18 hours. Finally, the turbidity of the sample and blank (contains 0.5 McFarland concentration of microbes) were measured at 600 nm by the Elisa-reader. Then, viability percentage of microbes was found by the below formula (17). Moreover, Persica mouthwash was used as the positive control.

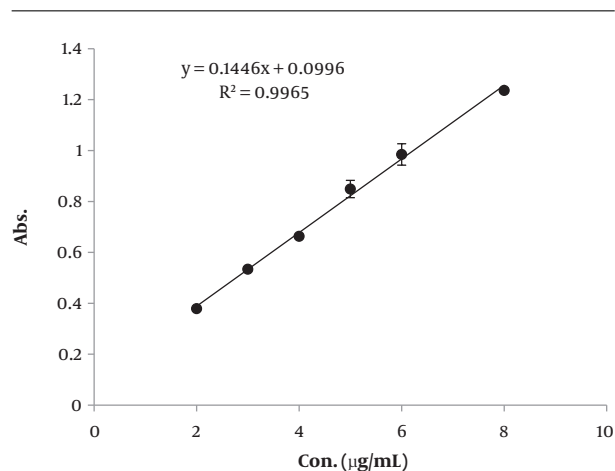


Figure 1. Calibration curve of tannic acid (n = 9)

$$\text{Cell viability\%} = \frac{\text{OD600 treated cell}}{\text{OD600 control cell}} \times 100 \quad (1)$$

3.11. Statistical Analysis

Statistical comparisons were done by a one-way analysis of variance (ANOVA) followed by Tukey's test. Differences were considered significant when $P < 0.05$.

4. Results

4.1. Tannin Colorimetric Assay

As mentioned in the methods, the colorimetric assay was used for tannin determination. Calibration curve validation is shown in Figure 1. The calibration curve equation and the linearity was as follows, absorbance = 0.1446 (concentration) + 0.0996 and 0.9965, respectively. Results indicated an average of $97.1 \pm 1.63\%$ and 98.8 ± 0.56 precision for all concentrations inter-daily and intra-daily. The average accuracy for all concentrations was $98.7 \pm 1.2\%$.

4.2. Tannin Determination in the Extract

The tannin concentration of extracted oak husk was determined using the analysis method and it was found to be $33.7 \pm 4.3\%$ of dry powdered extract (\approx equivalent to 337 mg/g dry extract).

4.3. Essential Oil Analysis

Identification of different components of *Zataria multiflora* volatile oil was done and their percentage was determined using their retention indices. The main constituents of *Z. multiflora* used in this study, were Thymol (44.23%), carvacrol (23.8%) and cymene (13.02%). The essential oil components were shown in Table 2.

The chromatogram of GC analysis of *Z. multiflora* is shown in Figure 2.

Table 2. Chemical Constituents of the Essential Oil of *Z. multiflora*

Compound	RT	KI	Percentage
α -pinene	6.552	938.3	1.54
Cymene	8.742	1018.4	13.02
1,8-cineole	9.013	1027.2	0.8
γ -Terpinene	9.905	1054.4	0.58
Linalool	11.077	1086.6	0.53
4-Terpineol	13.961	1165.3	0.53
α -Terpineol	14.367	1175.4	0.83
Thymyl methyl ether	16.053	1217.5	0.70
Carvacrol methyl ether	16.467	1228.3	0.87
Unknown	17.928	1264.3	0.62
Thymol	18.342	1274.06	44.23
Carvacrol	18.694	1282.1	23.8
Thymol acetate	20.488	1326.4	1.03
Durenol	21.218	1345.2	0.47
Trans-caryophyllene	23.994	1413.8	1.18
Valencene	24.761	1434.8	0.89
Ledene	26.834	1488.5	0.37
Unknown	29.493	1559.2	2.09
Longifolenaldehyde	29.700	1564.6	1.38
Unknown	30.575	1586.9	0.12
Unknown	31.566	1613.9	0.21
Unknown	32.288	1635.1	0.26
Unknown	32.738	1648.1	0.36
α -cyperone	35.372	1717.4	0.22
Unknown	40.617	1822.3	0.36
Unknown	41.455	1908.5	0.21
Total identified			92.97

4.4. Preparation of Mouthwash Formulations

As shown in Table 3, transmittance percentage, turbidity state, and phase state were reported for all formulations. Considering the absence of ethanol in the formulation, B7 was selected as the best base for preparing formulations. Final formulation ingredients are reported in Table 4.

4.5. Quality Control Tests for Selected Formulations

The formulations containing 1% and 2% tannin were excluded from the study because of precipitation after a few days. For other formulations, pH, tannin content, and essential oil yield were evaluated. pH and tannin content results are reported in Table 5. The yield percentage of the essential oil obtained from formulation F2 on day 0 and 45 was 90% and 86.6%, respectively.

4.6. Anti-Microbial Activity of Formulations

As indicated in Figures 3 - 7, viability percentage of four different microorganisms in presence of selected formulations compared with the control was calculated. According to usual mouthwashes dilution at the time of consumption, the antibacterial efficacy of two final selected formulations was examined at three different concentration (not diluted, 1:0 and 1:100 diluted). Formulation F1 was not entered in this test due to rejection in limit test experiments (data not shown).

5. Discussion

Oral hygiene is an important aspect of periodontal health. There is a balance in a person's oral microbial population. It is necessary to maintain this balance to prevent opportunistic microorganism proliferation (15). An optimum mouthwash has some advantages, such as having antiseptic effects on the mouth, washing the food residue on the gingival (gum) medium and teeth, reducing the mouth bacteria, masking and neutralizing halitosis, and introducing a good taste and sense of freshness in the mouth.

It has been shown that herbal mouthwashes do not cause change in the color of teeth or unpleasant taste. Reducing the plaque accumulation and gingival inflammation are other advantage of herbal mouthwashes in comparison to chlorhexidine mouthwash. Because of their minimum side effects, herbal mouth washes can be recommended for long term use (18). On the other hand, presence of ethanol in commercial mouthwashes seems to be problematic due to different reported side effects, such as xerostomia and oral and pharyngeal cancer induction

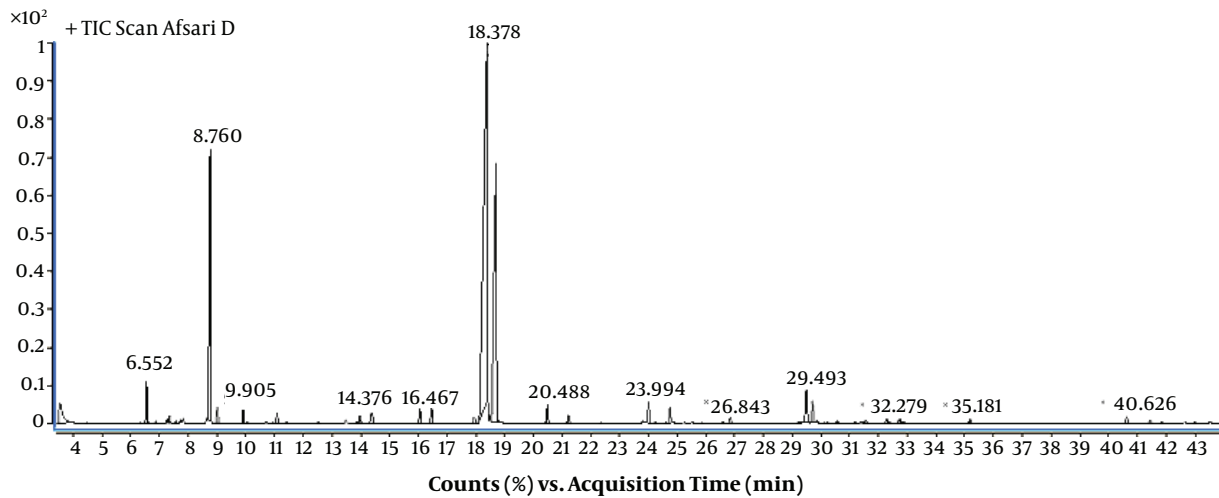


Figure 2. The chromatogram of GC analysis of *Zataria multiflora* volatile oil

Table 3. Different Quality Control Tests for the Base Formulations

Base	Transmittance, %	Turbidity State	Phase State	Ethanol Presence
B1	89.6	A little opaque	One-phase	+
B2	24.9	Opaque	One-phase	+
B3	91.2	Clear	Two-phase	+
B4	98.8	Clear	One-phase	+
B5	99.8	Clear	One-phase	+
B6	47.9	Opaque	Two-phase	-
B7	96.1	Clear	One-phase	-

Table 4. Different Mouthwash Formulations^a

Formulation	Tannin	Essential Oil	Base
F1	0.1	0.15	P.G:PEG400 (1:1)
F2	0.2	0.15	P.G:PEG400 (1:1)
F3	0.5	0.15	P.G:PEG400 (1:1)

^aValues are expressed as percentage.

(19), thus, elimination of ethanol may be a good strategy for mouthwash formulation. Therefore, the ultimate goal of the present study was to prepare a mouthwash with two beneficial natural products (Persian oak tannins and *Zataria multiflora* essential oil) in absence of ethanol.

Tannins are a group of polyphenols that precipitate microbial proteins and prevent the development of microorganisms. Polyphenols and tannins are the main substances in oak tree and are abundant in all parts of *Quercus brantii*. Tannins are biosynthetic materials, which have a

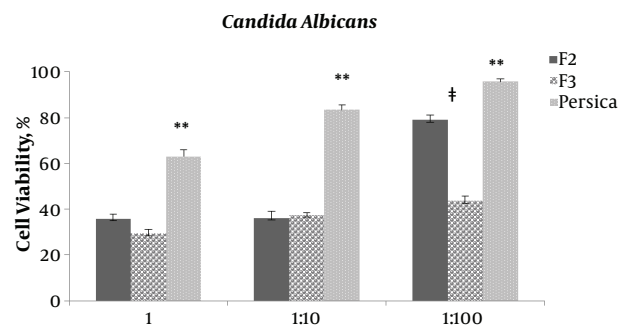
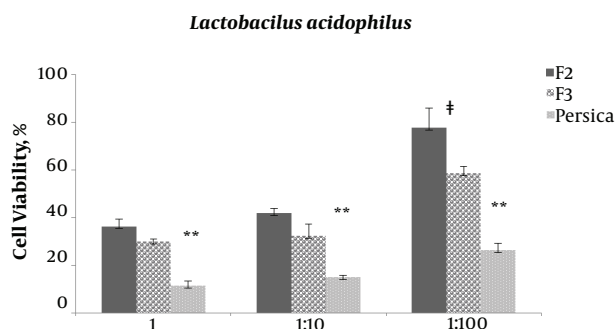
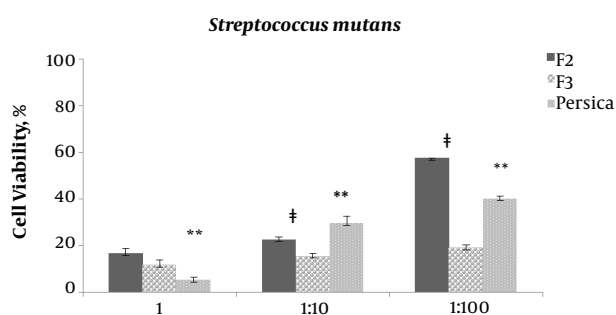
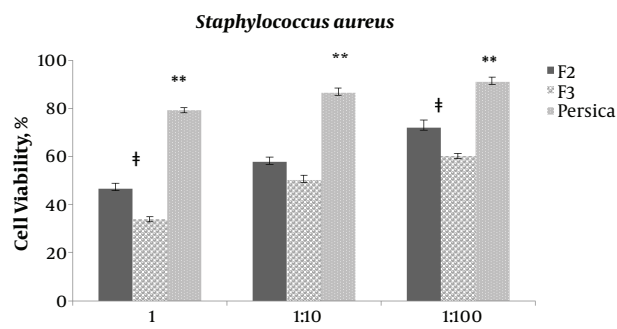
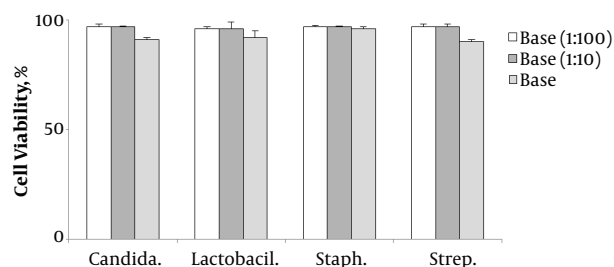


Figure 3. Cell viability percentage of *Candida albicans* in presence of F2, F3, and Persica mouthwash (the ** and † above charts show significant differences between F2 - F3 and Persica with selected formulations (F2 and F3))

potent antibacterial effect (13). Aslani et al. showed that the seed hull (Jaft) of *Q. brantii* contains considerable polyphenols (9). Ebrahimi et al. reported 162 to 648 mg/g of tannic acid/g dry extract for 27 populations of *Quercus brantii*,

Table 5. Quality Control Results of Three Final Formulations F1 - F3

	pH		Tannin, %	
	Day-0	Day-45	Day-0	Day-45
F1	5.3 ± 0.1	5.1 ± 0.2	101.4 ± 2.6	100.7 ± 3
F2	5.2 ± 0.2	5.1 ± 0.1	98.6 ± 1.3	97 ± 1.7
F3	5.5 ± 0.2	5.2 ± 0.1	100.9 ± 1.4	99 ± 1.6

**Figure 4.** Cell viability percentage of *Lactobacillus acidophilus* in presence of F2, F3, and Persica mouthwash (the ** and † above charts show significant differences between F2 - F3 and Persica with selected formulations (F2 and F3))**Figure 5.** Cell viability percentage of *Streptococcus mutans* in presence of F2, F3 and Persica mouthwash (the ** and † above charts show significant differences between F2 - F3 and Persica with selected formulations (F2 and F3))**Figure 6.** Cell viability percentage of *Staphylococcus aureus* in presence of F2, F3 and Persica mouthwash (the ** and † above charts show significant differences between F2 - F3 and Persica with selected formulations (F2 and F3))**Figure 7.** Cell viability percentage of different microorganisms after incubation with base composition of mouthwashes

however, the current results indicated 340 mg tannic acid /g dry extract, which is within the reported range (20).

Furthermore, *Z. multiflora*, is used as an important herbal medicine in Iranian traditional medicine for 1000 years to cure stomachache and agitation. It has several biological and pharmacological properties. There are many studies that have shown that *Z. multiflora* has antibacterial, antifungal, and antioxidant activities (21).

Antimicrobial activity of mouthwash solutions is important to ensure their efficacy in eliminating harmful periodontal bacteria, which leads to prevention of future

dental carries, gingivitis, and periodontitis (22). The most common causative dental plaque organisms, *Candida albicans*, *Lactobacillus acidophilus*, *Streptococcus mutans*, *Staphylococcus aureus* (22), were examined to evaluate selected mouthwashes.

According to the ANOVA test for *Candida albicans* F2 and F3, efficacy was similar for not diluted and 1:10 diluted ($P > 0.05$) solutions while in 1:100 dilution F3 was significantly ($P = 0.000$) more efficient than F2. It is interesting that at all concentrations, F2 and F3, even 1:10 and 1:100 diluted formulations, were significantly ($P = 0.000$) more efficient than Persica mouthwash.

For *Lactobacillus acidophilus* F2 and F3, efficacy was sim-

ilar for not diluted and 1:10 diluted ($P > 0.05$) solutions, while at the 1:100 dilution, F3 was significantly ($P = 0.011$) more efficient than F2. In not diluted, 1:10, and 1:100 diluted concentrations, Persica mouthwash was significantly ($P < 0.05$) more efficient than F2 and F3; however, F3 was more efficient than F2. It should be noted that Persica mouthwash was not diluted.

For *Staphylococcus aureus*, F2 and F3 efficacy was similar for 1:10 diluted ($P > 0.05$), while in not diluted and 1:100 dilution, F3 was significantly ($P = 0.002$ and $P = 0.003$) more efficient than F2. It is interesting that at all concentrations, F2 and F3, even 1:10 and 1:100 diluted formulations, were significantly ($P < 0.05$) more efficient than Persica mouthwash.

For *Streptococcus mutans*, F2 and F3 efficacy was similar for not diluted ($P > 0.05$) while in 1:10 and 1:100 dilution F3 was significantly ($P = 0.015$ and $P = 0.000$) more efficient than F2. In not diluted concentration, F3 had similar efficiency as Persica mouthwash ($P > 0.05$).

Results showed that the selected formulations of F2 and F3 (containing 0.2% and 0.5% tannin) had better antimicrobial activity against all examined microorganisms, except *Lactobacillus acidophilus*, compared with Persica.

F2 and F3 in not diluted and 1:10 diluted concentration had the same effect against *Candida albicans*, while F3 was significantly more effective than F2 in 1:100 diluted form, therefore, F3 was more affordable. This antibacterial effect pattern was similar for *Lactobacillus acidophilus* while it should be noted that the potency was less than Persica.

The best antibacterial activity against *S. aureus*, belonged to F3 with 0.5% tannin. Persica mouthwash (in not diluted concentration) was significantly more effective than the selected formulations against *S. mutans*. However, F2 and F3 had the same activity and significantly more efficacy than Persica mouthwash in 1:10 diluted concentration. In 1:100 diluted concentration, F2 was significantly more effective than Persica mouthwash and F3.

5.1. Conclusion

In this study, the mouthwash formulations, F2 and F3, which contained 0.2% and 0.5% tannin, were the best formulations with adequate stability. The results also showed anti-microbial activity in both mouthwash formulations. Therefore, these two preparations can be used for further studies to establish their efficacy and safety as antibacterial, anti-hemorrhage, and freshener herbal mouthwashes.

Footnotes

Authors' Contribution: Study concept and design, Shadab Dehshahri; acquisition of data, Afshin Afsari; analysis and interpretation of data, Shohreh Alipour, Shadab Dehshahri, Afshin Afsari; drafting of the manuscript, Shadab Dehshahri; critical revision of the manuscript for important intellectual content, Shohreh Alipour, Shadab Dehshahri; statistical analysis, Shohreh Alipour; study supervision, Shadab Dehshahri, Shohreh Alipour.

Financial Disclosure: Authors have not any financial interests related to the material in the manuscript

Funding/Support: The present work a pharm. D thesis that was part of a financially supported by Shiraz University of Medical Sciences

References

1. Araghizadeh A, Kohanteb J, Fani MM. Inhibitory activity of green tea (*Camellia sinensis*) extract on some clinically isolated cariogenic and periodontopathic bacteria. *Med Princ Pract.* 2013;**22**(4):368-72. doi: [10.1159/000348299](https://doi.org/10.1159/000348299). [PubMed: [23485656](https://pubmed.ncbi.nlm.nih.gov/23485656/)]. [PubMed Central: [PMC5586764](https://pubmed.ncbi.nlm.nih.gov/PMC5586764/)].
2. Sadeghi M, Bahramabadi R, Assar S. [Antibacterial effects of Persica and Matrica Herbal mouthwashes on common oral microorganisms: An in vitro study]. *J Mash Dent Sch.* 2011;**35**(2):107-14. Persian.
3. Sekita Y, Murakami K, Yumoto H, Amoh T, Fujiwara N, Ogata S, et al. Preventive effects of *Houttuynia cordata* extract for oral infectious diseases. *Biomed Res Int.* 2016;**2016**:2581876. doi: [10.1155/2016/2581876](https://doi.org/10.1155/2016/2581876). [PubMed: [27413739](https://pubmed.ncbi.nlm.nih.gov/27413739/)]. [PubMed Central: [PMC4931051](https://pubmed.ncbi.nlm.nih.gov/PMC4931051/)].
4. Manipal S, Hussain S, Wadgave U, Duraiswamy P, Ravi K. The mouthwash war - chlorhexidine vs. herbal mouth rinses: A meta-analysis. *J Clin Diagn Res.* 2016;**10**(5):ZC81-3. doi: [10.7860/JCDR/2016/16578.7815](https://doi.org/10.7860/JCDR/2016/16578.7815). [PubMed: [27437366](https://pubmed.ncbi.nlm.nih.gov/27437366/)]. [PubMed Central: [PMC4948542](https://pubmed.ncbi.nlm.nih.gov/PMC4948542/)].
5. Hambire CU, Jawade R, Patil A, Wani VR, Kulkarni AA, Nehete PB. Comparing the antiplaque efficacy of 0.5% *Camellia sinensis* extract, 0.05% sodium fluoride, and 0.2% chlorhexidine gluconate mouthwash in children. *J Int Soc Prev Community Dent.* 2015;**5**(3):218-26. doi: [10.4103/2231-0762.158016](https://doi.org/10.4103/2231-0762.158016). [PubMed: [26236682](https://pubmed.ncbi.nlm.nih.gov/26236682/)]. [PubMed Central: [PMC4515805](https://pubmed.ncbi.nlm.nih.gov/PMC4515805/)].
6. Sharma R, Hebbal M, Ankola AV, Murugaboopathy V, Shetty SJ. Effect of two herbal mouthwashes on gingival health of school children. *J Tradit Complement Med.* 2014;**4**(4):272-8. doi: [10.4103/2225-4110.131373](https://doi.org/10.4103/2225-4110.131373). [PubMed: [25379471](https://pubmed.ncbi.nlm.nih.gov/25379471/)]. [PubMed Central: [PMC4220507](https://pubmed.ncbi.nlm.nih.gov/PMC4220507/)].
7. Dabholkar CS, Shah M, Kathariya R, Bajaj M, Doshi Y. Comparative evaluation of antimicrobial activity of pomegranate-containing mouthwash against oral-biofilm forming organisms: An in vitro microbial study. *J Clin Diagn Res.* 2016;**10**(3):ZC65-9. doi: [10.7860/JCDR/2016/16478.7475](https://doi.org/10.7860/JCDR/2016/16478.7475). [PubMed: [27135005](https://pubmed.ncbi.nlm.nih.gov/27135005/)]. [PubMed Central: [PMC4843390](https://pubmed.ncbi.nlm.nih.gov/PMC4843390/)].
8. Ghassemi F, Momenzade M, Najafian M, Kargar Jahromy H. The effect of hydroalcoholic extract of Oak fruit husks on liver function in Rat (Wistar)The effect of hydroalcoholic extract of Oak fruit husks on liver in Rat (Wistar). *Pars of Jahrom Univ Med Sci.* 2014;**12**(3):7-1. doi: [10.29252/jmj.12.3.7](https://doi.org/10.29252/jmj.12.3.7).
9. Aslani A, Ghannadi A, Najafi H. Design, formulation and evaluation of a mucoadhesive gel from *Quercus brantii* L. and *coriandrum sativum* L. as periodontal drug delivery. *Adv Biomed Res.* 2013;**2**:21.

- doi: [10.4103/2277-9175.108007](https://doi.org/10.4103/2277-9175.108007). [PubMed: [23977649](https://pubmed.ncbi.nlm.nih.gov/23977649/)]. [PubMed Central: [PMC3748668](https://pubmed.ncbi.nlm.nih.gov/PMC3748668/)].
10. Babae N, Baradaran M, Mohamadi H, Nooribayat S. Therapeutic effects of Zataria Multiflora essential oil on recurrent oral aphthous lesion. *Dent Res J (Isfahan)*. 2015;**12**(5):456–60. [PubMed: [26604960](https://pubmed.ncbi.nlm.nih.gov/26604960/)]. [PubMed Central: [PMC4630710](https://pubmed.ncbi.nlm.nih.gov/PMC4630710/)].
 11. Momeni T. *Essential oils and their therapeutic effects*. 2nd ed. University of Tehran Press; 1991.
 12. Sedigh-Shams M, Badiee P, Adl A, Sarab MD, Abbaszadegan A, Nabavizadeh M. In vitro comparison of antimicrobial effect of sodium hypochlorite solution and Zataria multiflora essential oil as irrigants in root canals contaminated with *Candida albicans*. *J Conserv Dent*. 2016;**19**(1):101–5. doi: [10.4103/0972-0707.173212](https://doi.org/10.4103/0972-0707.173212). [PubMed: [26957804](https://pubmed.ncbi.nlm.nih.gov/26957804/)]. [PubMed Central: [PMC4760003](https://pubmed.ncbi.nlm.nih.gov/PMC4760003/)].
 13. Moghbel A, Farjzadeh A, Aghel N, Agheli H, Raisi N. The effect of green tea on prevention of mouth bacterial infection, halitosis, and plaque formation on teeth. *Iran J Toxicol*. 2011;**5**(14):502–15.
 14. Zaidan MR, Noor Rain A, Badrul AR, Adlin A, Norazah A, Zakiah I. In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. *Trop Biomed*. 2005;**22**(2):165–70. [PubMed: [16883283](https://pubmed.ncbi.nlm.nih.gov/16883283/)].
 15. Freires IA, Denny C, Benso B, de Alencar SM, Rosalen PL. Antibacterial activity of essential oils and their isolated constituents against cariogenic bacteria: A systematic review. *Molecules*. 2015;**20**(4):7329–58. doi: [10.3390/molecules20047329](https://doi.org/10.3390/molecules20047329). [PubMed: [25911964](https://pubmed.ncbi.nlm.nih.gov/25911964/)].
 16. Katdare A, Chaulal M. *Excipient development for pharmaceutical, biotechnology, and drug delivery systems: Informa healthcare*. 2006. 441 p.
 17. Kumar A, Selvakumar S. Antiproliferative efficacy of *Tabernaemontana divaricata* against HEP2 cell line and Vero cell line. *Pharmacogn Mag*. 2015;**11**(Suppl 1):S46–52. doi: [10.4103/0973-1296.157682](https://doi.org/10.4103/0973-1296.157682). [PubMed: [26109773](https://pubmed.ncbi.nlm.nih.gov/26109773/)]. [PubMed Central: [PMC4461967](https://pubmed.ncbi.nlm.nih.gov/PMC4461967/)].
 18. Biswas G, N A, Acharya S, Kumawat H, Vishnani P, Tambi S. Evaluation of the efficacy of 0.2% chlorhexidine versus herbal oral rinse on plaque induced gingivitis- a randomized clinical trail. *IOSR J Nurs Health Sci*. 2014;**3**(2):58–63. doi: [10.9790/1959-03255863](https://doi.org/10.9790/1959-03255863).
 19. Badran Z, Bories C, Verner C, Demoersman J, Soueidan A. [Update on side effects of alcohol-containing antiseptic mouthwashes]. *Schweiz Monatsschr Zahnmed*. 2010;**120**(7):603–9. French, German. [PubMed: [21545045](https://pubmed.ncbi.nlm.nih.gov/21545045/)].
 20. Ebrahimi A, Mehregan I, Nejad Sattari T, Assadi M, Larijani K. Population variability in quercus brantii lindl. Based on the acorn morphology and composition of phenolic compounds. *Appl Ecol Environ Res*. 2016;**14**(3):215–31. doi: [10.15666/aeer/1403_215231](https://doi.org/10.15666/aeer/1403_215231).
 21. Aghamohammadi A, Azadbakht M, Hosseinimehr SJ. Quantification of thymol content in different extracts of Zataria multiflora by HPLC method. *Pharm Biomed Res*. 2016;**2**(1):8–13. doi: [10.18869/acad-pub.pbr.2.1.8](https://doi.org/10.18869/acad-pub.pbr.2.1.8).
 22. Masadeh MM, Gharaibeh SF, Alzoubi KH, Al-Azzam SI, Obeidat WM. Antimicrobial activity of common mouthwash solutions on multidrug-resistance bacterial biofilms. *J Clin Med Res*. 2013;**5**(5):389–94. doi: [10.4021/jocmr1535w](https://doi.org/10.4021/jocmr1535w). [PubMed: [23976912](https://pubmed.ncbi.nlm.nih.gov/23976912/)]. [PubMed Central: [PMC3748664](https://pubmed.ncbi.nlm.nih.gov/PMC3748664/)].