Research Journal of Pharmacognosy (RJP) 7(4), 2020: 23-30
Received: 26 May 2020
Accepted: 26 Aug 2020
Published online: 1 Sep 2020
DOI: 10.22127/rjp.2020.113428

Original article

Comparative Effects of Mouthwash Containing *Trachypermum ammi* Oil with Chlorhexidine on Dental Plaque Induced Gingivitis

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Abstract

Background and objectives: The primary etiology for gingivitis is dental plaque resulting in gum inflammation. Beside mechanical procedures, chemical agents in mouthwash can help in removal of plaques. Oil of *Trachyspermum ammi* rich in thymol may provide beneficial effect in control of gingivitis; therefore, the present study aimed to compare the effect of mouthwash containing the oil with chlorhexidine to control dental plaque and inflammation of gingivae. **Methods:** Essential oil of the plant was analyzed using GC-MS and amount of thymol was assayed by GC method. Herbal mouthwash was prepared and applied in an open-label non-randomized study, adult female and male suffering of gingivitis (N=30) entered in two groups after oral prophylaxis. Examining for plaque index (PI), bleeding index (BI), and modified gingival index (MGI) were conducted after two weeks administration of the herbal mouthwash. **Results:** The herbal mouthwash was stable for two months with no physical change and microbial contamination (<10 CFU/mL). All patients completed the study and parameters were evaluated after two weeks. Scores of PI were reduced for 6.67 and 53.33% in the test and control groups, respectively ($p<0.05$). The mouthwash containing the oil reduced BI and MGI for 33.33 and 40.00% in the subjects, while in the group of chlorhexidine, 66.67 and 73.33% of patients benefited the drug to control BI and MGI, respectively ($p>0.05$). **Conclusion:** The effect of *T. ammi* oil in control of inflammation was comparable with chlorhexidine, while chlorhexidine reduced risks of PI, BI, and MGI higher than those receiving the herbal mouthwash.

Keywords: bleeding index; herbal mouthwash; modified gingival index; thymol; *Trachyspermum ammi*

Citation: Saffarpour A, Amini N, Keshtkar A, Mokhber-Dezfuli N, Manayi A. Comparative effects of mouthwash containing *Trachyspermum ammi* oil with chlorhexidine on dental plaque induced gingivitis. Res J Pharmacogn. 2020; 7(4): 23-30.

Introduction

Gingivitis is one of the most prevalent periodontal diseases that cause inflammation of gums. Bacterial biofilm surrounding the teeth leads to the most common form of gingivitis termed as plaque-induced gingivitis [1]. Poor oral hygiene can cause a cascade of events resulting in early loss of teeth. Swollen gum, bleeding gum, and halitosis are classic signs of gingivitis [2]. Mechanical methods including use of toothbrush and dental floss are

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considered cornerstone of periodontal therapy; however, the difficulty of achieving an ideal mechanical removal of dental plaque beside ubiquitous prevalence of gingivitis would suggest that control of periodontal biofilms through mechanical means is insufficient [3]. To control this challenging problem of oral cavity, some chemical agents have been employed such as chlorhexidine which is considered a gold standard in control of plaques. The di-cationic molecule, chlorhexidine has a broad spectrum of antimicrobial activity, which causes lysis and rupture of cell wall through binding with negatively charged molecules of the surface of microorganisms [4]. *Trachyspermum ammi* (L.) Sprague ex Turrill belongs to Apiaceae family and grows in the center, south and southeast of Iran. The most used part of the plant is its fruit, which is small and brown with pungent odor [5,6]. The plant is traditionally suggested to be effective against gastrointestinal problems, kidney and bladder stones [7]. Essential oil of *T. ammi* contains thymol as the major component that has been reported to possess antifungal, antibacterial, insecticidal, bronchodilator, and analgesic effects. Thymol with phenolic structure could change permeability of cell membrane to facilitate penetration of antibiotics [5]; therefore, preparations containing thymol have been applied for various infections. In the monograph of thyme, it is indicated that mouthwashes containing thymol have antibacterial activity and can reduce oral bacteria [8]. Oil of the plant which is rich in thymol along with other constituents may provide beneficial activity in control of oral bacteria in prevention of gingivitis. The present preliminary clinical trial compared an herbal mouthwash containing 0.25% *T. ammi* oil with chlorhexidine 0.2% for their effectiveness in controlling plaque index (PI), bleeding index (BI), and modified gingival index (MGI).

Materials and Methods

Ethical considerations

All participants received complete information about the aim of the study, its duration, and the possible side effects of the rinsing solutions. Informed consent was provided by patients, ethical clearance obtained by the ethical committee of Tehran University of Medical Sciences (Approval code: 8823120003) and ethical principles of Helsinki involving human subjects followed. The protocol of study was registered at www.irct.ir (Registration ID: IRCT2015062022833N1).

Plant material

The fruit of the plant was purchased from herbal shop in 2015 in Tehran, Iran. The sample of the fruit was identified and deposited at the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences with voucher number of PMP: 775. Essential oil of the fruit (450 g) was extracted by hydro-distillation method using Clevenger type apparatus (4 h). Anhydrous sodium sulfate (Merck, Germany) was used to dry the obtained oil. The essential oil was stored in dark sealed container at 4 ºC.

Gas chromatography-mass spectrometry (GC-MS) analysis

The composition of oil was determined by GC-MS on Agilent 6890 with MS instrument (Agilent, U.S.) equipped with a BPX5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm). Oven temperature was held at 50 °C for 5 min and raised to 300 °C at the rate of 3 °C/min for 75 min. Helium at flow rate of 0.8 mL/min was used as carrier gas with split ratio of 1/30. The detector was quadruple mass spectrometer with ionizing voltage 70 eV and an ionization current of 150 µA. Retention time of n-alkanes (C_8-C_{25}) was used to calculate retention indices of the components. By comparison of retention indices with those reported in the literature along with their mass spectra with the Wiley, and NIST libraries with Adams book [9], the components were identified.

Gas chromatography (GC)

The stability of the preparation and amount of major component of the oil was assayed using GC method. Thymol as the main identified component was selected to be quantifed in the *T. ammi* oil according to the standard calibration curve of thymol (12.5-100 µg/mL). The assay was performed using Dani Master (Dani, Italy) equipped with fused OV1 (SE54CB, 25 m×0.25 mm i.d., 0.25 µm film thickness) column and flame ionization detector (FID). Carrier gas was nitrogen with flow rate of 1 mL/min and split ratio of 1/20. Temperature was increased from 75 °C to 250 °C with rate of 15 °C/min for 42 min. Injector and detector temperature was 250 and 260 °C. Farnesol, a sesquiterpene, was used

as an internal standard to minimize variations. Calibration line was plotted by thymol/farnesol (internal standard) peak areas vs concentration of thymol as follows [10]:

$$Y=0.0341X+0.387,\ R^2=0.99$$

Preparation and stability of mouthwash of *T. ammi* oil

Herbal mouthwash was prepared using glycerin (15 mL), ethanol (2 mL), propylene glycol (2 mL), essential oil (0.25) mL and deionized water up to 100 mL [11]. To evaluate stability in use of the herbal preparation, its chemical stability was assayed according to the thymol amount in the mouthwash using GC at room temperature for two months. Physical properties like appearance, color, smell, and clarity of the herbal mouthwash were evaluated and total viable counts of herbal mouthwash were expressed as numbers of colony forming units per mL (CFU/mL) to test stability of the formulation [12].

Patients

Thirty patients with plaque-induced gingivitis were enrolled in the present open-label non-randomized study (15 in each group) in clinic of Faculty of Dentistry, Tehran University of Medical Sciences (2018). Lack of sufficient clinical supply caused protocol deviation in the present study, as it was designed randomized in the protocol while it was performed as a non-randomized clinical trial. Both, scaling and root planning were performed similarly for all the patients who participated in the study by a single periodontist. Treatment group was given mouthwash containing only oil of *T. ammi* and control group was treated with mouthwash of chlorhexidine gluconate 0.2% (Najo, Iran). Both groups were advised to use the mouthwashes twice daily 10 mL each time for one min and 14 days and mechanical control of biofilm through tooth brushing was allowed during the study [13,14]. Plaque index was evaluated at four sites buccal, lingual, mesial and distal wall of six teeth (12, 16, 24, 32, 36, and 44 according to the two digit system adopted by the FDI in 1970) in every patient after 14 days of treatment [15,16]. The probable side effects following the application of mouthwashes were also recorded during the study.

Inclusion and exclusion criteria

Inclusion criteria were age>18 years, 24 teeth, systematically healthy person with plaque-induced gingivitis who complain of gingival bleeding. The exclusion criteria were mouthwash use and antibiotics in past 3 months, allergic reactions to any of the components of any mouth rinse, orthodontical and prosthetic appliances, systematic disorders, and patients with established periodontitis, pregnancy, lactate women, and smoking.

Statistical analysis

The parameters and outcomes were provided as mean (standard deviation: SD or standard error: SE) and differences between groups after treatment were analyzed using fisher exact test, two-tailed. Two effect sizes of risk ratio (RR) and risk difference (RD) were used to compare the treatments of the groups and confidence intervals 95% (CI 95%) were calculated using binomial exact method [17,18]. P value less than 0.05 was considered as significant level. Statistical analyses were performed using Stata 3.1 (Texas 77845, USA).

Results and Discussion

The fruits of the plant yielded 3.5% yellow-colored oil with density of 0.97 mg/mL. Analysis of the oil of *T. ammi* using GC-MS resulted in identification of thirteen monoterpenes of which 90.7% were aromatic monoterpenes, 7.5% monoterpenes hydrocarbon, and 0.5% oxygenated monoterpenes. Thymol (74.2%), p-cymene (16%), and γ-terpinen (7.1%) were the main components of the oil in our previous study (table 1). Since thymol is the major part of the oil, the compound was quantified in the mouthwash of *T. ammi* oil to evaluate the stability in use of the preparation for two months at room temperature. According to the results, the respective amounts of thymol were 26.83 (0.85), 25.64 (0.54), and 24.51 (0.79) µg/mL in the mouthwash for 0, 1, and 2 months, which indicated stability in use of the mouthwash in an acceptable in an acceptable range. No physical changes in appearance, color, and odor of the herbal mouthwash were found for 2 months. Yeast and mold contaminations were in the acceptable range in

the herbal preparation for the period of the analysis (<10 cfu/mL) according to ISIRI 11169. *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aurous* were not found in the herbal preparation.

Table 1. Chemical composition of essential oil of *Trachyspermum ammi* fruit

NO.	Compound	RT[1]	KI[2]	[3]R-KI	Percent (%)
1	α-Thujene	11.3	926	923	0.1
2	β-Pinene	14.06	981	980	0.3
3	β-Myrcene	14.62	992	991	0.1
4	α-Terpinene	16.11	1021	1018	0.1
5	*p*-Cymene	16.63	1031	1027	16
6	Limonene	16.78	1033	1031	0.1
7	β-Phellandrene	16.89	1036	1053	0.1
8	γ-Terpinene	18.32	1063	1062	7.1
9	Terpinen-4-ol	24.71	1190	1177	0.2
10	α-Terpineol	25.50	1206	1189	0.1
11	Carvone	28.04	1260	1243	0.2
12	Thymol	30.19	1306	1290	74.2
13	Carvacrol	30.46	1312	1298	0.5
Monoterpenes hydrocarbons					7.9
Oxygenated monoterpenes					0.5
Aromatic monoterpenes					90.7
Total					99.1

1: Retention time; 2: Kovats index; 3: Reported-KI.

Among 43 referred persons, 30 patients, 66.7% male (N=20) and 33.3% female (N=10), enrolled the study with age range of 21-40 years old, all completed the entire study duration of 14 days (figure 1). There was no significant difference in age of participants between two groups, while the number of males who administered chlorhexidine was higher than those who were treated with *T. ammi* oil mouthwash.

Score of plaque index (PI) were reduced for 53.33% (SE: 12.88; 95% CI: 25.88, 78.73) in the chlorhexidine group and 6.67% (SE: 6.44; 95% CI: 0.17, 31.94) in the test group, *T. ammi* oil. The mouthwash containing 0.25% of *T. ammi* oil reduced BI and MGI for 33.33% (12.17) and 40.00% (12.64) in the subjects, while in the group of chlorhexidine, 66.67% (12.17) and 73.33% (11.41) of the patients benefited the drug to control BI and MGI, respectively (table 2).

According to the effect size of risk ratio (RR) for PI (RR: 0.12; 95% CI: 0.02, 0.55, p=0.01), it could be assumed that chlorhexidine caused 88% reduction in the risk of PI in subjects compared to those who taking herbal mouthwash. The 95% confidence of RR excludes null value (RR=1) indicating significant finding [18]. Similarly, according to the risk difference (RD) for PI outcome, chlorhexidine inhibited plaques better than *T. ammi* oil (RD: -0.46; 95% CI: -0.8, -0.13; p=0.01) and considered statistically significant since the value 0.0 is not in 95% CI of RD.

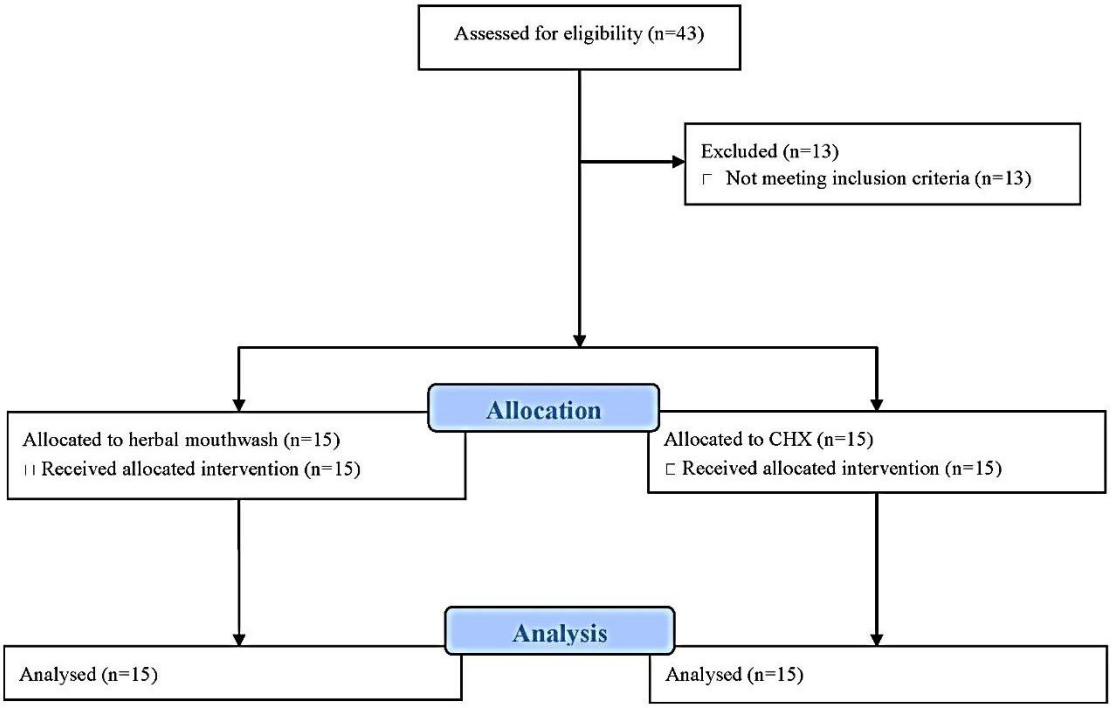

Figure 1. Consort flow diagram of clinical study for comparison of *T. ammi* mouth wash with chlorhexidine

Table 2. The obtained clinical data of *Trachyspermum ammi* oil and chlorhexidine mouthwashes including PI, BI, and MGI indexes after treatments with the calculated effect sizes

Outcomes	***T. ammi* oil**	**CHX**	**ES**	
	% Outcome (SE) (95% CI)		RR (95% CI)	RD (95% CI)
PI (PI<1)	6.67 (6.44) (0.17, 31.94)	53.33 (12.88) (25.88, 78.73)	0.12 (0.02, 0.55)	-0.46 (-0.8, -0.13)
BI	33.33 (12.17) (11.82, 61.61)	66.67 (12.17) (38.38, 88.17)	0.50 (0.23, 1.06)	-0.33 (-0.69, 0.03)
MGI	40.00 (12.64) (16.33, 67.71)	73.33 (11.41) (44.89, 92.21)	0.54 (0.28, 1.05)	-0.33 (-0.69, 0.02)

CHX: chlorhexidine; ES: effect Size; SE: standard Error; PI: plaque index; BI: bleeding index; MGI: modified gingival index; RR: risk ratio; RD: risk difference; CI: confidence interval

Considering the effect size of RR, chlorhexidine decreased risk of the bleeding and inflammation by 50 and 46% relative to those administered herbal mouthwash, the new intervention. Because the value 1.00 (no difference in risk) lies within the range of confidence intervals of RRs for both parameters of BI and MGI and 95% CI is wide, therefore effectiveness of these tested mouthwashes in control of bleeding and inflammation are not statistically significant (p=0.14).

Among the patients treated with chlorhexidine, four of them developed clearly visible staining (26.66% (11.41), CI 95%: 7.78, 55.10), while none of the patients in herbal mouthwash had tooth staining, however stain intensity and extent were not evaluated in our study. The herbal preparation caused burning sensation during rinsing the mouth that was reported by 6 patients (40.00% (12.64), CI 95%: 16.33, 67.71).

The present study was designed to evaluate effect of an herbal mouthwash, containing 0.25% *T. ammi* oil which was rich in aromatic monoterpenes specially thymol, in comparison with chlorhexidine for controlling gingivitis. In the previous studies, antibacterial activity of *T. ammi* oil was reported against several bacterial strains [6,7,19,20]. Antibacterial activity of thymol and thymol-rich essential oils was reviewed and capability of thymol to disturb bacterial plasma membrane was confirmed as a main mechanism of action of the compound against bacteria. The compound was also found to inhibit development of biofilm and inactivate mature biofilms [21]. Gingivitis involves plaque-induced inflammation of gums characterized by redness, swelling, and bleeding of the gingivae. Thus removal of microbial biofilm (plaque) by mechanical methods beside chemical agents like antimicrobial mouthwashes could promote adequate plaque control [22]. A systematic review and meta-analysis provided high-quality evidences that chlorhexidine mouthwash can reduce gingivitis in adjunct to regular mechanical oral hygiene.

Application of chlorhexidine may cause taste disturbance, irritation, mucosal erosion, burning sensation, tooth staining, and calculus build up [23]. Because of these adverse effects researchers investigate the therapeutic effects of herbal or natural mouthwashes in gingivitis and gum tissues [24,25]. Some studies examined anti-plaque and anti-gingivitis activity of mouthwashes containing essential oils and suggested that these preparations are effective in treatment of gingival inflammation with no significant difference with chlorhexidine [26,27]. However, similar to our study chlorhexidine reduced PI more than a mouthwash with combination of essential oils (CoolMint Listerine: 0.064% thymol, 0.092% eucalyptol, 0.060% methyl salicylate, and 0.042% menthol) [27]. A meta-analysis of long-term studies reveled that chlorhexidine mouthwash provided significantly more considerable efficacy regarding plaque control compared to essential oil containing mouth washes [28]. On the other hand, in an in vitro model, the mouthwash containing essential oils (CoolMint Listerine) demonstrated superior anti-plaque biofilm effect to amine fluoride/stannous fluoride, 0.07% cetylpyridinium chloride, and 0.05% cetylpyridinium chloride/0.05% chlorhexidine rinses, while showed comparable activity with chlorhexidine mouthwash [29]. Similarly, in comparison with 0.075% cetylpyridinium chloride, efficacy of an essential oil-containing mouth rinse was superior in control of PI and MGI in a short time trial [26].

The results of the present study are consistent with data of a meta-analysis that reviewed 20 studies and claimed mouth rinses containing essential oils provided about 60% anti-plaque effect of 0.12% chlorhexidine [30]. In the present study, chlorhexidine reduced the risk of plaque

88% more than the mouthwash of *T. ammi* oil maybe because of the higher concentration of the chlorhexidine mouthwash (0.2%) administered to the patients comparing to the previous studies.

Decrease in gingival inflammation is the ultimate goal of anti-plaque activity of a mouthwash to provide protection against destructive periodontal disease. No significant difference with respect to reduction of gingival inflammation was found between essential oil containing mouthwashes and chlorhexidine in long term application in a systematic review [28]. The anti-inflammatory of thymol and other monoterpenes were assessed through in vitro and in vivo tests and the results showed that thymol inhibited inflammation by reduction of elastase, inhibition of COX-1, and inhibition of lymphocyte proliferation [31]. Therefore, anti-inflammation property of the *T. ammi* mouthwash can be attributed to its monoterpene composition mostly thymol. In our study, 2% chlorhexidine reduced risk of BI and MGI indices about 50% higher than *T. ammi* oil mouthwash which was not statistically significant due to the sample size limitation. It was speculated that burning sensation with essential oils may cause patients to decrease rinsing time resulting in diminishing of their effectiveness [32] and in a similar way, 40% of the patients reported burning sensation in herbal mouthwash intervention in our study. According to the previous studies [30,33], bitter taste and formation of stain was reported with chlorhexidine. However, staining may be biased with the poor regular daily oral hygiene of those participants reported the side effect in the present study, because our study lasted for 2 weeks and staining by chlorhexidine appears after 4 weeks or longer [23].

The results of our study demonstrated that although chlorhexidine provided more considerable activity against dental plaque compared to the herbal mouthwash containing *T. ammi oil*, the herbal preparation showed comparable anti-gingivitis activity with chlorhexidine. Considering side effects of chlorhexidine which limited compliance of patients, our results suggested that in the management of periodontal diseases each product can have distinct role or their combination may provide more benefit with lower adverse effects.

Acknowledgements

This study was supported by Tehran University of Medical Sciences grant number of 93.04.169.28175.

Author contributions

Anna Saffarpour administrated technical and logistic support; Niloofar Amini contributed in acquisition of data; Abbas Keshtkar contributed in statistical experience; Najmeh Mokhber-Dezfuli cooperated in collection and assembly of data; Azadeh Manayi designed the study, provided final approval of the version to publish, and obtained funding.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

BI: bleeding index; CFU: colony forming units; CI: confidence intervals 95%; ES: effect size; FID: flame ionization detector; GC: Gas chromatography; GC-MS: gas chromatography-mass spectrometry; MGI: modified gingival index; PI: plaque index; RR: risk ratio; RD: risk difference; SD: standard deviation; SE: standard error