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In Vitro Comparison of Anti-Bacterial Effect of Shirazian and *T. deanesis* Essential oil and their Effective Ingredients Thymol and Carvacrol on *S. mutans* Strain

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ABSTRACT

Use of chemical mouthwashes to control caries can cause side effects, and disturb the balance of the buccal cavity. As a result, the use of natural materials such as plants has been recently considered. Considering the antibacterial features of Thymol and Carvacrol as the key ingredients of Thymus vulgaris and T. deanesis, respectively, this study aimed toward a comparative assessment of the antibacterial effects of T. vulgaris and T. deanesis and their effective ingredients Thymol and Carvacrol on S. mutans strain. This study was experimental. S. mutans strain was received from Iranian Institute of Pasteur, Thymol and Carvacrol were provided by Sigma-Aldrich and T.vulgaris and T.deanesis essential oils were obtained from the leaves of these two plants by the distillation method using Clevenger apparatus. The antibacterial effect was assessed by determining the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and zone of inhibition (ZOI) by macrodilution with disc diffusion at concentrations 15.3-100% of each four materials at 24, 48, and 72 h.Data were analyzed using SPSS 22 software and ANOVA (α=0.05). The MIC and MBC of T.vulgaris and T. deanesis essential oils and that of Thymol and Carvacrol on S. mutans strain were 6.25-6.25%, 6.25-3.125%, 3.125-3.125%, and 3.125-3.125%. The results revealed that ZOI increased significantly with the increasing concentrations (P <0.001). In T. deanesis, with a time increase from 24 to 72h, ZOI indicated a significant difference (P =0.043); however, in the other three substances, ZOI was not statistically significant over time (Carvacrol, P=0.254; Thymol, P =0.237, T. vulgaris, P =0.062). Moreover, the results revealed that ZOI of Thymol and Carvacrol not statistically significant (P =0.971), and ZOI of T. vulgaris and T. deanesis essential oil also were not significantly different (P =0.984); however, ZOI of Thymol and Carvacrol was higher in comparison to T.vulgaris and T.deanesis (P <0.05). The results of this study indicated that T.deanesis essential oil inhibited S.mutans strain growth in a time and concentration-dependent pattern; whereas, the T.vulgaris essential oil, Thymol and Carvacrol inhibited S.mutans strain growth in a concentration-dependent and time-independent pattern.

Keywords: Zone of inhibition, *Streptococcus mutans*, Anti-bacterial, *T. deanesis, T. vulgaris*, Thymol, Carvacrol

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INTRODUCTION

Dental caries is the most prevalent chronic bacterial disease observed in people worldwide. It forms through a complex interaction over time between acid-producing bacteria and fermentable carbohydrate, and the host factors including saliva and teeth [1, 2].

Dental caries is caused by the bacterial activity that can efficiently create an acidic environment to remove the dental minerals. This gelatinous mass of bacteria that binds to the surface of the tooth is called dental plaque [3].

Several (above 330) bacterial species may colonize the buccal cavity of the adults; however, only a small group of bacteria can produce acid and cause caries. The main strains involved in this process are Streptococci (*Streptococcus mutans* and *Streptococcus sobrinus*) and *Lactobacillus* [4, 5].

S. mutans is known to be the main etiologic factor of decay [6] and is naturally present in the mouth as a small group of oral microbial complexes [7].

The routine methods of decay prevention include mechanical methods (toothbrush—dental floss) and the use of chemical agents with antimicrobial features, such as mouthwashes, etc. The use of chemical agents may lead to microbial resistance and can also cause certain complications by disturbing the biological equilibrium of the mouth [8]. In recent years, due to the increasing resistance of pathogenic microorganisms to the chemical antimicrobials and the side effects of these compounds, researches on medicinal plants have been considered in order to discover new antibacterial sources [9].

The thymus plant of the *Labiateae* family contains about 230 species of grass and small shrubs worldwide, and the Mediterranean region is considered as the growth center of these species [10]. Thyme species are used extensively in several parts of the world as beverages (tea), food flavors (spices and seasonings), and herbal medicines [11]. Thyme also has antibacterial, antifungal, antiparasitic, antispasmic, and antioxidant effects [12]. Different species of thyme differ in the types and percentages of chemical compounds; therefore, the analysis of their compounds is particularly emphasized [13]. In 2004, Kalvandi *et al.,* [14] analyzed the essential oils of several thyme species, and the results revealed that the key ingredients of these essential oils include Thymol, Carvacrol, para-cymene, and gamma terpinene with different percentages. *Thymus vulgaris* and *Thymus deanesis* are two important species of thyme [15] T. deanesis cleak is from the Labiatae family, is naturally grown in Iran, and the largest distribution of this plant isin the Alborz and Zagros slopes (16, 17). Thyme is a small shrub with a height of 30–6 cm and has spear like leaves [17]. This plant reveals antibacterial, antifungal, and antiparasitic features [16]. According to Lahoji et al., the most important compounds of thyme are Thymol, Carvacrol, and paracymon [15], which vary according to the growth location, climatic conditions, and the harvesting time [8].

The other species of thyme is Zataria multiflora. This plant has a height of 80–400 cm, Green Willing to White and Scented, small leaves, and short petioles. It grows in Iran and Pakistan [18] and reveals features such as bacterial growth prevention, inhibiting the production of spores and toxins, and antiparasite and antifungal activities [19, 20]. The type and percentage of the compounds in the essential oil of *T. deanesis* are different from those of *T. vulgaris* [22]. The key ingredients of the essential oil of the T. deanesis are Thymol, Carvacrol, and paracymon (13), whereas that of *T. vulgaris* is Carvacrol, paracymon, Thymol, and Gamma terpinene [21].

Thymol and Carvacrol, the main components of the essential oils of the *Labiatae* family, are chemically similar [15]. Carvacrol has a chemical formula (2-methyl-1-5 (1-methyl)) and Thymol is its isomer [23] with a different position of the hydroxyl group [13]. These two compounds have antibacterial, antifungal, and antiyeast properties [22]. The antimicrobial activity of these two compounds is due to the cell membrane penetration, which can chelate with the surface of the membrane two compounds is -1-5 (1-metthe vital activity of the bacteria. Moreover, these compounds reveal their antimicrobial activity by creating a complex with the membrane proteins. [15]. Studies have reported that Thymol and Carvacrol can have synergistic, antagonistic, or ineffective effects [23-25]. Carvacrol is generally recognized as safe and its use in food products is considered [26]. In dentistry, Carvacrol is used as a dental canal disinfectant and in the treatment of

alveolar abscesses [27]. Thymol is used as an antibacterial agent with chlorhexidine to control decay [28].

Various studies have revealed that the essential oil of T. deanesis has an antibacterial effect against Gram negative and Gram positive bacteria [12, 29, 20]; however, to date, no study has investigated the antimicrobial activity of this essential oil on *S. mutans* strain. The antibacterial effect of T. *vulgaris* essential oil has been reported against Gram positive and Gram negative bacteria in the studies by Fournemiti *et al.,* [31] and Fazli *et al*., [32] Moreover, Owila *et al.,* [33] in her study reported the antibacterial effect of *T. vulgaris* on *S. mutans.*

In the previous studies, the antibacterial effects of Carvacrol and Thymol on different strains of bacteria have been investigated [34-36], and Botelho *et al.,* [37] have reported the antibacterial effects of Carvacrol and Thymol on S. mutans.

Considering the antibacterial features of Thymol and Carvacrol as the most important components of essential oil of T. vulgaris and T. deanesis and the possibility of interaction between these two substances (Thymol and Carvacrol) on each other as a synergistic effect or vice versa antagonistic, the purpose of this study was to investigate the bactericidal and bacteriostatic effects of *T. deanesis, T. vulgaris,* Carvacrol and Thymol, alone, and in comparison with each other on *S. mutans.*

MATERIAL AND METHOD

The study protocol that was approved by the Medical University of Isfahan in 2016 includes the following steps:

A: Preparation of the bacterial strain

The *S. mutans* ATCC 1683 strain was prepared from Iran Pastor Institute collection of fungus and bacteria.

B: Preparation the Thymol and Carvacrol

Thymol (T0501) and Carvacrol (W224502) were purchased from Sigma-Aldrich Chemical Company

C: Collecting plants and extracting the *T. vulgaris* (*Z. multiflora*) essential oil.

The *Z. multiflora* leaves were prepared from their natural habitats in Chaharmahal and Bakhtiari province. The collected leaves were air-dried at room temperature for 3 days. For preparation, the essential oil (150 gm) of the dried plant was fragmented in a mixer to obtain its powdered form. Then the essential oil was extracted using the Clevenger apparatus (made in glass-blowing unit of Iranian Research Organization for Science and Technology) by hydrodistillation and was dehumidified by sodium sulfate. The obtained essential oil was preserved at 4°C in sterile vials until further use.

D: Collecting plants and extracting essential oil of *T. deanesis*

The *T. deanesis* leaves were collected from their natural habitat in Chaharmahal and Bakhtiari. The collected leaves were air-dried at room temperature for 3 days. For preparation, the essential oil (150 gm) of dried plant was fragmented in mixer to form powder. Then the essential oil was extracted using the Clevenger apparatus (made in glass-blowing unit of Iranian research organization for science and technology) by hydrodistillation and was dehumidified by sodium sulfate. The obtained essential oil was preserved at 4°C in sterile vials until further use.

Identifying the constituent compounds of studied essential oil

Identification of chemical compounds existing in the essential oil was done using by gas chromatography (GC) and GC mass spectrometry (GC-MS). Agilent 7890 GC device with MS HP-5 column (30 m × 0.25 mm; 0.25 μm film thickness) and helium gas (purity 99/999%) was used as a carrier gas with a linear velocity of 0.8 ml/min. Oven temperature was set at 60°C and was then programmed to 280°C at a rate of 4°C/min. Hamilton syringe for injecting 0.1 ml from samples was used. Injector temperature was 300°C. Furthermore, the essential oils were injected to the GC-MS device. Agilent 5975 GC-MS was used with 70 eV ionization energy. For identifying the constituents, C5–C25 series of n-alkanes were injected to GC-MS under the mentioned conditions and the retention time was calculated. Identification was performed based on the retention indices and comparison to the standard mass spectra.

Preparation the bacterial suspension

18-24 h cultivation of S. mutans strain with 0.5 McFarland (1.5 × CFU/ml) was prepared in the brain heart infusion broth (BHI broth) (Merck, Darmstadt, Germany) medium.

Identification of MIC and MBC (through macrodilution method)

We prepared few sterile tubes and transported 1 cc of BHI broth culture (Merck, Darmstadt, Germany) to each tube. Furthermore, we then transported each essential oil or material with 100% concentration to the first tube. Then the other dilutions (10 concentrations) were prepared through serial macrodilution method. Following this, 1 cc bacterial suspension equivalent (1.5 × 108CFU/ml) bacteria were added to each tube and two tubes were selected, one of them was for positive growth control (culture with intended strain) and the other was considered as negative control (culture with essential oil). The tubes were incubated at 37°C for 18–24 h. Moreover, the results were evaluated through the turbidity test. The concentration of the first tubule in which growth was not observed, determined the minimum inhibitory concentration (MIC) of the bacterial growth.

After determining the MIC, we cultivated the culture from tubes with no turbidity on the BHI broth solid culture medium through linear method. Growth or absence of bacterial growth was assessed after incubation at 37°C for 18–24 h; the least concentration with no bacterial growth in it was reported as the minimum bactericidal concentration (MBC). This experiment was performed thrice for all four materials separately.

Determination of ZOI through the disc diffusion method

For performing this experiment, we took one loop of bacteria from 24 h bacterial suspension culture, which had turbidity equivalent 0.5 Mc Farland and then cultured it on blood agar culture medium uniformly. Moreover, other concentrations of herbal essential oil and effective materials were prepared through the serial macrodilution method. Sterile blank discs were placed on the solid culture medium in which the bacteria were cultivated. Furthermore, the plates were incubated at 37°C for 24, 48, and 72 h. The diameter of the zone of inhibition (ZOI) was measured by a caliper and was statistically analyzed. This experiment was repeated thrice for all four materials separately.

The findings were assessed using the statistical tests (one way ANOVA, two way ANOVA, and repeated measures) by the SPSS software (α = 0.05).

RESULTS

The components of T. deanesis and T. vulgaris essential oils, which were determined by GC-MS method, are displayed in Tables 1 and 2.

Table 1: *T. deanesis* essential oil components

Row	Chemical compounds	In term of percentage
1	α-Thujene	1.19
2	α-Pinene	1.28
3	β-Pinene	0.87
4	Myrcene	1.55
5	α-Terpinene	2.45
6	*p*-Cymene	12.3
7	1,8-Cineole	1.48
8	ɣ-Terpinene	13.76
9	Borneol	2.33
10	Thymol	46.51
11	Carvacrol	3.72
12	Thymyl acetate	0.98
13	Carvacryl acetate	0.55
14	β-Caryophyllene	2.52
15	Germacrene-D	0.37
16	Caryophyllene oxide	0.38
		92.24

Table 2: *T. vulgaris* essential oil components

Row	Chemical compounds	In term of percentage
1	Alpha-Pinene	1.23
2	a-Thujene	0.65
3	Myrcene	1.93
4	a-Terpinene	1.84
5	r-Cymene	13.7
6	l-Terpinene	21.6
7	Trans-sabinene hydrate	1.48
8	Linalool	1.37
9	Borneol	0.24
10	Thymol	44.6
11	Carvacro	2.35
12	a-Terpinenyl acetat	0.33
13	b-Caryophyllene	2.20
14	Trace components	5.53
15	Heavy components	0.90
		99.98

Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of four tested materials in %

The type of material tested	MIC	MBC
Thymol	3.125	3.125
Carvacrol	3.125	3.125
Thymus deanesis celak extract	3.125	6.25
Thymus shirazian celak extract	6.25	6.25

For evaluating the antibacterial effect of *T. deanesis* and *T. vulgaris* essential oils and their effective materials (Thymol and Carvacrol), the MIC, MBC, and ZOI of these materials were

determined. The amount of MIC and MBC for the four studied materials is displayed in Table 3.

Table 4: The average and the standard deviation of ZOI at different concentrations of Carvacrol extract

The mean growth inhibition zone (mm)	Carvacrol concentration (percent)
44.48 ± 2.01	**100**
32.62 ± 3.69	**50**
23.31 ± 1.25	**25**
12.14 ± 1.03	**12.5**
8.71 ± 1.34	**6.25**
7.42 ± 0.82	**3.125**
6.58 ± 0.28	**0**

Table 5: The average and the standard deviation of ZOI at different concentrations of Thymol extract

The mean growth inhibition zone (mm)	Thymol concentration (percent)
50.21 ± 2.72	**100**
30.82 ± 3	**50**
20.27 ± 3.10	**25**
12.73 ± 3.39	**12.5**
9.17 ± 2.76	**6.25**
7.40 ± 1.13	**3.125**
6.46 ± 0.09	**0**

Table 6: The average and the standard deviation of ZOI at different concentrations of T. deanesis extract

The mean growth inhibition zone (mm)	*Thymus deanesis* celak extract concentration (percent)
44.59 ± 1.16	**100**
27.64 ± 1.31	**50**
18.75 ± 0.56	**25**
11.66 ± 2.20	**12.5**
7.64 ± 1.34	**6.25**
6.77 ± 0.3	**3.125**
6.41 ± 0.1	**0**

Table 7: The average and the standard deviation of ZOI at different concentrations of T.vulgaris extract

The mean growth inhibition zone (mm)	Thymus shirazian celak extract concentration (percent)
42.6 ± 2.65	**100**
27.13 ± 0.6	**50**
17.87 ± 1.17	**25**
13.04 ± 0.71	**12.5**
8.24 ± 0.79	**6.25**
6.77 ± 0.1	**3.125**
6.40 ± 0	**0**

For evaluating the effect of the concentration on ZOI, the mean ZOI was determined in different concentrations of the four materials and compared statistically. In Tables 4–7, the average and the standard deviation of ZOI in S. mutans strain have been displayed in different concentrations of the four studied materials (Carvacrol, Thymol, *T. deanesis* essential oil, *T. vulgaris* essential oil).

For evaluating the effect of the concentrations of the four studied materials on the average of ZOI, we used two way ANOVA analyses. The results of this analysis indicated that the average of ZOI at different concentration of the four studied materials is considered to be statistically significant ($P < 0.001$). For comparing the effect of type of material on ZOI, the average of ZOI was determined in 100% concentration of all four studied materials and was compared statistically. The average and the standard deviation of ZOI is listed for 100% concentration of these materials in Table 8.

Table 8: The average and the standard deviation of ZOI in S. mutans, exposed to the four studied materials at 100% concentration

Standard deviation	Average inhibition	The type of material tested
2.01	44.48	Carvacrol
2.72	50.21	Thymol
1.16	44.59	*Thymus deanesis* celak extract
2.65	42.6	*Thymus shirazian* celak extract

Table 9: The average and the standard deviation of *S. mutans* ZOI at 100% concentration of all four studied materials at 24, 48, 72 h

The type of material tested	Mean and Standard eviation in first 24h	Mean and Standard eviation in 48h	Mean and Standard Deviation in 72h
Thymol	50.21 ± 2.72	50.60 ± 2.52	51.42 ± 3.82
Carvacrol	44.48 ± 2.01	44.74 ± 2.26	45.29 ± 2.06
Thymus shirazian celak extract	42.6 ± 2.65	43.21 ± 2.27	43.33 ± 2.29
Thymus deanesis celak extract	44.59 ± 1.16	45.00 ± 1.09	45.34 ± 1.25

One way ANOVA analysis and Tukey's test revealed that the bacterial ZOI in plates with Carvacrol and Thymol did not have statistically significant difference ($P = 0.971$) also zone of inhibition with *T. vulgaris* or *T. deanesis* has no statistically significant difference (P value=0.984);

however, the amount of ZOI in plates with Thymol and Carvacrol was more than that with *T. vulgaris* and *T. deanesis* ($P < 0.05$). For assessing the effect of passing of time on ZOI at 100% concentration of all four materials, their average was determined and compared statistically after 24, 48, and 72 h. In Table 9, the average and the standard deviation of *S. mutans* ZOI is displayed for all four studied materials at 24, 48, and 72 h.

For evaluating the effect of passing of time from 24 h to 48 and 72 h on ZOI, we used repeated measures ANOVA and Bonferroni test, and the results indicated that in T. deanesis there was statistically significant difference in ZOI with passing of time from 24 h to 72 h (P value = 0.043), but in the other three other materials there was no statistically significant difference in ZOI after passing of time (Carvacrol, P = 0.254; Thymol, P = 0.237; *T. vulgaris*, P = 0.062).

DISCUSSION

The null hypothesis of this study was: first, *T. vulgaris* and *T. deanesis* essential oils and their effective materials (Thymol and Carvacrol) had no effect on the *S. mutans* strain. Secondly, all four materials revealed similar effects on *S. mutans* strain. The null hypothesis of the present study was rejected based on the findings.

Thymol and Carvacrol are the most important phytochemical components of both *T. deanesis* and *T. vulgaris* essential oils. In different studies, the content of Thymol was reported as 4.2–85% and that of Carvacrol was reported as 2–52.3% for *T. deanesis* essential oil [38-40]. Moreover, the content of Thymol was reported as 44–60% and that of Carvacrol was reported as 2.2–4.2% for T. vulgaris essential oil [41]. In the present study, the concentrations of Thymol and Carvacrol were 46.51 and 3.72% for *T. deanesis* essential oil and were 44.6 and 2.35% for *T. vulgaris* essential oil. The amount of Thymol and Carvacrol in these two essential oils depending on the location and climate, cultivation area, harvest time, maintenance method, drying plant, and essential oil extraction method, is different [42-47]. *T. deanesis* has considerable antibacterial effect due to the high amount of phenol components, specially Thymol and Carvacrol.[48] To date, no study has assessed the antibacterial effect of this essential oil on *S. mutans.* In the present study, the inhibitory effect of *T. deanesis* was evaluated on the S. mutans for the first time and the results revealed that this essential oil inhibited the growth of S. mutans strain. The studies performed by Dadashpour *et al.* [49], Ghannadi *et al.* [30], and Teimouri [29],which assessed the antibacterial effect of *T. deanesis* essential oil on *Staphylococcus aureus, Echerischia coli*, and *Helicobacter pylori* indicated that this essential oil can inhibit the growth of the mentioned strains in similarity to the present study; however, the studies performed by Pirbalouti *et al*., [50] and Dadfar *et al*., [51] that evaluate the antibacterial effect of T. deanesis on Streptococcus iniae and Pseudomonas aeruginosa indicated that in contrast to present study, the essential oil of this thyme had no inhibitory effect on the studied strains. The difference in the bacterial strain and also the effective components of the studied essential oil is the possible reason for disagreement of the findings.

T. vulgaris is one of the most important components in inhibiting the bacterial growth as it contains more than 60 components with antibacterial and antioxidant effects [52].

The T. vulgaris antibacterial effect has been revealed on many species of Gram positive and Gram negative bacteria such as *Staphylococcus proteus, Salmonella Typhi, E. coli, Bacillus cereus*, and *Shigella*; however, the inhibitory effect against the Gram positive species is more obvious[31,32].
In the study performed by Feizabadi, Mahboubi *et al.,* [53], and Owlia *et al.,* [33] the inhibitory effect of this essential oil was revealed on the growth of *S. mutans* strain.

Owlia *et al.,* [33] reported that the amount of MIC and MBC was 10 and 25% for *T. vulgaris*; however, the present study reported that the amount of MIC and MBC was 6.25 and 6.25% for this essential oil, and the comparison between these two studies indicated that the antibacterial effect of *T. vulgaris* essential oil in the present study was more than that in Owlia's study.

Alternatively, Feizabadi and Mahboubi in 2009 [53] reported that MIC of T. vulgaris on *S. mutans* was equal to 1 mg/ml, and the comparison between this study and the present study (MIC = 6.25% equal to 29 mg/ml) indicated that the *T. vulgaris* in the mentioned study is more effective.
The difference in the bacterial strain and also the effective components of the studied essential oil is the possible reason for the disagreement of the

findings. In all the mentioned studies, *S. mutans* strain was assessed but its species were different. These species are different in structure, performance, and sensitivity to the antibacterial materials [54].

The present study findings indicated that Carvacrol has inhibitory and bactericidal effect on S. mutans; thus, the MIC and MBC was reported as 3.125%. Carvacrol as one of the main phenol components of *T. deanesis* and *T. vulgaris*, has an antibacterial effect.

Carvacrol reacted to the bacterial cytoplasmic membrane lipids due to its hydrophobic nature, and as a result by creating distance between the fatty acid chains of bacterial membrane, it increases the permeability and swelling and finally leads to death [55, 56].

In the previous studies, the antibacterial effect of Carvacrol was assessed on different strains of bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *P. aeruginosa*, *Salmonella typhi*, and *Staphylococcus enterica* [57-59]. In addition, some of the studies revealed the inhibitory and bactericidal effect of Carvacrol on the antibiotic resistant strains; Magi *et al*., in 2015 [60] demonstrated the Carvacrol antibacterial effect on Streptococcus group A that was resistant to erythromycin. Moreover, in the study performed by Nostro *et al.,* [61], the antibacterial effect of Carvacrol was reported on the methicillin resistant Staphylococcus.

Botelho et al. in 2007 [37], assessed the antibacterial effect of Carvacrol on the oral pathogens like Streptococcus. According to the findings of these studies, when the concentration of Carvacrol is 50 mg/ml (equal to 12.5%), the ZOI was reported as 8 mm, whereas in the present study, the ZOI was reported as 12 mm in the aforementioned concentration of Carvacrol. Hence, the Carvacrol used in the present study is more effective than the one in Botelho's study. The differences of S. mutans strains can be the possible reasons of this issue; in the present study, 1683 ATCC strains were evaluated, whereas Botelho studied 10239ATCC strains.

The other phenol components of *T. deanesis* and *T. vulgaris* include Thymol that is very similar to Carvacrol in structure, and the only difference is the location of its hydroxyl group.

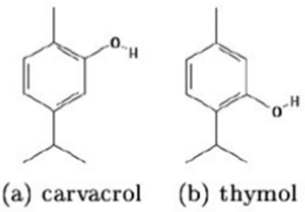

Figure 1: Chemical formula of Thymol and Carvacrol

Nevertheless, this small structural difference can affect the performances of these two materials, for instance, Carvacrol has synergic effect with ampicillin and nitrofurantoin but Thymol does not reveal any such effects [62].However about the inhibitory and bactericidal effect on the Streptococcus strain, our findings similar to the study performed by Botelho et al. [37], revealed that the antibacterial effect of these two materials (Thymol and Carvacrol) has no statistically significant difference (P = 0.971).Our results indicated that the antibacterial effect (measure of ZOI) of both T. deanesis and T. vulgaris essential oil had no statistically significant difference (P = 0.984), and Thymol and Carvacrol also revealed no statistically significant difference (P = 0.971); however, the antibacterial effect of Thymol and Carvacrol was more than that of T. vulgaris and T. deanesis ($P < 0.05$).

For assessing the possible reason of this issue, it is necessary to consider: (1) the percentage of Thymol and Carvacrol in the essential oil and (2) the interaction of these two materials.Considering that the study by Lambert *et al*., [56] in 2001 reported that Thymol and Carvacrol do not have synergistic or antagonistic effects and are neutral against each other, the antibacterial effect of *T. deanesis* and *T. vulgaris* essential oils, which contains a percentage of these compounds as Thymol (*T. deanesis* = 46.51% and T. vulgaris = 44.6%) and Carvacrol (*T. deanesis* = 3.72% and *T. vulgaris* = 2.35%) are less than their pure use. One of the limitations of the present study is the inability of this study to determine the minimum bacterial death time, and moreover, that the study was conducted in the laboratory. Therefore, it is suggested that in subsequent studies, the bacterial growth chart should be based on time and in vivo

CONCLUSION

In general, the results of this study revealed that the essential oil of *T. deanesis* with a concentration- and time-dependent pattern inhibited *S. mutans* strain growth, and the essential oil of T. vulgaris and the active ingredients of Thymol and Carvacrol with a time-dependent and time-independent pattern, respectively, inhibited *S. mutans* growth. Moreover, the antibacterial effect of Thymol and Carvacrol is higher than that of *T. vulgaris* and *T. deanesis*. Therefore, this plant can be considered as a potentially effective combination for use as a mouthwash. Therefore, further clinical studies are recommended.

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